nature portfolio

Peer Review File

A real-world observation of patients with glioblastoma treated with a personalized peptide vaccine

Editorial Note: This manuscript has been previously reviewed at another journal that is not operating a transparent peer review scheme. This document only contains reviewer comments and rebuttal letters for versions considered at *Nature Communications*.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

I thank the authors for responding to previous comments. The paper is much improved with the revised text as well as larger sample.

As previously mentioned, a combined OS is not informative, this includes the sub-analyses – e.g., multiple vaccine-induced responses vs. no/low. Given the discrepancy in survival - it would be helpful to separate the univariate and multivariate analyses (e.g., Table 2).

41 patients treated with glucocorticoids seems low for a clinical cohort of IDH-WT GBM patients. Why are 87 patients treated with glucocorticoids in Table 1, but only 41 are mentioned in the text?

The paragraph before safety starts with 87 patients as a denominator and then switches back to 97. Can the authors rephrase to make interpretation easier?

The authors mention that fewer pairs exist if they include EOR as a matching variable; however, it would be helpful to know if that analysis supports the one in the manuscript.

How is median follow-up time calculated? With the reverse Kaplan-Meier? Please include it in the methods section. Also, please include confidence intervals for all estimates (including medians).

The cohorts are repeatedly referred to by different names - it would help the reader if each cohort had one name and was referred to consistently.

On pages 6 & 7, the number of patients with pre-vaccination blood samples is stated as 84 and as 87. Please clarify.

Of the 7 high TMB patients - how many were newly diagnosed? What was the survival experience of those patients?

Reviewer #2 (Remarks to the Author):

The authors have responded sufficiently to some of my concerns, and have improved the manuscript.

However, it would be crucial to know all the details about the patients analyzed for Figure 3. Can the survival difference of no/low immune responders vs good immune responders be explained by differences in MGMT methylation status and/or primary/stable vs progress/recurrent status? A multiparameter analysis, at least a fair report of this crucial information is necessary.

Reviewer #3 (Remarks to the Author):

Comments to Author:

In this manuscript, Latzer et al. present the results of a personalized peptide vaccination in 173 patients with glioblastoma. They have comprehensively addressed many of the previous comments. They now demonstrate a median overall survival of 31.9 months, with a low rate of adverse events and have compared it to propensity-matched survival data of publicly available GBM cohorts. The authors should be commended for this impressive body of work which shows an exciting survival benefit in relation to this vaccine. However, there are aspects of the immune monitoring and vaccination protocol that require further clarity and additional relevant details to enhance the interpretability and generalizability of these results. Please see below for detailed comments.

Specific comments:

1. The study protocol is unclear from the text. Clarification could be achieved with the inclusion of a figure detailing the timepoints (i.e., intervals between vaccinations, definition of the end of the vaccine priming phase, and the immune monitoring timepoints). For instance, in the Immune Monitoring section, it is stated, “The median time from diagnosis to the end of the priming phase and the first immune monitoring (at 7th vaccination) was 13.5 months.” However, the text previously indicates that the priming phase concludes after four vaccinations. Thus, the sentence is challenging to interpret as the times of diagnosis, end of priming phase, and first immune monitoring are three distinct timepoints.

2. The authors note that 41 patients were treated with glucocorticoids but lack details on the dose and timing. This is inconsistent from the table which has 87 patients receiving glucocorticoid. This accounts for either 24% or 50% of the cohort depending on which number is correct. Does this imply that the remaining 76-50% never received glucocorticoids? This percentage seems unusually low for this patient population. Is this reflective of the standard practice at the participating centres? If so, could this variable be included in the propensity matching to ensure the groups compared for survival analysis are comparable.

3. Additional information regarding vaccine generation is needed, acknowledging that some aspects of peptide selection are proprietary. However, it would be beneficial to know the number of neoantigens selected per patient and whether this number was standardized.

4. The authors mention that 10% of patients did not generate any induced T-cell response. Is there an overlap between these patients and those who used glucocorticoids?

5. It is noted that of the patients with multiple timepoints, 27% had more than one positive timepoint. Have the authors evaluated for a memory T-cell response? The data suggests a lack of T cell memory formation in the majority of patients.

