nature portfolio

Corresponding author(s): SASKIA BISKUP

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# Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

## Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed | |
|---|---|---|
| ☐ | ☒ | The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☒ | ☐ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | The statistical test(s) used AND whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☐ | ☒ | A description of all covariates tested |
| ☒ | ☐ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ | For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ | Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | Patient data were collected and curated in tabular form. |
| Data analysis | Patient data were collected and curated in tabular form and imported into R Version 4.0.4. All survival statistics were computed using the "survival" package (reference 35, 36). Statistical significance of survival differences was computed using the log-rank test (function survfit). Kaplan-Meier curves were generated using the package "survminer" (reference 37). The median follow-up time was calculated with reverse Kaplan-Meier. All univariate statistics were computed using the Cox Proportional Hazards model implemented in function coxph. Continuous variables (HRD score, TMB) were tested both as-is and as binary variables using thresholds.<br>The patient's HLA type was identified using OptiType algorithm (reference 28). Selection of epitopes was performed using an in-house developed and proprietary neoantigen selection algorithm as previously described (reference 29).<br>FACS Data were analysed using FlowJo version 10.5.3 (FlowJo LLC, Ashland, AZ, USA).<br>The matching of our vaccine patients (treatment group) to patients from four public datasets (control group) was implemented using the MatchIt R package (reference 23). The balance of the propensity scores before and after matching was visually assessed using jitter plot and histograms. The balance of individual variables was evaluated using the standardized mean difference before and after the matching (variable balance plot). Multivariate Cox regression model was fitted with the 5 variables together with the treatment group variable. |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

With publication, the data collected, generated, and evaluated for this article can be provided by the corresponding author. The data are not publicly available because they contain information that compromise the privacy of the research participants. Information can be made available as de-identified data after both parties have signed a data access agreement. The publicly available data used in this analysis are available in the supplementary tables of the corresponding publications. The data published by Lakomy et al. (reference 19) were requested. The remaining data are available within the Article, Supplementary Information or Source Data file.

## Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

| | |
|---|---|
| Reporting on sex and gender | Findings apply to both sexes. Sex was determined based on patient documents, reflecting genetic (chromosomal) sex. Gender has not been collected. Univariate analysis has been performed with sex as covariate. |
| Reporting on race, ethnicity, or other socially relevant groupings | n/a |
| Population characteristics | Our report includes patients with histologically defined and molecularly confirmed IDH-wildtype GBM as well as patients with previously diagnosed GBM recently reclassified as diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype according to the 5th edition of the CNS WHO classification. At the time of first vaccination, 159 patients (92%) had received standard of care treatment with radiation therapy and temozolomide chemotherapy. Three patients had received radiotherapy only, five patients had received TMZ only, and one patient was treated with combined radiotherapy and lomustine chemotherapy. Eighty-seven patients were treated with glucocorticoids including dexamethasone for which timing and dosing were not comprehensively recorded. Other therapeutic strategies were applied at the discretion of the patients' primary treating physicians including standard of care agents as well as agents available on a compassionate use, non-approved basis. Detailed patient characteristics are outlined in Table 1 and in Supplementary Table 1. Median age at the start of the peptide vaccine treatment was 54 years (range: 9 to 87) and 68% of patients were male. Thirty patients came from Germany, 42 patients came from other European countries, 77 from the United States, and 24 from other countries. Median time from GBM diagnosis to first vaccination was 10.3 months (range: 3 to 54). |
| Recruitment | n/a |
| Ethics oversight | In this case, no ethics board approval and no official registration is needed. § 40 German Pharmacy Law (AMG) in conjunction with guidelines 2001/20/EG and 2005/28/EG, § 34 German Penal Code (StGB), Declaration of Helsinki of the World Medical Association (Article 37). An approval by the Institutional Review Board and ethics committees is not applicable [statement WD 9 - 3000 - 083/23 of the German Bundestag]. All patients provided informed consent for our personalized neoantigen vaccine therapy in an individual healing attempt and for the use of results for scientific research. The patient did not receive any compensation. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

# Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

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|---|---|
| Sample size | No sample size calculation was performed. We included all patients that received at least one vaccination at the cut-off date. |
| Data exclusions | All patients included in this analysis must have received at least one vaccination and provided a clinical history. |
| Replication | Not applicable to our analysis. |
| Randomization | Not relevant to our analysis. All patients included in this analysis received the neoantigen derived personalized peptide vaccine. |

| | |
|---|---|
| Blinding | Not relevant to our analysis. All patients included in this analysis received the neoantigen derived personalized peptide vaccine. |

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Antibodies |
| ☒ | ☐ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☒ | ☐ Animals and other organisms |
| ☐ | ☒ Clinical data |
| ☐ | ☒ Dual use research of concern |
| ☒ | ☐ Plants |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☐ | ☒ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

## Antibodies

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|---|---|
| Antibodies used | CD3-BV785 (clone UCHT1; BioLegend; dilution: 1/33), CD4-FITC (clone RPA-T4; BioLegend; dilution: 1/100), CD8-APC/Cyanine (clone SK1; BioLegend; dilution: 1/50), Zombi Aqua Dye (BioLegend; dilution: 1/200). IFN-BV421 (clone 4S.B3; BioLegend; dilution: 1/50), TNF-AlexaFluor700 (clone MAb11; BioLegend; dilution: 1/50), IL-2-PE/Cy7 (clone MQ1-17H12; BioLegend; dilution: 1/50) and CD154 – BV711 (clone 24-31; BioLegend; dilution: 1/25). |
| Validation | Antibodies have been validated by the manufacturer and have already been published by us in all of our manuscripts (reference 29, 30, 31, 32). |

## Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJEguidelines for publication of clinical research and a completedCONSORT checklist must be included with all submissions.

| | |
|---|---|
| Clinical trial registration | n/a |
| Study protocol | n/a |
| Data collection | One hundred seventy-three GBM patients were treated with a personalized neoantigen-derived peptide vaccine between 2015 and 2023. |
| Outcomes | Overall survival indicates the time between first diagnosis and date of death/cut-off date.<br>On-treatment survival is referring to the time between first vaccination and date of death/cut-off date.<br>T-cell responses monitored before the first vaccination and usually before the seventh vaccination. |

## Plants

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| Seed stocks | n/a |
| Novel plant genotypes | n/a |
| Authentication | n/a |

# Flow Cytometry

## Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

## Methodology

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| Sample preparation | Peripheral blood mononuclear cells (PBMC) including T-cells were isolated by Ficoll Hypaque and cryopreserved in MACS® Freezing Solution (Miltenyi Biotec) for later use. Cryopreserved PBMC were thawed and cells were cultured overnight to recover, stimulated with patient-individual mutated peptides and cultured 12 days in the presence of IL-2 and IL-7. For analysis, cells were restimulated for 12 ± 2 hours with peptides or incubated with DMSO (unstimulated negative control) or CytoStim™ (as unspecific positive control) in presence of Golgi-Plug (BD biosciences) at a concentration of 1 µl/ml. After restimulation, the final readout was an Intracellular Cytokine Staining (ICS). After cultivation, cells were washed twice followed by extracellular staining with fluorochrome-conjugated antibodies titrated to their optimal concentrations |
| Instrument | Novocyte 3005R cytometer (Agilent, Santa Clara, CA, USA) |
| Software | FlowJo version 10.5.3 (FlowJo LLC, Ashland, AZ, USA) |
| Cell population abundance | We used unsorted PBMCs. Cell viability was determined using Trypan blue staining. |
| Gating strategy | We included only cells that were constantly measured over time (1; Forward-scatter (FSC)-H versus Time). Herein, single (2; FSC-A versus FSC-H), viable (3; Zombie Aqua-negative cells), lymphocytes (4; FSC-A versus Side-scatter (SSC)-A) and CD3+ T-cells (5) were selected. CD3+ T-cells were further discriminated in CD4+ or CD8+ T-cells (6). Within both CD4+ (7-10) and CD8 + T-cells (11-14), we determined the production/expression of the functional markers IFN-γ (7, 11), TNF (8, 12), IL-2 (9, 13) and CD154 (10, 14). A detailed gating strategy can be found in Supplemental Figure 1. |

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.