**Supplementary Materials**

Supplemental Information for Methods

Generation of neoantigen peptides

Tumor neoantigens were predicted and prioritized by in-house bioinformatics pipeline iNeo-SUITE, which consists modules of sequencing read filtering, genome alignment, mutation calling, HLA typing, MHC affinity prediction, gene expression profiling, vaccine peptide sequence design and prioritization based on therapeutic potency. FastQC (v0.11.4) was used for sequencing data quality control. Reads with quality score below 15 or more than 4 N bases were discarded [[1](#_ENREF_1)] . The qualified reads were then mapped to the human reference HG38 (Human Genome version 38) by using Burrows-Wheeler Aligner software (BWA, v0.7.12). Next, comparing to normal sample, tumor somatic mutations were identified by integrating the mutation calling results from Mutect (v2.0), Varscan 2 (v.3.5.19) [[2-5](#_ENREF_2)], Strelka (1.0.11) and somatic-sniper (v1.0.5.0). Then, somatic mutation candidates were ranked based on their reliablity, and were further manually inspected in Integrative Genomics Viewer (IGV v.1.0.6) according to their alignment profile. Meanwhile, germline mutations in both normal and tumor samples were identified by GATK haplotypecaller. The database of Single Nucleotide Polymorphism (dbSNP) (https://www.ncbi.nlm.nih.gov/snp/) and 1000 Genome datasets were used to filter out high population frequency (PF) mutations (PF > 1%) from somatic mutation candidates. Next, the mutations were further annotated by Variation Effect Predictor (VEP, ensemble v89) [[5-7](#_ENREF_5)]. Using the reference sequences from IMGT datasets [[6](#_ENREF_6)], HLA typing and quantification was done by OptiType (v1.3.1), Polysolver (V4), PHLAT (release 1.1) and in-house software iNeo-HLA. Subsequently, the flanking sequence of peptide or the upstream sequence of peptide were extracted from human protein database for single nucleotide mutations or frame-shift and stop-loss mutations. To predict the neo-epitopes within those peptides, all possible segments that contain mutation-induced amino acid(s) were further extracted with length ranges from 8 to 16 amino acids (8-11 mer for HLA class I and 12-16 mer for HLA class II). The HLA class I neo-epitopes were predicted by in-house software iNeo-Pred, which is a deep-learning and machine-learning integrated predictor trained on datasets from IEDB and mass spectrometry (MS) profiling of HLA ligands. The HLA class II neo-epitopes were predicted by NetMHCIIPan (v4.0) [[8](#_ENREF_8)].

After identifying all the neo-epitope candidates, in-house software iNeo-PRIOR was used to rank mutations based on their therapeutic potency, which considered mutation prevalence, gene expression, affinity change, epitope number, and heterologous level of mutant peptide, etc. Since all these factor will contribute to the final therapeutic effect, a mathematical formula was designed to integrate all these factor into single socre for prioritization:

In this formula, *iNeo\_Score* refer to the score used for prioritization, Ag stands for mutation prevalence, E stands for the average gene expression obtained from TCGA database, H stands for the heterologous level of mutant peptide. Mi and Mii stand for the quality index which take affinity change and epitope number into account for epitopes presented by MHC I molecules and MHC II molecules respectively. Generally, we would choose mutations whose scores ranked at the top, and also consider the reliability of the mutation (manually examination and Sanger sequencing) and the gene function (whether the mutation was in an oncogenic or cancer-driver gene).

In-house software iNeo-DESIGN was applied to automatically design vaccine peptide sequences (length ranges from 15 to 30 amino acids) containing neo-epitopes of both HLA class I and II. The safety risk (potential peptide toxicity and bioactivity) and synthesis difficulty of the vaccine peptides were also evaluated and optimized accordingly.

Finally, the customized long peptides were manufactured by chemical synthesis at GMP-like standard and clinical-grade (bacteria-free, >95.0% purity and quantities of bacterial endotoxin less than 10 EU/mg).