6. At the data cut-off date, it is stated that patients received between 1 and 31 vaccinations, with 66% having received at least 7. Given that the initial timepoint for post-vaccination immune analysis is after 7 vaccinations, could the authors justify not limiting the included patients in this study to only those patients who have completed a minimum number of vaccinations, whether defined as finishing the priming phase or reaching the immune monitoring phase?

7. The authors mention in the methods that PBMCs were stimulated with peptides to assess the immune response. Can the authors confirm if each patient's samples were specifically

stimulated with the same pool of patient-specific peptides included in their personalized vaccine?

8. Was a humoral immune response assessed for? If there is a detectable response, it could serve as a useful a biomarker of response as it would be more feasible to monitor compared to purely a T cell response which requires cell culture and flow cytometry as conducted in this study.

Response to the Reviewers' Comments

Reviewer #1:

I thank the authors for responding to previous comments. The paper is much improved with the revised text as well as larger sample.

> *We thank Reviewer 1 for the positive feedback of our manuscript. Moreover, we appreciate the constructive suggestions that we have now addressed in the revised manuscript and below in the point-by-point response.*

As previously mentioned, a combined OS is not informative, this includes the sub-analyses – e.g., multiple vaccine-induced responses vs. no/low. Given the discrepancy in survival - it would be helpful to separate the univariate and multivariate analyses (e.g., Table 2).

> *We thank Reviewer 1 for raising this important point. Please note, that we have re-named the no/low group for better understanding as "immunological non-responders" (iNR) compared to "immunological responders" (iR) throughout the revised manuscript.*
>
> *Because we "only" have n=20 iNR, we refrained from a multivariate analysis. We have however compared the amount of recurrent and stable, and MGMT methylated and unmethylated patients within iNR vs iR. The amount was balanced, indicating that these parameters might not have biased the significant differences in OS and on-treatment survival. We have included a new table in the Supplementary Information (Supplementary Table 7) and the following sentence to the end of that paragraph:*
>
> *"MGMT status, recurrent status, and immunosuppressant intake (for example Dexamethasone) were not significantly associated with developing higher (> 0.1) immune responses (Fisher's exact test, P= 0.12, 0.32 and 0.62 respectively; Supplementary table 7)."*

41 patients treated with glucocorticoids seems low for a clinical cohort of IDH-WT GBM patients. Why are 87 patients treated with glucocorticoids in Table 1, but only 41 are mentioned in the text?

> *We apologize for being unclear here. At the time of first vaccination, 41 patients were treated with glucocorticoids. During the time of observation (before, during and after vaccination), 87 patients were treated with glucocorticoids. For better understanding, we will only mention the n=87 patients. We have corrected the corresponding section in the manuscript.*

The paragraph before safety starts with 87 patients as a denominator and then switches back to 97. Can the authors rephrase to make interpretation easier?

> *We apologize for this confusion. At least one post-vaccination blood sample for evaluation of vaccine-induced neoantigen specific T-cell response was available for 97 of 173 patients (56%). n=87 patients exhibited post-vaccination T-cell responses against at least one vaccinated neoantigen-derived peptide. For n=84 patients, an additional pre-vaccination time point is available. For better understanding, we will only mention the n=97 patients with available immune monitoring data at the 7th vaccination. We have deleted the other statement from the manuscript.*

The authors mention that fewer pairs exist if they include EOR as a matching variable; however, it would be helpful to know if that analysis supports the one in the manuscript.

We acknowledge that EOR is an important prognostic factor and therefore it is important to balance this factor between the cohorts using propensity score matching (PSM). We first assessed the data availability among the four public clinical datasets:

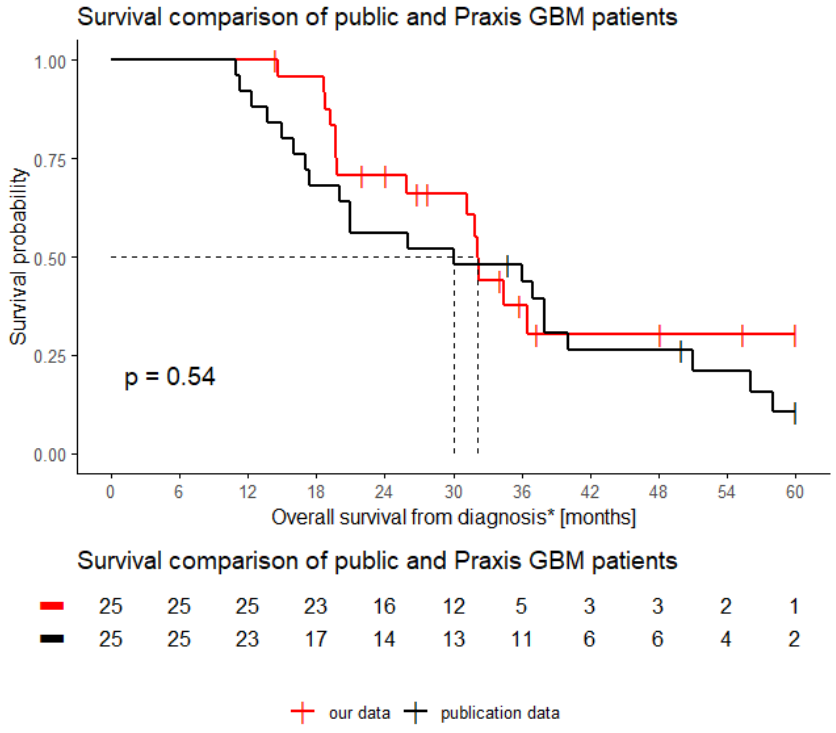
Publication	Resection data
GLASS Consortium, Nature 2019	43 patients had total resection, 14 patients had subtotal resection
TCGA, Cell 2013	Not available
MSKCC, Clin Cancer Res 2019	Not available
Lakomy, Frontiers in Oncology 2020	30 patients had total resection, 34 patients had subtotal resection, 9 patients received partial resection or biopsy

Within our cohort, based on the data provided by the patients, 26 patients had total resection, 12 patients had partial or subtotal resection, and 7 patients received only biopsy.

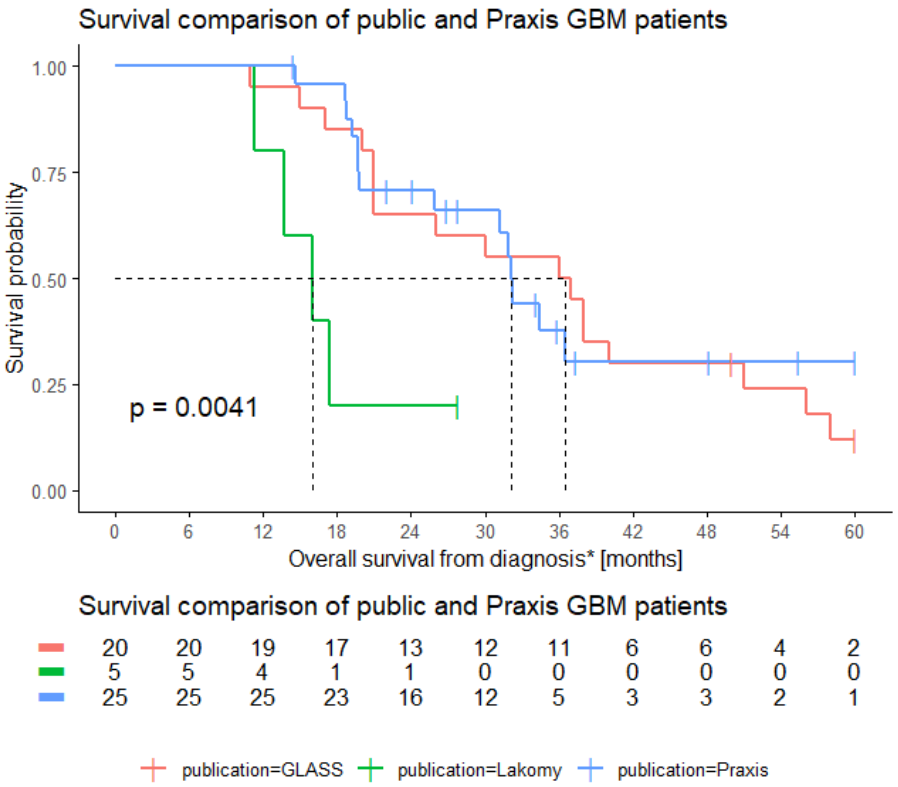
The EOR of brain tumor was categorized into biopsy (no reduction of tumor volume), partial (1-79%), subtotal (>80%), near-total (> 95%), or total resection (Karschnia et al., 2021, PMID: 33819718).

