

Reporting Summary

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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
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
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

single cell seq: Chromium Single Cell V(D)J Reagent kit v1 chemistry (10x Genomics), sequenced on HiSeq2500 rapid and HiSeq4000 platforms (Illumina).
TCRB seq: hsTCRB Kit (Adaptive Biotechnologies), sequenced on Illumina MiSeq.
Flow Cytometry: BD FACSDiva version 8.0 (FACSAria IIu for sorting) or version 9.0 (FACSCanto II for CD8+ LIL analysis), Attune Nxt software version 2.7 (Attune Nxt for peripheral immune monitoring), and BD FACSuite version 1.3 (Lyric for recall assay)

Data analysis

single cell seq: processed with cellranger pipeline (version 3.1.0) with GRCh38 genome assembly (version 3.0.0, 10x Genomics). analysis of filtered matrices with Seurat.transformation and normalization with sctransform (Seurat version 3.2.0). Gene expression analysis of clusters with MAST (<https://github.com/RGLab/MAST>) using R version 3.6.3.
TCRB seq: Data processing (demultiplexing, trimming, gene mapping) was done using Adaptive Biotechnologies' proprietary platform. Data was visualized using the Treemap Visualization package version 2.4.2 (<https://cran.r-project.org/web/packages/treemap/index.html>).
Flow cytometry: FlowJo V.10.5.0.
exploratory statistical analysis: GraphPadPrism V.9.0.0.

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 Research [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 list of figures that have associated raw data
- A description of any restrictions on data availability

single cell RNA and VDJ sequencing data that are associated with Figure 4 and ED Figures 11, 12 have been deposited in the NCBI Sequence Read Archive with the accession codes SRR12880623 and SRR12880624, and are publicly accessible. TCRB sequencing data that are associated with ED Figures 10, 12 is available at <https://clients.adaptivebiotech.com/immuneaccess>

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](https://www.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.

Sample size	Sample size estimation was primarily based on the accuracy requirements for the primary endpoint immune response (responder rate) to the IDH1 peptide vaccine. sample size was adjusted for non-evaluable patients (see Data Exclusions). Estimation that 70% of patients evaluable for immunogenicity testing will be evaluable for all time points (Macdonald D, Cascino T, Schold SJ, et al: Response criteria for phase II studies of supratentorial malignant glioma. J Clin Oncol 8:1277-1280, 1990.) 21 patients sufficient for immunogenicity testing with all time points, i.e. 30 evaluable patients to be enrolled. due to expected dropout rate of 20% (due to progression or other reasons), 39 patients to be recruited.
Data exclusions	Of all 32 patients treated (= safety data set), 2 patients were excluded from immunogenicity testing, because they were not evaluable, because not enough time points were eligible to immunogenicity testing. A patient was pre-defined to be evaluable if study completed until and incl. visit 7, received at least 4 vaccinations and all blood samples collected for immunogenicity testing, OR received 6 vaccinations and baseline plus 2 blood samples collected for immunogenicity testing.
Replication	Findings concerning patient outcomes and immunogenicity at defined time points, and all related analyses using patient blood samples or derivatives thereof cannot be reproduced due to sample limitations. TCR testing using TCR14 expressing Jurkat T cell line was reproduced three times with similar outcome.
Randomization	Phase 1 study, i.e. All patients received IDH1 vaccination. In addition, they received standard of care treatment prior to enrollment as decided by the local investigator and the patient. 3 types of standard of care treatment resulted in 3 treatment groups, all receiving the exactly same trial related intervention. for PLA, tissue slides of primary tumors were selected based on availability. for TCRB deep seq, PBMC samples were selected based on availability. for recall assay, samples were selected based on availability and MSS (all available samples within top 10 highest MSS) and cells from 2 aliquots of the same sample were pooled before allocation to stimulus treatment and staining. for recall assay, ID08 LIL ELISpot, ID08 CD8+ LIL FACS, and TCR14 NFAT reporter assay, cells were allocated to stimulus treatment randomly. for all flow cytometry (incl. FACS), cells were allocated to different stainings (full stain panels, staining controls) randomly.
Blinding	single arm, open label trial, i.e. neither patients nor clinical nor immunogenicity investigators were blinded concerning IDH1 vaccination (=trial related intervention). With respect to standard of care treatment groups, immunogenicity investigators were blinded. All primary endpoint analyses were done in a blinded fashion. Exploratory analyses e.g. immunological phenotyping, were performed non-blinded with respect to immune response detectable in the sample, because samples for these analyses were selected based on immune response.

