**Title**
**Actively personalized vaccination trial for** **newly diagnosed glioblastoma**

**Authors**

Norbert Hilf1,#, Sabrina Kuttruff-Coqui1,#, Katrin Frenzel2, Valesca Bukur2, Stefan Stevanovic3, Cécile Gouttefangeas4,3, Michael Platten5,6,7, Ghazaleh Tabatabai8,3, Valerie Dutoit9, Sjoerd H. van der Burg10,4, Per thor Straten11,4, Francisco Martínez-Ricarte12, Berta Ponsati13, Hideho Okada14, Ulrik Lassen15, Arie Admon16, Christian H. Ottensmeier17, Alexander Ulges1, Sebastian Kreiter2,4, Andreas von Deimling5,6, Marco Skardelly8, Denis Migliorini9, Judith R. Kroep10, Manja Idorn11, Jordi Rodon12,19, Jordi Piro13, Hans S. Poulsen15, Bracha Shraibman16, Katy McCann17, Regina Mendrzyk1, Martin Löwer2, Monika Stieglbauer3, Cedrik M. Britten4,2,20, David Capper5,6,21, Marij J.P. Welters10,4, Juan Sahuquillo12, Katharina Kiesel1, Evelyna Derhovanessian2, Elisa Rusch3, Lukas Bunse5,6, Colette Song1, Sandra Heesch2, Claudia Wagner1, Alexandra Kemmer-Brück2, Jörg Ludwig1, John C. Castle2,22, Oliver Schoor1, Arbel D. Tadmor18, Edward Green7,6, Jens Fritsche1, Miriam Meyer1, Nina Pawlowski1, Sonja Dorner1, Franziska Hoffgaard1, Bernhard Rössler1, Dominik Maurer1, Toni Weinschenk1, Carsten Reinhardt1, Christoph Huber2, Hans-Georg Rammensee3, Harpreet Singh1, Ugur Sahin², Pierre-Yves Dietrich9, Wolfgang Wick5,6,\*

**Affiliations**

1Immatics Biotechnologies GmbH, Tübingen, Germany; 2BioNTech AG, Mainz, Germany; 3Eberhard Karls Universität Tübingen, Tübingen, Germany, and German Cancer Consortium (DKTK), German Cancer Research Center Partner Site Tübingen, Tübingen, Germany; 4CIMT/CIP - Association for Cancer Immunotherapy, working group Cancer Immunoguiding Program, Mainz, Germany; 5University Hospital Heidelberg, Heidelberg, Germany; 6German Cancer Consortium (DKTK), German Cancer Research Center, Heidelberg, Germany; 7Medical Faculty Mannheim, Mannheim, Germany; 8University Hospital Tübingen, Tübingen, Germany; 9Geneva University Hospital, Geneva, Switzerland; 10Leiden University Medical Center, Leiden, The Netherlands; 11Center for Cancer Immune Therapy (CCIT), Department of Hematology, University Hospital Herlev, Denmark, and Department of Immunology and Microbiology, University of Copenhagen, Denmark; 12Vall d’Hebron University Hospital, Barcelona, Spain; 13BCN Peptides S.A., Barcelona, Spain; 14University of California San Francisco, San Francisco, USA, and Parker Institute for Cancer Immunotherapy, San Francisco, USA; 15Ringhospitalet, Copenhagen, Denmark; 16Technion - Israel Institute of Technology, Haifa, Israel; 17University of Southampton, Southampton, UK; 18TRON - Translational Oncology at the University Medical Center of Johannes Gutenberg University GmbH, Mainz, Germany; 19current affiliation: U. T. M. D. Anderson Cancer Center, Houston, USA; 20current affiliation: Glaxo-Smith-Kline, Oncology R&D, Stevenage, UK; 21current affiliation: Charité, University Medicine Berlin, Berlin, Germany; 22current affiliation: Agenus Inc., Lexington, USA; #Contributed equally; \*Corresponding author

**Patients with glioblastoma (GB) so far do not sufficiently benefit from recent breakthroughs with checkpoint inhibitors (CPI)1,2,** **for which high mutational load and responses to neo-epitopes are regarded essential3. GB tumours show limited intra-tumoral immune cell infiltration4 and carry 30-50 non-synonymous mutations only5. Exploitation of the full repertoire of tumour antigens - non-mutated and neo-epitopes – may offer more effective immunotherapies, especially for tumours with low mutational load. In the first-in-human trial GAPVAC-101, the Glioma Actively Personalized Vaccine Consortium (GAPVAC) integrated both tracks highly individualized into standard treatment to optimally exploit the limited target space for patients with newly diagnosed GB. Fifteen HLA-A\*02:01+ or -A\*24:02+ patients were treated with a warehouse-based vaccine (APVAC1) targeting non-mutated antigens followed by APVAC2, targeting preferentially neo-epitopes. Personalization was based on mutations, transcriptome, and immunopeptidome of the individual tumours. The GAPVAC approach was feasible and vaccinations adjuvanted by poly-ICLC (polyriboinosinic-polyribocytidylic acid-polylysine carboxymethylcellulose) and GM-CSF (granulocyte macrophage colony stimulating factor) displayed favourable safety and excellent immunogenicity: Non-mutated APVAC1 antigens induced sustained central memory CD8+ T-cell responses. APVAC2 induced predominantly T helper cell type 1 (TH1) CD4+ T-cell responses against predicted neo-epitopes.**

Targeting of selected neo-epitopes by vaccination in melanoma – a tumour showing many mutations – has demonstrated high immunogenicity and clinical efficacy6,7. However, only a minority of mutations is processed to human leukocyte antigen (HLA)-presented neo-epitopes and can be targeted by T cells8-10. For cancers with few mutations, this is likely a limitation for antigen-unspecific immunotherapies as CPI, as these tumours may frequently lack sufficient targetable neo-epitopes. Nonetheless, clinical and radiographic responses to CPI have been observed in GB patients with a hypermutator phenotype11,12, suggesting that GB is in principle susceptible to immunotherapy13. T-cell responses to non-mutated, over-presented antigens have likewise demonstrated encouraging clinical data in GB14-16 and may perfectly supplement targeting of neo-epitopes to achieve broad anti-tumour responses.

To establish a warehouse of pre-manufactured, synthetic peptides directly available for ‘off-the-shelf’ formulation, non-mutated HLA-presented antigens were identified from 30 GB specimens using the XPRESIDENT® technology17; preferred antigens were either frequently over-presented18 or tumour-exclusive, but presented only occasionally on tumours. Finally, 33 HLA-A\*02:01-binding and 26 HLA-A\*24:02-binding peptides (predominantly 9 amino acids (aa); Supplementary Table 1) were selected into the warehouse, favouring candidates with good immunogenicity (see Online Methods). To address for every GAPVAC-101 patient the relevance of each warehouse peptide as tumour antigen, the individual tumour’s HLA immunopeptidome and transcriptome were characterized by mass spectrometry (MS) and microarray analysis, respectively (Fig. 1). Pre-vaccine T-cell reactivity completed the dataset used to generate a patient-specific ranking of the warehouse peptides (exemplarily shown in Extended Data Fig. 1a-d). The peptide PTP-013 ranked high for patient 05: It showed tumour-specific over-presentation (Extended Data Fig. 1b) and messenger ribonucleic acid (mRNA) over-expression of the source gene (Extended Data Fig. 1c). Pre-vaccination peripheral blood mononuclear cells (PBMC) of patient 05 contained CD8+ T cells specific for PTP-013 - a pre-requisite for a later immune response (Extended Data Fig. 1d). Actively Personalized Vaccines 1 (APVAC1) were then composed of the seven best-ranking HLA class I peptides, two peptides binding promiscuously to different HLA-DR (class II) molecules (‘pan-DR’ antigen) and a viral marker peptide (Extended Data Fig. 2a). All APVAC1 compositions were different (n=16; Supplementary Table 2), also illustrated by the selection frequencies of HLA-A\*02:01 warehouse peptides for HLA-A\*02:01+ patients (n=13; Extended Data Fig. 1e), supporting the warehouse concept for personalization. Median definition time from enrolment to start of APVAC1 manufacturing was 52 (42-74) days, median production time until release of APVAC1 was 41 (33-51) days, enabling the planned vaccination start during the 1st maintenance temozolomide (TMZ) cycle.