Given the available resection data, we propose to include only patients who received total resection into the PSM to avoid either introducing biases due to incomplete data or performing matching with missing factor levels in one of the cohorts.

Taken together with other matching variables, we could perform a PSM between 25 patients from our cohort and 64 patients from publica data who survived longer than the median months from diagnosis to 1st vaccination of our 25 patients. We performed nearest neighbor 1-1 matching between the two patient groups. The results showed that the 2 groups did not have survival difference:



However, after a closer look at the matched control patients, 19 patients came from the GLASS cohort and 6 patients came from the Lakomy cohort. The former one is a cohort with high selection bias where patients were selected from 35 hospitals requiring that each patient have high quality DNA sequencing data in at least 2 time points (primary or recurrent). The Lakomy cohort instead reflects real-world survival status. If we separate the matched patients from the Lakomy and the GLASS papers, one can clearly observe the survival difference:



Therefore, we could not gain more insightful conclusions by including the resection into the matching mainly because of the following limitations:

- *Around 80% of our patients did not provide accurate EOR data.*
- *The public clinical datasets often did not provide EOR data. If we include only the datasets with EOR data, then there is a higher selection bias towards patients who survived long (as shown in the 2nd figure).*

How is median follow-up time calculated? With the reverse Kaplan-Meier? Please include it in the methods section. Also, please include confidence intervals for all estimates (including medians).

Yes, the median follow-up time is calculated with reverse Kaplan-Meier. We have included the requested information in the Methods section and Supplementary Table 5.

The cohorts are repeatedly referred to by different names - it would help the reader if each cohort had one name and was referred to consistently.

We apologize for being inconsistent here. We have changed the name of the cohorts in the revised manuscript. "our cohort" refers to the patient treated with a personalized neoantigen-derived peptide vaccine. The "matching" cohort/patients refers to the patients from public datasets selected after propensity score matching. "Primary" refers to patient treated prior to progression. "Recurrent" refers to patient treated after progression.

On pages 6 & 7, the number of patients with pre-vaccination blood samples is stated as 84 and as 87. Please clarify.

> *We apologize for this confusion. Pre-vaccination blood samples were available for 84 patients. At least one post-vaccination blood sample for evaluation of vaccine-induced neoantigen specific T-cell response was available for 97 of 173 patients (56%). 87 patients exhibited post-vaccination immune responses against at least one vaccinated neoantigen. As stated above, pre-existing T-cell responses were not further analyzed in this manuscript. We therefore decided to remove this information from the manuscript in order to improve understanding.*

Of the 7 high TMB patients - how many were newly diagnosed? What was the survival experience of those patients?

> *Of the seven patients with high TMB, six patients had a stable disease (primary) at the first vaccination and 1 patient had a progression (recurrent). The overall survival for the primary patients (in months): 22.3, 32.0, 34.4, 34.4, 35.0 and 65.1. For the recurrent patient, the overall survival is 48.0 months. The on-treatment survival (from start of the vaccination) for the primary patients (in months): 16.4, 21.1, 27.9, 28.6, 29.3 and 54.7. For the recurrent patient, the on-treatment survival is 21.5 months.*

Throughout the manuscript, we have made minor changes in order to improve understanding. These changes are likewise marked, but not further explained here.

Reviewer #2:

The authors have responded sufficiently to some of my concerns, and have improved the manuscript.

> *We thank Reviewer 2 for this encouraging statement. We have addressed the comments in the revised manuscript and in the point-by-point response below.*

However, it would be crucial to know all the details about the patients analyzed for Figure 3. Can the survival difference of no/low immune responders vs good immune responders be explained by differences in MGMT methylation status and/or primary/stable vs progress/recurrent status? A multiparameter analysis, at least a fair report of this crucial information is necessary.

