# 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.*[1] 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

[1] 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.

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.

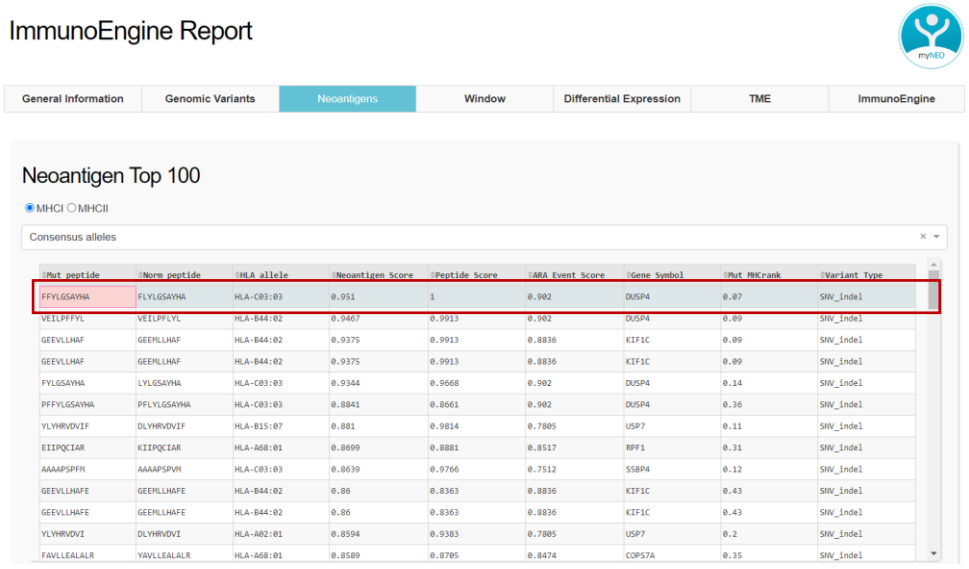
The **Differential Expression tab** allows more careful investigation of the expression level of known cancer genes, MHC related genes, CTA genes and affected genes in the top 100 neoantigens. The percentile expression plot on top visualises if genes are highly (red) or lowly (blue) expressed compared 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.

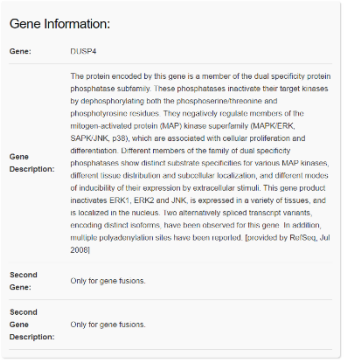
## Use Case Example

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




| Mut peptide | Norm peptide | HLA allele | Neoantigen Score | Peptide Score | ARA Event Score | Gene Symbol | Mut MHCrank | Variant Type |
|---|---|---|---|---|---|---|---|---|
| FFYLGSAYHA | FLYLGSAYHA | HLA-C03:03 | 0.951 | 1 | 0.902 | DUSP4 | 0.07 | SNV_indel |
| VEILPFFYL | VEILPFLYL | HLA-B44:02 | 0.9467 | 0.9913 | 0.902 | DUSP4 | 0.09 | SNV_indel |
| GEEVLLHAF | GEEMLLHAF | HLA-B44:02 | 0.9375 | 0.9913 | 0.8836 | KIF1C | 0.09 | SNV_indel |
| GEEVLLHAF | GEEMLLHAF | HLA-B44:02 | 0.9375 | 0.9913 | 0.8836 | KIF1C | 0.09 | SNV_indel |
| FYLGSAYHA | LYLGSAYHA | HLA-C03:03 | 0.9344 | 0.9668 | 0.902 | DUSP4 | 0.14 | SNV_indel |
| PFFYLGSAYHA | PFLYLGSAYHA | HLA-C03:03 | 0.8841 | 0.8661 | 0.902 | DUSP4 | 0.36 | SNV_indel |
| YLYHRVDVIF | DLYHRVDVIF | HLA-B15:07 | 0.881 | 0.9814 | 0.7805 | USP7 | 0.11 | SNV_indel |
| EIIPQCIAR | KIIPQCIAR | HLA-A68:01 | 0.8699 | 0.8881 | 0.8517 | RPF1 | 0.31 | SNV_indel |
| AAAAPSPFM | AAAAPSPVM | HLA-C03:03 | 0.8639 | 0.9766 | 0.7512 | SSBP4 | 0.12 | SNV_indel |
| GEEVLLHAFE | GEEMLLHAFE | HLA-B44:02 | 0.86 | 0.8363 | 0.8836 | KIF1C | 0.43 | SNV_indel |
| GEEVLLHAFE | GEEMLLHAFE | HLA-B44:02 | 0.86 | 0.8363 | 0.8836 | KIF1C | 0.43 | SNV_indel |
| YLYHRVDVI | DLYHRVDVI | HLA-A02:01 | 0.8594 | 0.9383 | 0.7805 | USP7 | 0.2 | SNV_indel |
| FAVLLEALALR | YAVLLEALALR | HLA-A68:01 | 0.8589 | 0.8705 | 0.8474 | COPS7A | 0.35 | SNV_indel |