For APVAC2, one of the following strategies was applied to select two peptides for Good Manufacturing Practice (GMP) synthesis and to formulate the vaccine (Extended Data Figs. 1f, 2a): I. Mutation-containing peptides confirmed as HLA ligands by MS; II. mutation-containing peptides with a predicted high likelihood for HLA class I binding and immunogenicity (usually 19mers, mutation at position 10), but not MS-confirmed as HLA ligands; III. if none of the two strategies identified suitable neo-epitopes, non-mutated GB-associated HLA class I epitopes identified in the individual immunopeptidome, but not part of the APVAC1 warehouse, were selected (9 to 10 aa). A median of 36 (19-84) somatic, non-synonymous mutations were detected by next generation sequencing within the analysed GB specimens from GAPVAC-101 patients (Table 1, Supplementary Table 3). None of these 643 genomic mutations was identified in the HLA class I and II peptidomes of the respective patients (n=15) employing high-sensitivity MS, while the identical process identified a mutated HLA-presented peptide in another patient with hypermutated GB, but not participating in GAPVAC-101 (data not shown). Consequently, the selection of the two APVAC2 peptides was based on the second and third strategies presented above, preferring predicted, mutation-containing neo-epitopes (example in Extended Data Fig. 1g). Eleven patients received APVAC2 compositions with a total of 20 *de novo* synthesized peptides (14 mutated, 6 non-mutated; Extended Data Fig. 1h, Supplementary Table 4). Median definition time for APVAC2 was 96 (85-114) days and median production time 86 (63-138) days.

APVAC1 and APVAC2 were applied independently intradermally during TMZ maintenance therapy to HLA-A\*02:01+ or -A\*24:02+ positive patients with newly diagnosed GB following gross total resection and standard chemoradiotherapy with TMZ (see Extended Data Fig. 2b for a detailed vaccination schedule). GM-CSF (i.d.) and poly-ICLC (s.c.) were co-applied as adjuvants and may enhance immune responses in a synergistic fashion19. Clinical trial authorization (NCT02149225) was obtained in five European countries based on a standardized drug definition and GMP manufacturing process, leading to variable vaccine compositions for each patient (Extended Data Fig. 9c) and potentially serving as a model for future trials.

Primary endpoints were safety, tolerability, immunogenicity, and operational feasibility of the concept. Of 58 screened patients (Extended Data Fig. 2e), 33 failed eligibility criteria and nine were not enrolled due to limited GMP production capacity (1-2 patients / month). Sixteen patients were enrolled before entering chemoradiotherapy. Baseline characteristics of these patients reflect a standard GB patient population (Table 1). Fifteen patients received APVAC1 (median: 12 applications) and eleven patients received APVAC2 (median: 10 applications; Extended Data Fig. 2c).

All vaccinated patients experienced study drug-related adverse events (AE; Supplementary Table 5) within expectations. Mild to moderate injection site disorders were most frequent, consistent with the expected mechanism of action of the vaccinations. Eleven patients experienced AE ≥ grade 3, mainly bone marrow suppression, frequently observed in patients undergoing chemoradiotherapy with TMZ. Two patients experienced an anaphylactic reaction after receiving vaccinations, potentially related to repeated application of GM-CSF20. Patient 09 required high-dose steroid treatment due to potentially immune-associated grade 3 brain oedema.

*Ex vivo* multimer analysis revealed vaccine-induced CD8+ T-cell responses to at least one APVAC1 HLA class I peptide in 12 out of 13 patients (92.3%; median: 3 responses, Fig. 2a). These responses persisted usually for several months. Presence of specific precursor T cells in the patients’ blood was a strong selection criterion for APVAC1. Consequently, APVAC1-reactive T cells were frequently observed pre-vaccination, usually at low frequency and of naïve phenotype. After vaccination, APVAC1-specific T cells shifted to a memory phenotype and in most cases showed increased frequency (Fig. 2b). For single specificities, the magnitude of *ex vivo* measured CD8+ T-cell responses was up to 0.02% of CD8+ T cells. Increase in Programmed Cell Death Protein 1 (PD-1) expression was low to moderate, not indicative of T-cell exhaustion (Extended Data Figs. 3a-b). In total, 45 of 87 (51.7%) vaccinated, immune-evaluable APVAC1 peptides were immunogenic (Figs. 2a, 4; Supplementary Table 2).

APVAC1-induced PTP-013-reactive CD8+ T cells cloned from post-vaccination PBMC of patient 16 (‘PTP T cells’) killed peptide-loaded target cells with high avidity (Fig. 2c; further avidity data in Extended Data Fig. 3d). These T cells also lysed the GB cell line L2 (Fig. 2d), that endogenously expresses the source gene of PTP-013 (PTPRZ1), demonstrating the functional capacities of APVAC1-induced T cells and the natural HLA-presentation of PTP-013. The dominant NLGN4X-001-specific T-cell receptor (TCR) from the same patient was cloned and re-expressed in healthy donor PBMC. T cells transfected with this TCR (‘NLGN4X T cells’) showed cytotoxicity against the NLGN4X+ GB cell lines L2 (Fig. 2e) and P2XX (Extended Data Fig. 3e) while the NLGN4Xneg GB cell line U87MG was only killed after transfection with NLGN4X (Extended Data Fig. 3f; gene expression data for used cell lines in Extended Fig. 3g). Nine out of 13 patients (69.2%) showed CD4+ T-cell responses to one or both non-mutated pan-DR antigens in APVAC1 (*ex vivo* intracellular cytokine staining [ICS]; example shown in Fig. 2f) with an overall immunogenicity of 50% for these peptides (Fig. 4). The responding T cells were of heterogeneous phenotype, but frequently TH1 (Fig. 2a, Fig. 4). The ratio of cumulative APVAC1-specific CD8+ memory T cell frequencies post- vs. pre-vaccination for a patient was defined as ‘memory cell induction factor’ (MCIF) (Fig. 2a). MCIF was higher in patients lacking a predominant TH2 or suppressive CD4+ T-cell response to APVAC1 (Extended Data Fig. 4d). Regulatory T cell (Treg) frequencies pre-vaccination (Extended Data Fig. 4a) were predictive for the biological activity of APVAC1: Low Treg levels were found in patients with MCIF above median and were associated with a higher proportion of interferon (IFN)-γ producing APVAC1-specific CD4+ T cells (Extended Data Figs. 4e-f).

Eight of ten patients (80%) evaluable for APVAC2 immunogenicity developed neo-epitope-specific, dominantly CD4+ T-cell responses (pan-ICS assay after *in vitro* sensitization (IVS); Extended Data Fig. 4h). Eleven of the 13 (84%) vaccinated, mutated APVAC2 peptides induced a CD4+ T-cell response (as exemplarily shown in Fig. 3a). These responses were predominantly of TH1 phenotype and multi-functional (Fig. 4). For two neo-epitope-specific CD4+ T-cell responses, recognition of the corresponding wildtype sequences was tested, revealing for one response cross-recognition and for the other neo-epitope specificity (Extended Data Fig. 7a), in line with the broad spectrum of wildtype cross-recognition previously reported6. Five neo-epitope-specific CD4+ T-cell responses came with CD8+ T-cell responses to nested, mutated HLA class I-restricted epitopes (Extended Data Fig. 4h, example in Fig. 3b). None of the mutated APVAC2 peptides evoked an isolated CD8+ T-cell response. Noteworthy, the 14-M09-specific CD8+ T-cell response measured by pan-ICS was confirmed by *ex vivo* multimer staining using a predicted, mutated HLA-A\*02:01 ligand contained in the 19mer (Fig. 3c).

Fig. 4 displays the favourable immunogenicity observed for GAPVAC-101. For the six non-mutated antigens within APVAC2, an immune response was observed only once (patient 08). Noteworthy, these antigens were immunized without prior knowledge about their immunogenicity (in contrast to APVAC1 peptides), arguing for the importance of immunogenicity as selection criterion for HLA class I antigens.