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input type="checkbox"/>	<input checked="" type="checkbox"/> Human research participants
<input type="checkbox"/>	<input checked="" type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input type="checkbox"/>	<input checked="" type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used

Flow cytometry antibodies:

anti-CD3-FITC (clone UCHT1, cat # 300452), anti-CD4-Alexa Fluor700 (clone RPA-T4, cat # 300526), anti-CD8-PerCP (clone RPA-T8, cat # 301030), anti-CD11b-BV510 (clone M1/70, cat # 101263), anti-HLA-DR-PE-Cy7 (clone L243, cat # 307616), anti-CD14-BV711 (clone M5E2, cat # 301838), anti-CD16-PE/Dazzle594 (clone 3G8, cat # 302054), anti-CD25-BV605 (clone BC96, cat # 302632), anti-CD33-APC (clone P67.6, cat # 366606), anti-CD127-BV421 (clone A019D5, cat # 351310), anti-Foxp3-PE (clone 206D, cat # 320108) (all BioLegend), anti-CD45-eFluor450 (clone 2D1, cat # 48-9459-42, eBioscience), anti-CD3-PE (clone HIT3a, cat # 300308, BioLegend), anti-CD3-BV510 (clone HIT3a, cat # 564713), anti-CD4-BV605 (clone SK3, cat # 566908), anti-CD8-APC-H7 (clone SK1, cat # 560179), anti-IFNgamma-BV421 (clone 4S.B3, cat # 564791), all BD Biosciences, anti-TNFalpha-APC (clone MAb11, cat # 502912, Biolegend), anti-IL17-PE (clone N49-653, cat # 560486), anti-IL4-PerCP-Cy5.5 (clone 8D4-8, cat # 561234), anti-CD25-BV711 (clone 2A3, cat # 563159), anti-CD127-FITC (clone HIL-7R-M21, cat # 560549), anti-FoxP3-PE (clone 259D/C7, cat # 560046), anti-IL10-APC (clone JES3-19F1, cat # 554707), anti-CD3-BV510 (clone HIT3a, cat # 564713) (all BD Biosciences), anti-CD8-PerCP-Cy5.5 (clone RPA-T8, cat # 45-0088-42), anti-TNFalpha-APC (clone MAb11, cat # 17-7349-82), and anti-IFNgamma-eFluor450 (clone 4S.B3, cat # 48-7319-42) (all ebioscience).

PLA antibodies:

anti-IDH1R132H (clone H09, cat # DIA-H09, Dianova), anti-HLA-DR (clone EPR3692, cat # ab92511, Abcam).

ELISA antibodies:

mouse anti-IDH1R132H (clone H09, cat # DIA-H09, Dianova), sheep anti-mouse IgG-HRP (polyclonal secondary, cat # NXA931, Amersham) and goat anti-human IgG-Fc-HRP (polyclonal secondary, cat # A80-104P, Bethyl Laboratories, Inc.).

Validation

all antibodies were titrated prior to use as advised by supplier in the material data sheet or used according to manufacturer's instructions, and, for flow cytometry, scaled up according to cell numbers.

Flow cytometry antibodies: each lot is quality control tested by extra- or intra-cellular flow cytometry using positive control samples and appropriate isotype control stainings.

anti-IDH1R132H (clone H09, cat # DIA-H09, Dianova): approved for in vitro diagnostic use with IHC in Europe.

anti-HLA-DR (clone EPR3692, cat # ab92511, Abcam): tested and guaranteed application for IHC on FF/PFA fixed paraffin-embedded sections and immunofluorescence.

secondary ELISA antibodies: Bethyl: By immunoelectrophoresis and ELISA this antibody reacts specifically with human IgG. Cross reactivity with IgM, IgA and light chains is less than 0.1%. This antibody may cross react with IgG from other species. Amersham: Horseradish peroxidase (HRP) conjugated antibodies are highly species specific antibodies optimized for use with Amersham ECL Western blotting detection reagents.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)

Jurkat delta 76, obtained from TRON gGmbH

Authentication

authenticated using the Multiplexion STR profiling and compared to normal Jurkat cells

Mycoplasma contamination

cells were regularly tested for mycoplasma contamination and tested negative at all time points

Commonly misidentified lines
(See [ICLAC](#) register)

none.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

full details for all patients incl. non-evaluable patients, are provided in Table 1 of the manuscript. 62.5 % male, 37.5% female. mean age was 40.4 (SD 8.95) years. 71.9 % received radiotherapy (RT) plus chemotherapy with temozolomide (TMZ), 9.4 % received TMZ alone, 18.8% received RT alone. 65.6% patients had WHO grade 3, 34.4% patients had WHO grade 4 astrocytomas. 71.9 % of tumors located at frontal lobe. 53.1 % received complete resection, 37.5 % received subtotal resection, 9.4 % received biopsy.