> *See also comment 1 from Reviewer 1.*
>
> *We have analyzed the distribution of MGMT and recurrence status (and immunosuppressant intake) within the iR and iNR*. The distribution was balanced. We assume that these three parameters did not have an influence on the "success" of vaccination. We included a statement at the end of the "T-cell response and outcome" section and the new Supplementary Table 7.*
>
> **Please also note, that we have re-named the no/low group for better understanding as "immunological non-responders" (iNR) compared to "immunological responders" (iR) throughout the revised manuscript.*

Throughout the manuscript, we have made minor changes in order to improve understanding. These changes are likewise marked, but not further explained here.

Reviewer #3:

In this manuscript, Latzer et al. present the results of a personalized peptide vaccination in 173 patients with glioblastoma. They have comprehensively addressed many of the previous comments. They now demonstrate a median overall survival of 31.9 months, with a low rate of adverse events and have compared it to propensity-matched survival data of publicly available GBM cohorts. The authors should be commended for this impressive body of work which shows an exciting survival benefit in relation to this vaccine. However, there are aspects of the immune monitoring and vaccination protocol that require further clarity and additional relevant details to enhance the interpretability and generalizability of these results. Please see below for detailed comments.

We thank Reviewer 3 for the positive feedback of our manuscript, and the evaluation of its relevance to the field. Moreover, we appreciate the constructive criticism that we have now addressed in the revised manuscript and below in the point-by-point response.

Specific comments:
1. The study protocol is unclear from the text. Clarification could be achieved with the inclusion of a figure detailing the timepoints (i.e., intervals between vaccinations, definition of the end of the vaccine priming phase, and the immune monitoring timepoints). For instance, in the Immune Monitoring section, it is stated, "The median time from diagnosis to the end of the priming phase and the first immune monitoring (at 7th vaccination) was 13.5 months." However, the text previously indicates that the priming phase concludes after four vaccinations. Thus, the sentence is challenging to interpret as the times of diagnosis, end of priming phase, and first immune monitoring are three distinct timepoints.

We apologize for being unclear here. As suggested by Reviewer 3, we have included a figure in the Supplementary Information (Supplementary Figure 5) showing vaccination and blood draw time points. We thank Reviewer 3 for this hint.

We have furthermore removed the term "Priming phase" from the manuscript for better understanding. The mentioned sentence has been modified to: "The median time from diagnosis to the first immune monitoring (at 7th vaccination) was 13.6 months."

2. The authors note that 41 patients were treated with glucocorticoids but lack details on the dose and timing. This is inconsistent from the table which has 87 patients receiving glucocorticoid. This accounts for either 24% or 50% of the cohort depending on which number is correct. Does this imply that the remaining 76-50% never received glucocorticoids? This percentage seems unusually low for this patient population. Is this reflective of the standard practice at the participating centres? If so, could this variable be included in the propensity matching to ensure the groups compared for survival analysis are comparable.

See also statement 2 from Reviewer 1

We apologize for being unclear here. At the time of first vaccination, 41 patients were treated with glucocorticoids. During the time of observation (before, during and after vaccination), 87 patients were treated with glucocorticoids. For better understanding, we will only mention the n=87 patients. We have corrected the corresponding section in the manuscript.

We understand that the influence of glucocorticoids like Dexamethasone is very interesting in this context. However, glucocorticoid intake is not yet recognized as an independent factor that influences survival but rather a necessary medication to alleviate severe headaches. It may have more influence on the immune responses rather than the overall survival.

Additionally, among the four public clinical datasets, only the Lakomy, Frontiers in Oncology 2020 publication provided binary glucocorticoid usage data. Given potential differences in the dosage, duration, and timing of glucocorticoid administration, and limited amount of public data for the matching, including this variable would significantly reduce the amount of data and introduce more variance. For these concerns, we did not include this variable for the matching.

3. Additional information regarding vaccine generation is needed, acknowledging that some aspects of peptide selection are proprietary. However, it would be beneficial to know the number of neoantigens selected per patient and whether this number was standardized.

We thank Reviewer 1 for raising this important point. Please note that we have detailed information regarding peptide selection and vaccine generation in the Supplementary file. We originally refrained from adding this information into the main text because it might be out of scope.