Tumour-infiltrating lymphocytes (TIL) were isolated and cultured for all but one patient at initial surgery, applying a harmonized TIL isolation and expansion protocol for GB specimens (see Online Methods). Expanded TIL comprised CD4+ and CD8+ T cells (Extended Data Figs. 8a-c). Analysis of TCR clonotypes in tumours and corresponding expanded TIL cultures (n=3) revealed relatively few amplified TCR clonotypes in glioma tissue, in line with recent flow cytometry-based data21, but in contrast to previous observations for TIL in other indications22,23. Interestingly, even fewer TCR clonotypes expanded *in vitro* (Extended Data Fig. 8d). Not a single APVAC reactivity was detected in any pre-vaccination TIL cultures from GAPVAC-101 patients or other GB patients (n=30 total, data not shown), suggesting that spontaneous T-cell immunity against GB is rare, but may be inducible by vaccination.

Some centrally confirmed radiographic responses were reported (Fig. 5a). However, in the months after chemoradiotherapy and maintenance TMZ such changes are frequently seen. Patients that received vaccinations (n=15) had a median overall survival (OS) of 29.0 months from diagnosis and a median progression-free survival (PFS) of 14.2 months (Extended Data Figs. 9a-b; Table 1). Patient 08 with a resolved pseudo-progression and an OS of >38.9 months experienced a favourable immune response pattern, with CD8+ T-cell responses to five APVAC1 class I peptides, TH1 CD4+ T-cell responses to both APVAC1 pan-DR peptides, and a combined CD8+ and TH1 CD4+ T-cell response to an APVAC2 neo-epitope. The tumour was re-resected upon recurrence 26.4 months after diagnosis and demonstrated high infiltration by T cells with a favourable CD8+/Treg ratio (Fig. 5b). The tumour contained CD4+ T cells directed to the APVAC1 pan-DR peptide PTP-010 (Fig. 5c), corresponding to a multi-functional, TH1-dominated reactivity in the periphery (Fig. 5d).

The warehouse-based APVAC1 process resulted in truly individual drug compositions, justifying the complex personalization. The failure to identify any of the 643 genomic mutations in the HLA peptidome of the patients may be partly due to the limited sensitivity of the MS approach. However, rates below 1% were reported previously for the transition of mutations into HLA class I ligands9,24 suggesting that the GAPVAC-101 data may partially reflect high attrition of mutations from the genetic level to actual HLA presentation of mutated peptides. This hypothesis is supported by the finding that during immunological validation of mutations, TIL were found reactive against 1.2% of mutations only25. Noteworthy, the same MS approach as applied here was able to identify mutated HLA ligands in human tumour tissue9. With the uncertainty of neo-epitope predictions, targeting of a higher number of mutations - if not all - would be favourable8. For GB and other low-mutation tumour types, additional exploitation of the non-mutated antigen pool is justified, and our personalized warehouse-based peptide vaccine approach showed to be well suited to address these antigens.

General superiority of neo-epitopes to induce CD8+ T-cell responses *versus* non-mutated class I peptides was not observed (Fig. 4), although data must be compared with caution since peptide format, analysis methodology, and availability of immunogenicity pre-testing differed between APVAC1 and APVAC2. Our CD8+ T-cell reactivity compared favourably with previous trials and particularly with responses seen with a vaccine that shared several antigens with the GAPVAC warehouse16 (Extended Data Fig. 4g). Improved immunogenicity may be attributable to the immune modulators, poly-ICLC plus GM-CSF (GAPVAC-101) *versus* GM-CSF alone (previous trial). Nevertheless, immune response induction needs further optimization.

The ACT IV trial with similar eligibility criteria demonstrated a median OS of 20 months26 and may come closest to a reference cohort, to which a median OS of 29 months in the GAPVAC-101 trial compares favourably with all limitations of comparing small early phase to phase III trials. Further efforts are required to decrease process complexity and duration, but the achievements of the presented trial certainly warrant further studies to understand how anti-tumour immunity can be leveraged to achieve ultimately clinical benefit for GB patients.

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**Supplementary Information** is available in the online version of the paper.

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**Author contributions** C.B., H.-G.R., H.O., H.S., N.H., P.-Y.D., S.K.-C., S.H., U.S., W.W. developed the GAPVAC concept trial design. De.M., F.M.-R., G.T., J.K., J.R., J.S., P.-Y.D., U.L., W.W. recruited and treated patients. A.T., C.S., J.C., J.F., K.F., M.L., N.H., O.S., S.H., S.K., S.K.-C., T.W., V.B. composed the APVAC vaccines. B.P., H.-G.R., J.P., M.S., S.D., S.S. contributed to APVAC manufacturing. A.U., C.G., C.W., Do.M., E.G., E.R., L.B., M.P., M.W., R.M., S.v.d.B. contributed to immune response analyses. C.G., E.R., M.I., M.W., P.t.S., S.v.d.B., V.D. contributed the TIL analyses and together with C.B., G.T., M.S., J.R.K., H.S.P., P.-Y.D., and De.M. contributed to the TIL protocol. K.McC., K.F., V.B., A.U., R.M., U.S., C.O. contributed further biomarker data. J.L., F.H., K.K., N.H., P.-Y.D., W.W. evaluated the clinical data. A.U., C.G., C.W., J.L., H.O., K.K., N.H., P.-Y.D., R.M., S.v.d.B., S.K.-C., V.D., W.W. wrote the manuscript. All other authors contributed significantly to design, conduct or analysis of the GAPVAC-101 trial or the scientific concept. For detailed contributions of shown experiments see Supplementary Notes.

**Author information** The authors declare competing financial interest as detailed in the Supplementary Notes. Correspondence and request for materials should be addressed to W.W. (wolfgang.wick@med.uni-heidelberg.de).

**Table 1** **** **Baseline characteristics and key outcomes for enrolled patients (n=16).**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Pat. | Gender | Age | KPS1 | HLA | Non-syn.mutations | MGMTpromoterhyper-methyl. | Number ofvaccinations(APVAC1 / 2) | Peptides vaccinated 🡪 immunogenic | OS3[months] | PFS3[months] |
| APVAC1Class I | APVAC1Class II | APVAC2² |
| 01 | m | 25 | 90 | A\*02:01 | 36 | ir | 10 / 7 | 7 → 4 | 2 → 1 | m/n → 1 | 24.8+ | 22.2 |
| 02 | f | 48 | 80 | A\*02:01 | 35 | - | 13 / 10 | 7 → 3 | 2 → 1 | m/n → 1 | 11.8 | 4.4 |
| 034 | m | 40 | 80 | A\*24:02 | 72 | - | 0 / 0 | - | - | - | (8.7) | (8.7) |
| 04 | m | 61 | 90 | A\*02:01 | -5 | - | 16 / 0 | 6 → 3 | 2 → 2 | - | 30.8 | 14.2 |
| 05 | m | 47 | 100 | A\*02:01 | 20 | - | 14 / 11 | 7 → 3 | 2 → 1 | m/n → 0 | 26.7+ | 14.5 |
| 06 | m | 27 | 100 | A\*24:02 | 19 | - | 8 / 1 | 7 → 0 | 2 → 2 | m/- → ne | 29.0 | 17.9 |
| 07 | f | 35 | 80 | A\*02:01 | 23 | - | 14 / 10 | 7 → 3 | 2 → 2 | m/m → 2 | 14.6 | 4.6 |
| 08 | m | 41 | 100 | A\*24:02 | 34 | + | 16 / 16 | 6 → 5 | 2 → 2 | m/n → 2 | 38.9+ | 26.4 |
| 09 | f | 55 | 90 | A\*02:01A\*24:02 | 76 | - | 4 / 0 | 7 → ne | 2 → ne | - | 13.9 | 5.8 |
| 10 | m | 70 | 70 | A\*02:01 | 38 | - | 8 / 0 | 6 → 5 | 2 → 0 | - | 31.5+ | 30.4+ |
| 11 | f | 67 | 80 | A\*02:01 | 48 | - | 5 / 0 | 7 → ne | 2 → ne | - | 8.8 | 8.8 |
| 12 | m | 57 | 100 | A\*02:01 | 50 | - | 12 / 9 | 7 → 1 | 2 → 1 | n/- → 0 | 19.3 | 10.8 |
| 13 | f | 50 | 90 | A\*02:01 | 38 | nt | 14 / 11 | 7 → 7 | 2 → 0 | m/m → 2 | 16.9 | 5.3 |
| 14 | f | 70 | 90 | A\*02:01 | 84 | + | 12 / 8 | 6 → 5 | 2 → 0 | m/m → 2 | 14.6 | 5.7 |
| 15 | m | 60 | 80 | A\*02:01 | 35 | + | 11 / 8 | 7 → 1 | 2 → 1 | m/n → 1 | 34.1+ | 33.4+ |
| 16 | f | 57 | 90 | A\*02:01 | 35 | + | 16 / 15 | 7 → 5# | 2 → 0 | m/m → 1 | 27.5+ | 27.4+ |
|  | f: 44%m: 56% | Median:52.5 yrs. | Median:90 | A\*02:01+: 81.3%A\*24:02+:25% | Sum: 643Median: 36Mean: 43 | 28.6%MGMThyper-methyl. | Median: 12 / 10(n=15 / 11) | 92%immuneresponder(n=13) | 69%immune responder(n=13) | 80%immuneresponder(n=10) | Median: 29.0 m(n=15) | Median: 14.2 m(n=15) |