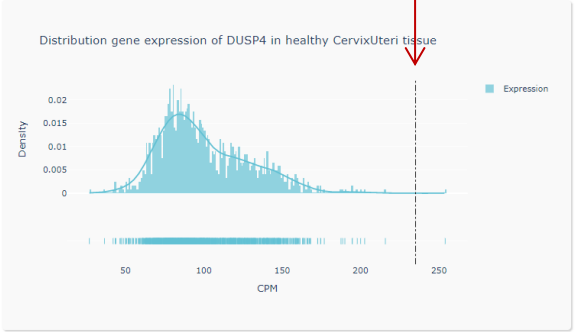
**Note**: it should be noted that FFYLGSAYHA is considered the best neoantigen targeting a consensus HLA-type, more specifically HLA-C03:03. If we want to select a neoantigen directed against another HLA-type, the dropdown menu on top can be used.




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.

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.

| Gene | Percentile | TPM | CPM |
|---|---|---|---|
| ADCK1 | 0.5 | 9.24 | 8.71 |
| AK3 | 0 | 19.48 | 31.64 |
| ANKRA2 | 0.02 | 8.56 | 7.55 |
| BRAF | 0 | 5.68 | 20.69 |
| CHD8 | 0.23 | 17.28 | 83.4 |
| CHKA | 1 | 30.08 | 33.93 |
| COG4 | 0.86 | 39.85 | 73.86 |
| COPS7A | 0.71 | 68.75 | 74.77 |
| CSE1L | 0.89 | 46.76 | 111.03 |
| DLG1 | 0.99 | 50.64 | 97.64 |
| DST | 0 | 32.78 | 165.67 |
| DUSP4 | 1 | 61.26 | 235.16 |
| DYM | 0.91 | 33.58 | 43.12 |
| FAM84B | 0.28 | 2.5 | 8.22 |
| FASTKD1 | 0.78 | 11.6 | 20.34 |