**IFN-γ enzyme-linked immunospot (ELISpot) assay**

Peripheral blood (10-30 mL) was collected from each patient, followed by the isolation of peripheral blood mononuclear cells (PBMCs) by Ficoll/Hypaque density-gradient centrifugation (GE Healthcare). IFN-γ ELISpot assays were performed with Human IFN-γ precoated ELISpot kit (DAKEWEI). Briefly, after adding 200μL serum-free medium into each well, the plate was incubated at room temperature for 5-10 minutes before discarding the solution. 100 μL cell suspension was added to each well at a density of 2×105 cells per well, followed by the addition of 5-10 μg/mL neoantigen peptides into experimental wells respectively or 2 μg/mL of CEF peptide into positive control wells. Then the mixtures were incubated at 37 °C for 16-24 hours. 200 μL pre-cooled deionized water was added into each well to lyse at 4 °C for 10 minutes. The plates were washed for 6 times before the addition of 100 μL biotin-labeled antibody and then incubated at 37 °C for 1 hour. After washing the plates, 100 μL enzyme-labeled avidin working solution was added into each well and incubated at 37 °C for 1 hour. AEC solution mix was then added into each well after washing the plates, and the plates were kept in dark for 25 minutes at room temperature before adding deionized water to stop reaction. ELISpot plate was then placed in an automatic plate reader to adjust appropriate parameters, spot count, and statistical analysis. The samples with more than 100 spots after noise subtraction (based on negative control group) were considered to be strong positive results, while samples with 8 to 20 spots were considered to be weak positive results.

Cytometric analysis of T-lymphocyte activity through surface biomarker

Antibodies were purchased from Biolegend, as shown in Table A. PBMCs were isolated, and T cells were labeled following manual instruction. In brief, the corresponding antibodies were added into a blank flow tube, mixed with 100 μL T cell sample thoroughly, and then incubated in dark for 15 minutes. 2 mL of erythrocyte lysate (Zhejiang Bozhen Biotechnology Co., Ltd.) was added into the sample, mixed entirely, and then incubated in dark for 10 minutes. The sample was centrifuged at 500×g for 5 minutes, and 1620 μL of supernatant (440 μL remained) was removed. Next, 10 μL of absolute count microspheres was added into the tube and mixed well. The sample was ready for cytometric analysis.

Table A: Antibodies for flow cytometry

|  |  |  |
| --- | --- | --- |
| Fluorescence | Antibody | Clone |
| FITC | CD279（PD-1） | EH12.2H7 |
| PE | CD197（CCR7） | G043H7 |
| PerCP/Cy5.5 | CD4 | OKT4 |
| PE/Cy7 | CD45RA | HI100 |
| APC | CD38 | HB-7 |
| A700 | CD8 | SK1 |
| APC/Cy7 | CD3 |  |
| BV421 | HLA-DR |  |
| BV510 | CD45 |  |
| BV605 | CD152（CTLA-4） | BNI3 |

**Cytometric Bead Array (CBA) Analysis of Cytokines**

The concentrations of serum cytokines were measured by CBA, according to the manufacture’s protocol (Hangzhou Saiji Biotechnology Co., Ltd). Th1/Th2 cytokine kit was applied. In brief, 25 μL solution of captured microspheres was added into a blank flow tube, followed by the addition of 25 μL buffer solution of microspheres. The mixture was incubated in dark for 30 minutes. 25 μL fluorescence detection reagent and 25 μL serum were added successively. The solution was vortex-mixed and then incubated in dark for 2.5 hours. After the addition of 1mL of PBS solution, the sample was centrifuged at 200×g for 5 minutes. Following the removal of supernatant, 100 μL PBS solution was added to resuspend the sample. The samples were tested by flow cytometer, and the acquired data were analyzed using FlowJo V10 software.

**References**

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Supplementary Tables

Supplementary Table S1. The treatment process of patients before and after neoantigen vaccine therapy

Supplementary Table S2. QC metrics of whole-exome sequencing for each patient

Supplementary Table S3. Overview of identified neoantigens for each patient

Supplementary Table S4. Summary of the number of identified somatic mutations, predicted neoantigens and synthesized vaccine peptides

Supplementary Table S5. HLA allotypes in both normal and tumor cells for each patient

Supplementary Table S6. Summary of designed and synthesized peptides for each patient

Supplementary Table S7. The concentration of INF-γ in peripheral blood for each patient

Supplementary Table S8. Mutation of KRAS for each patient