**Footnotes:**

1Karnofsky Performance Score before 1st vaccination; ²Composition of vaccinated APVAC2: m = mutated antigen, n = non-mutated antigen; 3OS and PFS from diagnosis, ‘+‘ = censored; 4Patient dropped out before 1st vaccination (not included in OS/PFS analysis); 5Tumor material necrotic; mutations could not be analyzed; #Only 1 post-vaccination time point evaluable for APVAC1 responses; MGMT = O6-methylguanine-DNA methyltransferase; nt = not tested; ir = inconsistent results; ne = not evaluable.

**Figure legends**

**Figure 1** **** **GAPVAC concept of active personalization**. Non-mutated antigens (APVAC1) and preferentially mutated, *de novo* antigens (APVAC2) were targeted. Vaccinations (triangles) in the GAPVAC-101 trial superimposed to standard chemoradiotherapy (CRT) and maintenance TMZ cycles (T). Several pre-treatment biomarkers and immune response data were evaluated. For soluble HLA (sHLA) analysis see27.

**Figure 2** **** **Non-mutated APVAC1 induces sustained immune responses.** **a**, Summary of immune responses to APVAC1 (n=13 evaluable patients). Total APVAC1-specific CD8+ T cell frequencies are shown (P = pre-treatment; 1, 2, 3, … = post-treatment; MCIF = memory cell induction factor). For CD4+ T-cell responses, dominant phenotypes are indicated. **b**, CD8+ T-cell response in patient 02 against NLGN4X-001 evaluated pre- and post-treatment (Post1 to 3). Percentages of specific CD8+ T cells are indicated. Phenotyping: Percentage of cells in the quadrants correspond to differentiation phenotypes (colour code as in a; see also Extended Data Fig. 4b). **c**, Avidity determination of PTP-013-reactive CD8+ T cells from patient 16 (Vital-FR assay; E:T = 10:1; mean ± SD; n=3 replicates; flow cytometry data in Extended Data Fig. 3c). Half-maximal effective concentration (EC50) ± standard error is indicated. **d,** PTP T cells show cytotoxicity against the PTPRZ1+ GB cell line L2. **e**, NLGN4X T cells lysed NLGN4X+ L2 cells. **d**, **e**, Lactate dehydrogenase (LDH) release assays, n=3 replicates; median ± range; HD T cells = healthy donor-derived T cells, not target-specific. **f**, CD4+ T-cell response to the pan-DR peptide BIR-002 in patient 07 (flow cytometry data in Extended Data Fig. 5). For assays with limited patient material, see statistics section.

**Figure 3 ** **Neo-epitope-targeting APVAC2 induces predominantly TH1 CD4+ T-cell responses.** **a**, Exemplary TH1 CD4+ T-cell response to mutated peptide 16-M06. **b**, Exemplary CD8+ T-cell to mutated peptide 15-M05. **a**, **b**, pan-ICS after IVS (flow cytometry data in Extended Data Fig. 6). **c**, CD8+ T-cell responses to a mutated epitope (HMKVSVYLL) contained in the 19mer peptide 14-M09 (*ex vivo* 2D multimer assay, flow cytometry data in Extended Data Fig. 7b). For assays with limited patient material, see statistics section.

**Figure 4 ** **Summary of APVAC-induced responses.** Typical amino acid (aa) lengths for APVAC peptide categories are indicated.

**Figure 5 ** **Clinical outcome and intra-tumoral T cells in patient 08.** **a**, Swimmer plot for enrolled patients (n=16). **b**, Immunohistochemical staining of patient 08’s tumour tissues primary and at 1st re-resection. Stained cells per mm² tumour tissue section are indicated (n=4 independent regions analysed). **c**, **d**, CD4+ T-cell response against the APVAC1 pan-DR peptide PTP-010 in TIL at re-resection (c; ICS) and in PBMC of patient 08 (d; IFN-γ ELISpot; tested once in n=2 replicates; for ICS data see Extended Data Fig. 4c). **c**, n=5 independent experiments with 6 TIL cultures. Results provided from one of n=3 positive cultures.

**Online Methods**

**APVAC1 warehouse.** For APVAC1 warehouse setup, a total of 30 HLA-A\*02+ and HLA-A\*24+ GB tumour samples was analysed in 2012/13 for their immunopeptidomes by MS revealing several thousands of different HLA-A\*02:01 and A\*24:02-presented peptides applying the XPRESIDENT® antigen discovery platform17,28. From this repertoire, strongly tumour-associated candidates were identified, i.e. peptides exclusively found on tumours or highly over-presented as compared to the immunopeptidome of a panel of normal tissue types. Expression analysis of source gene mRNA in GB samples in comparison to normal tissue expression and immunogenicity analysis in the blood of healthy donors was used as supportive data to select for 33 A\*02:01 and 26 A\*24:02 warehouse peptides (Supplementary Table 1).

**GAPVAC-101 phase I trial design.** Between 2014 and 2018, a single-arm, open-label first-in-human trial (NCT02149225) was conducted at six study centres in Germany, Switzerland, The Netherlands, Spain, and Denmark in accordance with the Declaration of Helsinki, International Conference on Harmonization/Good Clinical Practice guidelines and all other applicable regulatory and ethical requirements. The trial was approved by the responsible Ethics Committees and regulatory agencies as detailed in the Supplementary Notes. All patients provided written informed consent. Pseudonymization of patients was performed at the clinical centres. Patient identification logs were kept strictly confidential by the investigators. HLA-A\*02:01+ and/or HLA-A\*24:02+ patients with newly diagnosed GB after gross total resection as judged by a post-operative scan within 48 h post-surgery, KPS ≥70% and a life expectancy >6 months were enrolled (see clinicaltrials.gov for details on eligibility criteria). From the initial surgery, a tumour tissue specimen was freshly frozen for later immunopeptidome, mRNA expression and mutanome analysis. If possible, a further tumour tissue sample was used freshly for isolation of tumour-infiltrating lymphocytes (TIL, see below). Moreover, plasma samples were collected pre- and post-surgery for analysis of the soluble HLA ligandome27. Before start of standard CRT with TMZ, a leukapheresis was performed (target cell yield: 4.8 x 109 white blood cells) and peripheral blood mononuclear cells (PBMC) were isolated for patient-individual immune repertoire analysis (see below). APVAC1 and APVAC2 vaccines for each patient were designed and manufactured starting during standard of care CRT29. Vaccinations with APVAC1 (400 µg per peptide i.d., 11 vaccinations scheduled) started on day 15 of the 1st adjuvant TMZ cycle (see Extended Data Fig. 2b for schedule details). GM-CSF (75 µg i.d.) and poly-ICLC (1.5 mg s.c.) were used as immune modulators and were applied near the APVAC injection site. APVAC2 vaccines were applied starting on day 15 of the 4th adjuvant TMZ cycle (400 µg per peptide i.d., 8 vaccinations scheduled), usually leading to co-application of APVAC1 and APVAC2 at three visits (Fig. 1). After completion of the regular APVAC vaccinations or after progression, it was possible to continue vaccinations if in the patient’s best interest and if medication was still available. Continued vaccinations beyond the regular schedule were performed in 4-weekly intervals. Nine patients received vaccinations under this “Continued vaccination” option. Exposure to standard therapy was not altered for patients in the GAPVAC-101 trial (Extended Data Fig. 2d). Primary endpoints of the trial were safety and tolerability, feasibility of the personalized approach and biological activity.