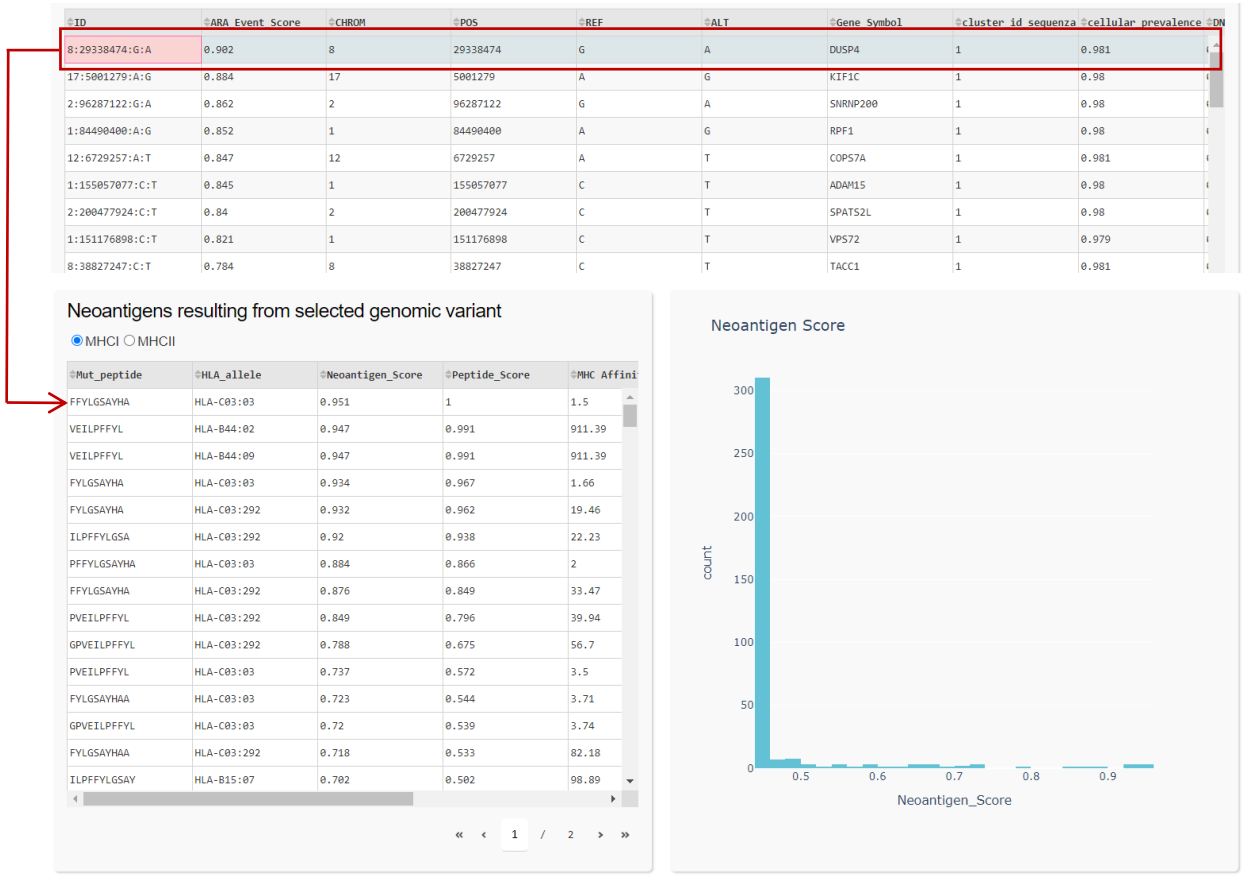


If we switch back to the **Neoantigen tab**, we can see that the selected neoantigen has a very high ARA Event Score indicating that the variant is a high-confidence variant with a high expression. Indeed, if we examine the variant information box, we can see a high allelic frequency (AF), both on DNA 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_MHCrank | 1.45 |
| Mut_peptide | FFYLGSAYHA |
| Mut_MHCAffinity | 1.5 |
| Mut_MHCrank | 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 | 0.902 | 8 | 29338474 | G | A | DUSP4 | 1 | 0.981 | |
| 17:5001279:A:G | 0.884 | 17 | 5001279 | 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 | COPS7A | 1 | 0.981 | |
| 1:155057077:C:T | 0.845 | 1 | 155057077 | C | T | ADAM15 | 1 | 0.98 | |
| 2:200477924:C:T | 0.84 | 2 | 200477924 | 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 | |