Recruitment

patients were recruited at 8 trial centers in Germany (see ED Table 1), based on molecular and clinical inclusion criteria: presence of a histologically confirmed IDH1R132H+ glioma (with or without measurable residual tumor after resection or

biopsy) with absence of chromosomal 1p/19q co-deletion and loss of nuclear ATRX expression in the tumor tissue (subgroup of molecular astrocytoma without positive prognostic factors). In addition, inclusion criteria were: patients received standard of care treatment (RT+TMZ, TMZ alone, or RT alone) prior to enrollment; at least 18 years old; women of child-bearing potential (WOCBP) must provide a negative pregnancy test within 72 h prior to start of IDH1 vaccination; WOCBP and their partners must use birth control method (failure rate below 1% per year). exclusion criteria included: concomitant treatment with dexamethasone (or equivalent) > 2 mg/day, Karnofsky Performance Status (KPS) < 70, progressive (incl. pseudoprogression) or recurrent disease after standard of care treatment, experimental treatment of the tumor; grade 2 or higher CTCAE v4.0 laboratory values for hematology, liver, or renal function. For complete list of exclusion criteria, please refer to the study protocol. Patients agreeing to trial methods are normally well-informed and motivated to comply with study procedures, which may influence results in a way of better interpretability.

Ethics oversight

The study was approved by the national regulatory authority (Paul-Ehrlich Institut) and the institutional review board at each study site (Ethikkommission), namely: Ethikkommission der medizinischen Fakultät Heidelberg (Heidelberg), Ethik-Kommission Albert-Ludwigs-Universität Freiburg (Freiburg), Ethik-Kommission des Landes Berlin (Berlin), Ethik-Kommission der Medizinischen Fakultät der Universität Duisburg-Essen (Essen), Ethik-Kommission der Medizinischen Fakultät "Carl Gustav Carus" (Dresden), Ethikkommission des Fachbereichs Medizin der Goethe-Universität Frankfurt am Main (Frankfurt), Ethik-Kommission an der Medizinischen Fakultät der Eberhard-Karls-Universität und am Universitätsklinikum Tübingen (Tübingen). The study was conducted in accordance with the Good Clinical Practice guidelines of the International Conference on Harmonisation. All participants provided written signed informed consent.

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

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration

Study protocol

Data collection

Outcomes

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

Sample preparation

peripheral immune monitoring of PBMC: PBMC were isolated by ficoll gradient centrifugation from heparin blood, frozen in 50% freezing medium A (60% X-Vivo 20 + 40% FCS) and 50% medium B (80% FCS + 20% DMSO) and stored in liquid nitrogen at -140°C until analysis.

recall assay of PBMC: PBMC were thawed, rested for 4 h in X-Vivo medium, and seeded into 96-well U-bottom plates. 2x10⁶ PBMCs were stimulated with 2 µg peptide per well using IDH1R132H(p123-142), MOG(p35-55) as negative control, or CEFT peptide pool (concentration, jpt) as positive control for 2 h before adding 10 µg/ml brefeldin A (Sigma-Aldrich, order no. B6542) and 1x GolgiStop (BD Bioscience). Cells were incubated additional 12 h

Leukocytes from lesion: tissue was dissected into small pieces (2x2mm) and transferred to 24-well tissue culture treated plates – 3 pieces per well in 2 ml human TIL medium (RPMI1640 (Pan Biotec) + 10% Human serum (Sigma Aldrich) + 2mM L-glutamine +1.25 µg/ml Amphotericin B (both Gibco) + 1000U/ml IL-2 (Proleukin)) containing 30ng/ml anti-human CD3 (clone OKT-3, (eBioscience)). Medium was exchanged every 2-3 days and tissue pieces removed on day 7. LILs that migrated out of the tumor into the medium were further expanded until day 14 and cryopreserved as above.

Instrument

peripheral immune monitoring of PBMC: Attune Nxt (Thermo Fisher Scientific); recall assay of PBMC: Lyric (BD); sorting of T cells: FACSAria IIu (BD); CD8+ LIL testing: Canto II (BD)

Software

collection: Attune Nxt software version 2.7 (Thermo Fisher Scientific) (peripheral immune monitoring); BD FACSDiva software version 8.0 (sorting of T cells) or version 9.0 (CD8+ LIL testing); BD FACSuite version 1.3 (recall assay).
analysis: FlowJo version 10.5.0.