**APVAC1 selection and manufacturing.** The patients’ fresh frozen tumour samples underwent central pathology review to enrich for necrosis-free tumour pieces with high tumour cell content. Tumour cell enriched specimens were lysed and HLA class I and class II associated peptides were isolated and analysed by MS as previously described17. Briefly, HLA peptide extracts were separated by reversed-phase chromatography (nanoAcquity UPLC system, Waters, USA) using ACQUITY UPLC BEH C18 columns (75 µm × 250 mm, Waters). Mass spectrometry was performed on Orbitrap LTQ Velos or Fusion mass spectrometers (Thermo Fisher Scientific, USA) by data-dependent (DDA) acquisition using collision-induced dissociation (CID) as well as higher-energy collisional dissociation (HCD). Further, genomic DNA and mRNA was isolated from the same lysate for mutanome and gene expression analyses, respectively. mRNA was quality-controlled and analysed by gene microarray analysis using Affymetrix Human Genome (HG) U133 Plus 2.0 oligonucleotide microarrays (Affymetrix). Finally, the patient’s PBMC were assessed for precursor T cells specific for the APVAC1 warehouse peptides as previously described30. Briefly, CD8+ T cells were stimulated *in vitro* under limited dilution conditions (usually 12 wells per peptide) using artificial antigen presenting cells. Wells with >1% specific CD8+ T cells as per multimer staining after 3 weeks of culture were counted as positive. If no patient individual immunogenicity data were available (patient 13), the percentage of positive wells in healthy donors was used as surrogate. For each patient a ranking score was calculated for each warehouse peptide, considering the individual peptide presentation data, mRNA expression data and immunogenicity data (see Supplementary Methods 1: ‘APVAC1 selection process’ for details). In brief, the different parameters were transformed into probability scores (values between 0 and 1) using a Bayesian method. The rank score of a warehouse peptide constitutes a probabilistic score ensuring a strong tumour association as well as sufficient immunogenicity for highly ranked peptides. For each patient, the seven best-ranked peptides were selected into the personalized APVAC1 composition (as shown exemplarily for patient 05 in Extended Data Fig. 1a) together with a viral marker peptide (HBV-001 or HCV-002) and BIR-002/PTP-010 as invariant pan-HLA-DR (class II) binding peptides (PTP-010 was replaced by BCA-005 for two compositions due to stability issues). Finally, APVAC1 compositions were reviewed and approved by a Target Selection Committee (TSC). The peptides were formulated into single-dose vials and released according to GMP at the manufacturing site of the Department of Immunology, University of Tübingen (Germany).

**APVAC2 selection and manufacturing.** For APVAC2, tumour-specific somatic mutations were analysed by BioNTech AG (Mainz, Germany) from the deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) samples extracted from high quality enriched tumour material as compared to DNA from normal blood cells from the same patient using Next Generation Sequencing (NGS). Whole exome sequencing libraries were prepared using Agilent’s SureSelect V6 XT Kit. From the tumour RNA, poly(A)-based RNA sequencing libraries were prepared using Illumina’s TruSeq. For samples with a tumour content above 40%, whole exome and RNA sequencing libraries were prepared in singletons and sequenced paired-end for 50 nucleotides (nt) on an Illumina HiSeq 2500, aiming for a minimum of 150 million reads per library. For samples with a tumour content below 40%, library preparation and sequencing were done in duplicates. All mutanome-related data analysis steps were coordinated by a software pipeline. For the DNA libraries, a minimum of 150 × 106 paired-end 50 nt reads and for the RNA libraries a minimum of 75 × 106 paired-end 50 nt reads were required. For mutation detection, DNA reads were aligned to the reference genome hg19 with bwa31. Exomes from tumour and matched normal samples were analysed for single nucleotide variants. Loci with putative homozygous genotypes in the normal samples were identified and filtered to retain high confidence calls for single nucleotide variants. Remaining sites were further inspected for a putative homozygous or heterozygous mutational event. Suspected sites were filtered to remove potential false positives. The final list of single-nucleotide variants was comprised of high confidence homozygous sites in the normal samples and high confidence heterozygous or homozygous mutational events in the tumour samples. Genomic coordinates of identified variants were compared with the University of California Santa Cruz (UCSC) known genes transcript coordinates to associate the variants with genes, transcripts, potential amino acid sequence changes and the RNAseq-derived expression values. For RNAseq, RNA reads were aligned to the hg19 reference genome and transcriptome using bowtie32, and gene expression was determined by comparison with UCSC known genes transcript and exon coordinates, followed by normalization to reads per kilobase million (RPKM) units33. High sensitivity liquid chromatography tandem MS (LC-MS/MS) data were analysed against a personalized proteome library containing all single-nucleotide variants to identify HLA class I- and class II-presented, mutation-containing peptides. MS-confirmed mutations containing HLA epitopes would have gained priority for APVAC2 (but were not detected). As second track, predicted variants were selected from the identified single-nucleotide variants by first removing of non-sense variants and filtering by non-zero exon- and transcript expression and then sorting by exon expression followed by HLA class I binding prediction score using a stable sorting algorithm. HLA binding affinity was predicted via the Immune Epitope Database (IEDB) T-cell prediction tools (version 2.5, IEDB-recommended mode)34 using all variant-containing 8-11mers for HLA-A/B binding estimations. Out of all predictions for a single variant, the best IEDB consensus score was associated with the respective variants. Potential immunogenicity was assessed by calculating the difference of the predicted HLA binding affinity of the mutant peptide and the wild type counterpart as well as the absolute predicted HLA binding and the variant expression, in analogy to a previously published algorithm35. A shortlist of up to 46 single-nucleotide variants (SNV) was selected for confirmation by Sanger sequencing. For primer design, genomic sequences flanking the mutation sites were extracted from the reference genome and used as input for the primer3 software36,37. The output primer pairs were aligned to the reference genome using BLAT38. Primer pairs with alignments to off-target loci were removed, and the remaining optimal primer pair was returned for each input site. Sanger sequencing was performed by amplifying each selected mutated locus from tumour tissue and white blood cell DNA by polymerase chain reaction (PCR) (15 min at 95 °C for the initial activation followed by 35 cycles of 30 s at 94 °C for denaturation, 30 s at 60 °C for annealing, 30 s at 72 °C for extension, and 6 min at 72 °C for the final extension). Each PCR product was quality controlled using a QIAxcel (Qiagen) device and purified via ExoI/AP treatment. Sanger sequencing was performed by Eurofins/MWG (Germany). From the confirmed mutations, only the highest-ranking ones as judged by HLA binding, expression and immunogenicity were considered for APVAC2 as 19mer peptides (mutation flanked by 9 amino acids of the natural sequence on each side to cover all potential mutation-containing HLA binding 9-10mers while minimizing the number of contained potential HLA binders that do not contain the mutation). As a third track, HLA class I peptidome data were assessed for tumour-associated non-mutated peptides not contained in the warehouse. Highly promising candidates were integrated into the prioritized ranking (without immunogenicity pre-testing). The TSC selected the most promising candidates from all APVAC2 tracks to a ranked shortlist with up to four candidates. The final selection also considered variant allele frequency, tissue distribution of wildtype source gene expression and confirmation of mutations by Sanger sequencing. Manufacturability of the four candidates was tested under non-GMP conditions. If successful, the two highest-ranking APVAC2 peptides were *de novo* synthesized, formulated under GMP conditions and filled into single-use vials at the manufacturing site of Department of Immunology, University of Tübingen (Germany). For patients 01, 02, 06, 12, and 15, low yield and/or purity of an APVAC2 peptide candidate required replacement by backup candidates or removal of one peptide (patients 06, 12). For patient 06, first application of APVAC2 was delayed by one TMZ cycle due to delays in manufacturing. In all other cases, GMP manufacturing of APVAC2 was completed in time.

**Clinical assessments and feasibility assessment.** Safety in this trial was analysed based on the occurrence of adverse events which were categorized according to US National Cancer Institute’s Common Terminology Criteria for adverse events (NCI-CTCAE) version 4.0 and summarized on Medical Dictionary for Regulatory Activities (MedDRA) version 18.0 preferred term level and according to their potential relation to study drugs (APVAC vaccines, GM-CSF, poly-ICLC) and TMZ treatment. Operational feasibility was assessed based on success rates and duration of APVAC1 and APVAC2 definition and manufacturing processes. Magnetic resonance imaging (MRI) scans were taken according to European Society of Medical Oncology (ESMO) guidelines39 and evaluated based on the criteria defined by the Neuro-Oncology Working Group (RANO)40 taking special care in the aspect of potential pseudo-progression which may be frequent in immunotherapy trials due to immune cell infiltration into the GB tumour14.