Neoantigens resulting from selected genomic variant

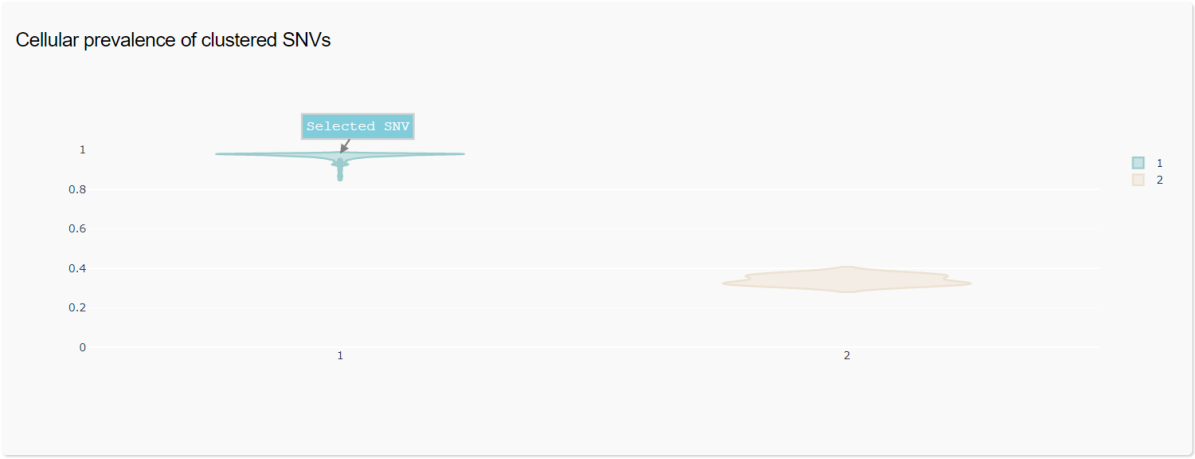
MHCI MHCII

| Mut_peptide | HLA_allele | Neoantigen_Score | Peptide_Score | MHC Affini |
|---|---|---|---|---|
| FFYLGSAYHA | 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 |
| FYLGSAYHA | HLA-C03:03 | 0.934 | 0.967 | 1.66 |
| FYLGSAYHA | HLA-C03:292 | 0.932 | 0.962 | 19.46 |
| ILPFFYLGSA | HLA-C03:292 | 0.92 | 0.938 | 22.23 |
| PFFYLGSAYHA | HLA-C03:03 | 0.884 | 0.866 | 2 |
| FFYLGSAYHA | 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 |
| FYLGSAYHAA | HLA-C03:03 | 0.723 | 0.544 | 3.71 |
| GPVEILPFFYL | HLA-C03:03 | 0.72 | 0.539 | 3.74 |
| FYLGSAYHAA | HLA-C03:292 | 0.718 | 0.533 | 82.18 |
| ILPFFYLGSAY | HLA-B15:07 | 0.702 | 0.502 | 98.89 |

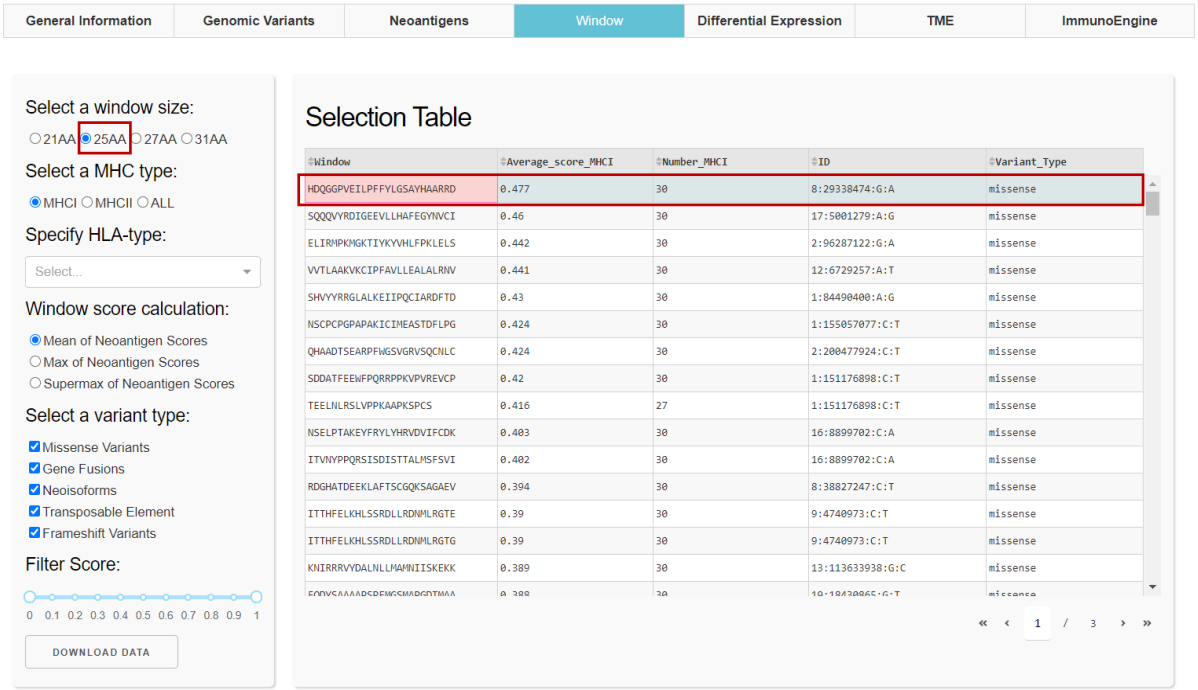
« ‹ 1 / 2 › »

Neoantigen Score

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.



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.