Cell population abundance

relevant post-sorted cell fractions, i.e. CD3+ T cells, were subjected to scRNAsequencing. based on RNA expression signatures, after further removal of stressed cells (by expression of heat shock proteins), 99.42% of cells were T cells. Non-T cells were excluded from analysis.

Gating strategy

peripheral immune monitoring of PBMC:

gated on lymphocytes based on size and granularity (FSS vs SSC) --> gated on single cells --> gated on live cells, i.e. dead stain negative cells --> gated on: A. CD3+ --> CD8+/CD4+ --> CD4+ gated on CD25+ CD127 --> gated on FoxP3+ cells = Treg, and B. CD33+ --> gated on a. CD11b+ HLA-DR+ --> CD14-/CD16- cells = monocytes, and b. CD11b+/HLA-DR- --> CD14+ cells = Mo-MDSC

recall assay:

gated on lymphocytes based on size and granularity (FSS vs SSC) --> gated on single cells --> gated on live cells, i.e. dead stain negative cells --> gated on CD3+ --> gated on A. CD4- CD8+ --> gated on IFNgamma+ and TNFalpha+, and B. CD4+ CD8- --> gated on a. IFNgamma+ and TNFalpha+, b. IL-4+ and IL-17+, and c. CD25+ CD127- --> gated on FoxP3+ --> gated on IL10+

sorting of T cells:
gated on lymphocytes based on size and granularity (FSS vs SSC) --> gated on single cells --> gated on live cells, i.e. dead stain negative cells --> gated on CD45 and CD3 double positive cells. live, CD45, CD3 gates based on FMO controls.

CD8+ LIL testing:

gated on lymphocytes based on size and granularity (FSS vs SSC) --> gated on single cells --> gated on live cells, i.e. dead stain negative cells --> gated on CFSE+ LIL (to distinguish from REP antigen-presenting cells) --> gated on CD3+ CD8+ --> gated on A. IFNgamma+ and B. TNFalpha+

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

Magnetic resonance imaging

Experimental design

Design type

n/a. Structural MRI

Design specifications

At least 6 MRI scans were acquired for each patient (pre study; clinical screening, visit 7,10,12,13)

Behavioral performance measures

n/a. MRI used solely for diagnosis of disease progression.

Acquisition

Imaging type(s)	T2-w; FLAIR; contrast enhanced T1-w
Field strength	3 Tesla
Sequence & imaging parameters	Parameter T2-w (TSE); TE: 88; TR: 5.280; Flip angle: 180; FOV: 230 × 230; Matrix size: 256 × 173; Slice thickness: 5; No. of averages 1; In-plane resolution 0.7 Orientation Axial (2D) Duration 1:15 (min:s) Parameter FLAIR; TE: 135; TR: 8.500; Flip angle: 170; FOV: 230 × 170; Matrix size: 320 × 216; Slice thickness: 5 No. of averages 1 In-plane resolution: 1 Orientation Axial (2D) Duration 2:52 (min:s) T1 (mpRAGE); TE: 3.57; TR: 1.770; Flip angle: 15; FOV: 256 × 256; Matrix size: 320 × 272; Slice thickness: 1 No. of averages: 1; In-plane resolution 1; Orientation sagittal(3D); Duration 3:27 (min:s)
Area of acquisition	whole brain scan
Diffusion MRI	<input type="checkbox"/> Used <input checked="" type="checkbox"/> Not used

Preprocessing

Preprocessing software	n/a
Normalization	n/a
Normalization template	n/a
Noise and artifact removal	n/a
Volume censoring	n/a

Statistical modeling & inference

Model type and settings	n/a
Effect(s) tested	n/a
Specify type of analysis:	<input type="checkbox"/> Whole brain <input type="checkbox"/> ROI-based <input checked="" type="checkbox"/> Both
Anatomical location(s)	tumor volumes were manually assessed by the imaging core lab using RANO criteria
Statistic type for inference (See Eklund et al. 2016)	n/a
Correction	n/a

Models & analysis

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Functional and/or effective connectivity
<input checked="" type="checkbox"/>	<input type="checkbox"/> Graph analysis
<input checked="" type="checkbox"/>	<input type="checkbox"/> Multivariate modeling or predictive analysis