**Immune response assessment and biomarker analyses.** At different time-points pre- and post-vaccination (Fig. 1), PBMC for immunomonitoring were isolated within 8 h from venipuncture from patients’ sodium heparin blood by standard Ficoll-Hypaque density gradient centrifugation. Cells were cryopreserved in serum-free medium until standardized assessment of immune responses (Immatics). Thawed PBMC samples were labelled with CD8 microbeads (Milteny Biotec) and the CD8+ T cell fraction was separated from CD8neg cells by MultiMACSTM device according to manufacturer instructions. Both cell fractions were rested overnight.

**HLA class I *ex vivo* 2D multimer assay.** UV-exchange was used to create peptide:MHC (pMHC) monomers loaded with specific APVAC peptides as previously described41. Anti-β2-microglobulin enzyme-linked immunosorbent assay (ELISA) was performed to confirm efficiency of peptide exchange. For each peptide to be tested, two pMHC multimers of the same specificity but with two different fluorochromes were combined as previously described42. All APVAC and control pMHC multimer solutions for a given patient were combined to one personalized pMHC multimer staining cocktail. Potential aggregates were removed by centrifugation. Staining was performed as previously described43. In brief, between 1.5x106 and 5x106 CD8+ T cells were treated first with Life/Dead near infrared (Life Technologies), followed by patient-individual 2D multimer staining18 (each pMHC multimer at a concentration of 1-5 µg/mL) and surface staining with antibodies CD8-FITC (fluorescein isothiocyanate; SK1, BioLegend), CD279/PD-1-BV786 (EH12.1, BD); CD197/CCR7-phycoerythrin (PE)-CF594 (150503, BD); CD45RA-AF700 (HI100, BioLegend) and CD4 (RPA-T4, BD), CD14 (61D3, ebioscience), CD16 (3G8, BD) and CD19 (HIB19, BD) antibodies for dump channel (all PE-Cy5). All washing steps were carried out in phosphate buffered saline (PBS), 2% foetal calf serum (FCS), 2 mM ethylenediaminetetraacetic acid (EDTA), 0.01% azide. Stained cells, fixed with FACS Perm2 solution (BD), were acquired on a LSRII SORP flow cytometer (BD) and analysed by FlowJo software version 10.1 (Tree Star, Ashland, USA). 2D multimer-positive cells had to be positive for the corresponding fluorochrome combination, but negative for any other fluorochrome as determined by Boolean gating42. The gating strategy is shown in Extended Data Figure 10a. The presence of a vaccine-induced response was determined according to pre-defined criteria43: Briefly, frequencies of 2D multimer-positive cells among CD8+ T cells had to be at least twofold above the corresponding frequency before vaccination and had to form a clustered population, discrete from multimer-negative cells. Staining against viral control antigens was performed for each donor: Influenza-derived peptide FLUM-001 (GILGFVFTL, HLA-A\*02) and/or Epstein-Barr virus (EBV)-derived peptide EBV-014 (TYSAGIVQI, HLA-A\*24) as positive controls; human immunodeficiency virus (HIV)-derived peptides HIV-001 (ILKEPVHGV, HLA-A\*02) and/or HIV-015 (RYPLTFGW, HLA-A\*24) as negative controls. Viral marker peptides (HBV-001 or HCV-002) were co-vaccinated in APVAC1 and used as vaccination controls44,45. For each stain experiment, two healthy donors (one HLA-A\*02+, one HLA-A\*24+) were used as external assay controls. Patient 09 and 11 were vaccinated with APVAC1 but were not evaluable, as they left the study before post-vaccination PBMC were available. For patient 16, only one post-vaccinations time point was available and was included post-hoc into the APVAC1 immune response analysis. The memory cell induction factor (MCIF) for patients was calculated as follows: The sum of frequencies of all APVAC1-specific CD8+ T cells of central memory (CD45RAneg CD197+), effector memory (CD45RAneg CD197neg), and effector memory RA (CD45RA+ CD197neg) phenotypes was calculated per assay time point. In case that no event was detected, 0.1 event per antigen specificity was assumed. Next, the ratios of these memory cell frequencies post-vaccination vs. pre-vaccination were calculated for each post-vaccination time point. The MCIF for a patient was defined as the maximum of these ratios. For patient 04, BCA-002- and PLEKHA4-001-reactive T cells were present before vaccination and did not expand, resulting in a low MCIF (Fig. 2a).

***Ex vivo* HLA class II ICS assay.** After overnight rest, between 1.7 and 5.1x106 CD8neg T cells per well were incubated with antigenic peptide (10 µg/mL) in the presence of monensin and brefeldin A per manufacturer’s instructions (both BD) for 6 h at 37°C. Cells of each assay time-point were stimulated with the corresponding APVAC1 class II peptides. Furthermore, cells were stimulated with MOCK peptide (solvent only) as internal negative and CMV-002 (LPLKMLNIPSINVH)/CEFT peptide pool MHC Class II (Panatecs, Germany) as positive control. Additionally, in each assay two healthy donors were used as external controls and stimulated using MOCK, HIV-001/HIV-015 as negative and a pool of CMV-002/EBV-014/influenza-derived FLUM-001 (GILGFVFTL)/CEFT peptides as positive controls. After stimulation, CD8neg cells were treated with Life/Dead near infrared (Life Technologies), followed by surface staining using a CD8-BV570 (RPA-T8, BioLegend). After a washing step in PBS, 2% FCS, 2 mM EDTA, 0.01% azide, cells were fixed using FACS Perm2 solution (BD) and intracellular staining was carried out using PermWash (BD) and CD3-BV711 (OKT3, BioLegend), CD4-BV605 (OKT4, BioLegend), CD154-FITC (TRAP1, BD), IFN-γ-PE-Cy7 (4S.B3, BD), interleukin (IL)-2-BV510 (MQ1-17H12, BioLegend), IL-4-BV421 (MP4-25D2, BioLegend), IL-5-PE (JES1-39D10, 1:10 BD), IL-10-allophycocyanin (APC) (JES3-9D7, Miltenyi) and tumour necrosis factor (TNF)-α-AF700 (MAb11, BD). Cells were acquired and analysed as described above. Gating and analysis of vaccine-induced responses was performed according to recommendations of the CIP46 (Extended Data Fig. 10b). Out of 7 cytokines/markers, all 27=128 possible combinatorial patterns were calculated using Boolean gating. To be defined as a vaccine-induced response, the frequency of cytokine positive cells within one combinatorial pattern had to be at least twofold over the frequency in the corresponding MOCK control and the frequency before vaccination.

**HLA-class I and class-II pan-ICS assay.** PBMC samples were incubated with 0.5 U/mL benzonase (Merck, Germany). APVAC2- or control peptide-specific CD4+ and CD8+ T cells were amplified by a single round of IVS for 12 days using 2.5x106 PBMC per well, 10 µg/mL of antigenic peptide and 20 U/mL IL-2 (Novartis, Germany). Samples of each assay time point of a given patient were stimulated with a pool of all patient-specific full length APVAC2 peptides as well as separately with a viral peptide pool (FLUM-001/CMV-002/EBV-014/CEFT pool) serving as positive control for IVS. As inter-assay control, two healthy donor controls were included in each individual assay. After 12 days, cells were re-stimulated (0.275 - 1.7x106 cells per well) with 10 µg/mL antigen peptide in the presence of monensin, brefeldin A and CD107a-FITC (H4A3, BD) for 6 h at 37 °C. Re-stimulation was either performed with individual full-length APVAC2 peptides or pools of all possible nonamers and predicted HLA class I epitopes derived from a given parental APVAC2 peptide (if applicable). MOCK peptide control and stimulation with viral peptide pool as positive control were included. Stimulated cells were surface stained using CD8-BV605 (SK1, BioLegend) before fixation with FACS Perm2 solution (BD). Intracellular staining with antibodies CD4-BV785 (RPA-T4, BioLegend), CD154-BV421 (TRAP1, BD), IFN-γ-PE-Cy7 (4S.B3, BD), IL-2-BV510 (MQ1-17H12, BioLegend), IL-5-PE (JES1-39D10, BD), IL-10-BV650 (JES3-9D7, BD), IL-17-BV570 (BL168, BioLegend), IL-21-APC (3A3-N2, BioLegend) and TNF-α-AF700 (MAb11, BD) was carried out in PermWash buffer (BD). Cells were acquired and analysed as described above to assess cytokine positive cells among CD4+ and CD8+ T cells. Gating was performed according to recommendations of the CIP (Extended Data Fig. 10c). Using Boolean gating, 77 of 512 combinatorial patterns were selected reflecting the cytokine expression profiles of TH cell subsets according to recent literature47. To be defined as vaccine-induced response, frequency of cytokine positive cells within one combinatorial pattern had to be at least 4-fold over the frequency of the corresponding MOCK control and the frequency before vaccination.