We aimed to vaccinate 20 peptides. However, for some patients less peptides could be selected due to reduced presence of mutations, HLA binders, synthesizable peptides, etc. Finally, the median number of peptides selected was 19 (mean 16.4).

4. The authors mention that 10% of patients did not generate any induced T-cell response. Is there an overlap between these patients and those who used glucocorticoids?

See also statement 1 Reviewer 2.

We have now analyzed the distribution of immunosuppressant intake (and MGMT and recurrence status) within the iR and iNR. The distribution was balanced. We assume that these three parameters did not have an influence on the "success" of vaccination. We included a statement at the end of the "T-cell response and outcome" section and the new Supplementary Table 7.*

**Please also note, that we have re-named the no/low group for better understanding as "immunological non-responders" (iNR) compared to "immunological responders" (iR) throughout the revised manuscript.*

5. It is noted that of the patients with multiple timepoints, 27% had more than one positive timepoint. Have the authors evaluated for a memory T-cell response? The data suggests a lack of T cell memory formation in the majority of patients.

It is assumed that our assay, using 12-day in-vitro expansion in presence of low dose IL-2 and IL-7, is primarily expanding functional memory T-cells – neither naïve nor late-differentiated TEMRA cells. Data supporting this hypothesis have recently been generated by us using viral antigens (Zelba et al., 2021, PMID: 33298615). Therefore, we assume that only effector memory T-cells can be detected. We have included this information in the manuscript.

Our "endpoint" was a measurable T-cell response at the 7th vaccination. Therefore, for better understanding, we have removed the sentence about the 27% from the manuscript.

6. At the data cut-off date, it is stated that patients received between 1 and 31 vaccinations, with 66% having received at least 7. Given that the initial timepoint for post-vaccination immune analysis is after 7 vaccinations, could the authors justify not limiting the included patients in this study to only those patients who have completed a minimum number of vaccinations, whether defined as finishing the priming phase or reaching the immune monitoring phase?

The original analysis included only patients with available immune monitoring data (51 patients). The reviewers questioned the interpretation of the results based on the small cohort. Therefore, we simply included all patients that have received at least one vaccination at the cut-off date. Please note that these patients have de facto no influence on long-term on-treatment survival.

7. The authors mention in the methods that PBMCs were stimulated with peptides to assess the immune response. Can the authors confirm if each patient's samples were specifically stimulated with the same pool of patient-specific peptides included in their personalized vaccine?

Yes, the patient's PBMCs were each stimulated with their personalized neoantigen-derived peptides. If cell numbers were low, some peptides were measured as a pool. We use a Hamilton pipetting robot to circumvent pipetting errors.

8. Was a humoral immune response assessed for? If there is a detectable response, it could serve as a useful a biomarker of response as it would be more feasible to monitor compared to purely a T cell response which requires cell culture and flow cytometry as conducted in this study.

We thank the reviewer 3 for this comment. No, we did not measure humoral immune responses. Although it might be easier to access at first glance, an easy high-throughput method might only be able to detect antibodies against the "linear" peptide, not a peptide presented on HLA. We can only assume if such antibodies might serve as a good biomarker. Nevertheless, storage of plasma is planned for future studies.

Throughout the manuscript, we have made minor changes in order to improve understanding. These changes are likewise marked, but not further explained here.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

Thank you for addressing all of my concerns.

Reviewer #3 (Remarks to the Author):

The authors have addressed many of the comments in their revised manuscript and should be commended on their efforts to improve the manuscripts clarity. There remains one point that the authors should re-consider.

With regards to comment #3 about including additional information about peptide generation and number of peptides per patient – this reviewer acknowledges there is additional information within the supplement as well as references to other publications with more details about the peptide development. However, the novelty of this trial revolves around the peptide vaccine and therefore including more details about the peptide generation, number of peptides per patient, etc. would fit well within the scope of the manuscript and be of interest to the readership of this article. Therefore, this reviewer suggests that these details be included within the main text in the methods section.

Response to the Reviewers' Comments

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We thank Reviewer 3 for this clarification. We included theses details within the main text in the Methods section as well as further details in the Supplementary Information.