**IFN-γ ELISpot (enzyme-linked immunospot assay).** Detailed protocols have been described previously48,49. Briefly for Fig. 5d, PBMC from patient 08 were thawed and expanded during 12 days in the presence of 5 µg/mL peptide and 2 ng/mL recombinant human IL-2 (R&D, MN). Cells were harvested and tested in duplicates by IFN-γ ELISpot with 2.5 µg/mL peptide, 10 µg/mL phytohaemagglutinin (PHA)-L as positive control, or 10% dimethyl sulfoxide (DMSO) as MOCK. After 26 h incubation, spots were revealed with 5-bromo-4-chloro-3-indolyl-phosphate (BCIP)/ nitro blue tetrazolium chloride (NBT) and analysed with an ImmunoSpot Series 6 ELISpot Reader (C.T.L. Europe, Germany).

**TIL expansion protocol.** A harmonized protocol for TIL expansion was established during the preclinical phase of the project. First, information on protocols established at each clinical centre for TIL isolation and *in vitro* expansion were compared; based on this survey, three IL-2 concentrations (150, 1,000 and 6,000 IU/mL) and the addition of a CD3 signal (soluble CD3 Ab or beads coated with CD3/CD28 Ab) were tested for expanding glioma TIL. Cell outgrowth, cell yields and distribution of T- and natural killer (NK)-cell subsets were determined for altogether 24 gliomas at four centres. T cell expansion was similar in the presence of 6,000 IU/mL and 1,000 IU/mL IL-2, but lower with 150 IU/mL. CD3+ CD4+ TCRαβ+ cells were prominent in most cultures, which is different when compared to what is found for melanoma (Extended Data Fig. 8a). In some individual cultures, a stronger expansion of CD8+ T cells was observed (Extended Data Fig. 8c). Hence, we recommend culturing several individual cultures rather than pooling all TIL. CD3 stimulation clearly increased cell yield as compared to IL-2 alone (Extended Data Fig. 8b), as observed previously by others50. In 2/2 tumours tested, the ratio CD4+/CD8+ T cells did not change significantly over the weeks of culture (3 tests at one-week intervals, not shown). Based on the results obtained in these optimization experiments, a concentration of rhIL-2 of 1,000 IU/mL and the addition of a CD3 antibody were finally chosen. The final GAPVAC harmonized protocol for GB TIL expansion which was afterwards applied during the clinical phase is provided as Supplementary Method 2 (‘Harmonized GAPVAC TIL isolation and expansion protocol from glioblastoma tumour specimens’).

**Functional testing of TIL.** TIL peptide-specificity testing was performed using ICS (for HLA class I peptides and HLA class II or long peptides) and HLA-multimer staining (for HLA class I peptides only). Both assays were harmonized during the pre-clinical phase by organizing successive proficiency panels (n=4 in total) based on those published by the Immunoguiding program of the Association for Cancer Immunotherapy (CIP-CIMT)46,51,52. For Fig. 5c, TIL were thawed, counted and allowed to rest in the presence of 1 µg/mL DNAse I. Cells were then washed and stimulated with peptide (10 μg/mL) or 10% DMSO in the presence of monensin and brefeldin A (10 μg/mL, Sigma). After overnight stimulation, cells were washed and stained with Zombie Aqua (Biolegend), and antibodies CD4-APC-Cy7 (BD), CD8-PE-Cy7 (Beckman Coulter) and CD3-BV711 (Biolegend) for 20 min at 4 °C, fixed and permeabilized (Cytofix/Cytoperm, BD) and stained with anti-CD154-APC, anti-IFN-γ-FITC, anti-IL-2-PE (all BD) and anti-TNF-Pacific Blue (Biolegend) for 20 min at 4 °C. Cells were washed and acquired on a LSR Fortessa SORP equipped with Diva v6.1.2 (BD). Results were analysed with FlowJo version 10. The gating strategy was: time histogram, FSC-A/FSC-H (singlets), FSC-A/Zombie Aqua (living cells), FSC-A/SSC-A (lymphocytes), FSC-A/CD3 (CD3+ lymphocytes), CD4/CD8 (CD8neg).

**TCR clonotype analysis.** RNA was isolated from tumour specimens and TIL cultures using the NucleoSpin® RNA kit (MACHEREY-NAGEL, Germany) according to manufacturer’s instructions. RNA was reverse-transcribed to complementary DNA (cDNA) using SuperScript® VILO™ cDNA Synthesis Kit (ThermoFisher Scientific). TCR clonotype mapping was carried out as previously described53. In short, cDNA was amplified by PCR using a primer panel amplifying the 24 beta-chain variable region (BV) families of the TCR in DNA fragments suitable for TCR clonotype mapping by electrophoresis on a denaturing gradient gel (DGGE) containing 6% polyacrylamide and a gradient of urea (Sigma Life Science) and formamide (Sigma-Aldrich) from 20% to 80%. Gels were run at 160-175 V for 4.5 h in TAE (0.04 M Tris-acetate, 0.001 M EDTA) and kept constantly at 56 °C. Gels were stained with cyber green and visualized under UV light.

**TCR identification and *in vitro* expansion.** APVAC1-specific T cells were sorted from post-vaccination PBMC of patient 16. TCR sequences were obtained by single-cell rapid amplification of cDNA-ends (RACE) PCR and subsequent sequencing of the α and β chains54. In parallel, sorted T cells were stimulated *in vitro* for 3 weeks using PHA (2µg/mL), IL-2 (300 IU/mL), IL-7 (10 ng/mL) and IL-21 (10 ng/mL) and were subsequently used in Vital-FR killing assays.

**TCR cloning.** The VDJ regions of the TCR were combined *in silico* with murine constant regions modified to include an additional disulphide bond, as described previously55. The α and β human/mouse chimeric TCR chains were codon optimised *in silico* and linked by a furin cleavage site and P2A sequence to form a polycistronic transcript. Constructs were synthesised by ThermoFisher Scientific or Eurofins Genomics, before being cloned downstream of an EF1a promoter in a mammalian transient expression vector using golden gate assembly.

**Cloning of over-expression constructs.** cDNA of NLGN4X in pDONR vector was provided by Dr. Rainer Will of the DKFZ GPCF Vector and Clone Repository. An internal ribosomal entry site (IRES) sequence was appended to the NanoLuc® luciferase sequence using tailed primer PCR, and the resulting PCR product was inserted into pDONRTM P2rP3 using GatewayTM BP cloning. The phosphoglycerate kinase (PGK) promoter was inserted into pDONRTM P4-P1r using tailed primer PCR and GatewayTM BP cloning. Expression vectors combining the PGK promoter, NLGN4X or NRCAM full length cDNA, and the IRES-NanoLuc® luciferase marker were assembled using Multisite GatewayTM LR cloning.

**Electroporation of T cells and transfection of target cells.** T cells were isolated from PBMC from HLA-A2\*01-positive healthy donors using the MagniSortTM Human T cell Enrichment Kit according to manufacturer’s instructions. T cells were kept in TexMACSTM without phenol red and activated with 155 U/mL human IL-7, 290 U/mL human IL-15, and T Cell TransActTM. Vector DNA was prepared using a ZymoPURE II plasmid maxiprep kit (Zymo Research). Plasmid DNA was electroporated using a Neon electroporator (ThermoFisher Scientific; pulse voltage 2,200 V, pulse width 20ms, pulse number =1, cell density = 5x106). Transfection of HLA-A2\*01-positive U87MG cells was done using FuGENE® HD (Promega).

**Cytotoxicity assays.** Avidity of APVAC1-specific T cells as shown in Fig. 2c was analysed using the Vital-FR assay56. In brief, T cells were co-cultured with K562-A2 cells loaded with titrated amounts of test peptide (carboxyfluorescein succinimidyl ester (CFSE)-labelled) and K562-A2 cells loaded with an irrelevant peptide (FarRed-labelled). After 20 h, numbers of CFSE- and FarRed-labelled cells were assessed by flow cytometry and specific lysis was calculated. For peptide titration experiments as shown in Extended Data Fig. 3d, T2 cells were stained with Vybrant® DiD and T-cell clones with CFSE. Co-culture (4 h) was performed with increasing concentrations of peptide (effector-to-target ratio [E:T] = 10:1). Cell death was measured by addition of 7-aminoactinomycin D (7AAD) and detection of 7AAD+ Vybrant® DiD+ T2 cells by flow cytometry. For NanoLuc® luciferase-based killing assays, TCR-engineered T cells and transfected U87MG target cells (NLGN4X, NRCAM) were co-cultured for 9 h. Supernatants were carefully removed to remove cell debris. After addition of new medium, the Nano-Glo® Luciferase Assay System (Promega) was used according to manufacturer’s instruction to measure NanoLuc® luciferase expression. For LDH-based killing assays, autologous (NLGN4X-001-reactive, PTP-013-reactive) and HLA-A2\*01+ T cells were rested overnight and co-cultured for 9 h with HLA-A2\*01+ glioma-initiating cell lines (L2 or P2XX). CytoTox96® Non-Radioactive Cytotoxicity Assay (Promega) was used according to manufacturer´s instructions to quantify LDH-release. Absorbance was measured at 490 nm.

**Assessment of Treg frequencies.** Cryopreserved PBMC samples were thawed and 1.5x106 PBMC were stained with the following antibodies: CD3-pacific blue (clone SK7, Biolegend), CD4-APC.Cy7 (clone SK3, Biolegend), CD25-APC (clone M-A251, Biolegend), CD45RA-PerCP.Cy5.5 (clone HI100, Biolegend), CD127-PE.Cy7 (clone HIL-7R-M21, BD), FoxP3-Alexa488 (clone 259D/C7, BD), Ki67-PE (clone Ki67, Biolegend) and the live/dead marker Zombie Aqua (Biolegend). Cell surface antibody staining was performed in PBS/0.5% BSA/0.01% azide for 30 min at 4 °C. Intranuclear staining was conducted with the True-Nuclear Transcription Factor Buffer set (Biolegend) according to the manufacturer’s instructions; antibody staining was for 40 min at 4 °C. Stained cells were acquired on a BD FACSCanto II and analysed using FlowJo software version 10.4. At least 500,000 events were acquired per sample. Gating and enumeration of Treg was based on CD25, CD127 and Foxp3 expression (Extended Data Fig. 10d), as previously described57.

**Statistical analyses.** Clinical data collection and management was carried out using SAS (version 9.3; SAS Institute, NC). Where applicable, data were reported as the median (indicating the range from minimum to maximum value occurred) and arithmetic mean ± standard deviation, as specified (R software package, 3.4.1). Dichotomous variables were evaluated by Fisher’s exact test while continuous variables were compared by two-sided t test or Mann-Whitney U test, as indicated. OS and PFS (both calculated from primary tumour diagnosis) were assessed by Kaplan-Meier analysis using Prism 7 (GraphPad Software Inc., CA). An independent data safety monitoring board oversaw the study. Patient PBMC were limited in this study. If not stated otherwise and owing to limited sample availability, immune response assessments could only be performed once and were not repeated. As detailed in the sections for *ex vivo* 2D multimer, *ex vivo* ICS and pan-ICS, various internal and external controls were included and per assay type, all patient assays were done with the same batches of critical reagents (e.g. staining antibodies). Further, all patient assays were performed according to detailed standard operating procedures.

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**Data Availability Statement**

Raw data that support the findings of this study in Figs. 2, 3 and 4, Extended Data Figs. 1, 3, 4, 5, 6, 7, 8, and 10 are available from the corresponding author upon reasonable request. Fig. 5, Extended Data Fig. 9, Table 1 and Suppl. Table 5 are patient outcomes data from the clinical study. These will be available upon reasonable request to the corresponding author from the GAPVAC-101 study CRFs. Supplementary Tables 1-4 represent raw data and should be used to recapitulate the findings demonstrated in this study. MS data for sequence identifications for APVAC warehouse peptides has been deposited at PeptideAtlas with the dataset identifier PASS01284.

**Extended Data Figure Titles and Legends**

**Extended Data Figure 1** **** **APVAC1 and APVAC2 processes lead to truly personalized vaccine formulations.** **a**, Summary of biomarker data used to define APVAC1 for patient 05. Immunogenicity: Percentage of positive wells (n=12) with patient-derived PBMC. Peptide presentation: Shown are *Detection by MS* (+/-) on the tumour of patient 05; *Exclusivity:* absence (+/-) from normal tissue samples (n=394); individual *over-presentation ratio* on HLA peptidome level on patient 05’s tumour vs. average of normal tissues or vs. the limit of detection for the respective peptide if never found on normal tissues; *mRNA over-expression ratio* of the source genes in patient 05’s tumour vs. average of normal tissues (n.d. = no data, genes not covered by used microarray). Data were integrated into a rank score as basis for selection into APVAC1 (see Online Methods for details). **b**, Peptide presentation of PTP-013 on normal tissues (n=394, not detected), GB specimens (identified on 7 of n=33 independent samples, median indicated), and the tumour of patient 05 as analysed by MS. For patient 05, mean signal and signal range of n=5 replicate MS runs are shown. **c**, mRNA expression of PTPRZ1 (source gene of PTP-013) analysed by mRNA microarray analysis in samples of pooled normal tissue mRNA, GB tumours (n=26, median indicated) and tumour of patient 05. n.d. = no data. **b,c**, BLV blood vessels, BRA brain, DIG digestive glands, FRP female reproductive organs, HRT heart, HEM hematopoietic cells, HOG hormonal glands, INT intestine, KID kidney, LIV liver, LYM lymphatic tissue, MRP male reproductive organs, NER nervous system, RES respiratory, SKM skeletal muscle, SKI skin, STO stomach/oesophagus, URI urinary system, OTH other. **d**, Immunogenicity pre-testing data for PTP-013 in pre-treatment PBMC of patient 05. Cells (1x106 per well, n=12 wells tested) were stimulated with each A\*02 warehouse peptide separately. After *in vitro* stimulation, wells were analysed for peptide-specific CD8+ T cells using 2D multimer staining. Representative positive (7 out of 12) and negative (5 out of 12) wells are shown. **e**, Number of selections into APVAC1 compositions for the 33 A\*02 warehouse peptides (n=16 patients). **f**, APVAC2 selection process for mutated neo-epitopes. **g**, The four APVAC2 candidates entering test synthesis for patient 11 are exemplarily shown (patient dropped out before being vaccinated with APVAC2). Best predicted HLA class I epitopes are underlined. Red = mutated position. **h**, APVAC2 composition overview for GAPVAC-101 patients vaccinated with APVAC2 (n=11).

**Extended Data Figure 2 ** **APVAC composition, vaccination schedule, exposure to drug and standard therapy, and course of patients. a**,Composition of personalized APVAC1 and APVAC2 drug products. **b**, Vaccination schedule for APVAC1 and APVAC2 and the immunomodulators. Note that APVAC1 and APVAC2 vaccinations started independently on day 15 of the 1st and 4th adjuvant TMZ cycle, respectively. In the default schedule, both APVACs were co-applied at the 9th APVAC1 / 4th APVAC2 vaccination, and at all 4-weekly applications following the 10th APVAC1 / 7th APVAC2 vaccination. In case of delayed or failed APVAC productions alternative schedules were in place to optimally align vaccinations with standard therapy (not shown). Any assessments at a vaccination visit, including blood draws for PBMC isolation, were performed prior to vaccinations. **c**, Patient exposure to study drugs. **d**, Exposure to standard therapy. Statistics on number of applications, dose and time lines between therapy phases are provided for all enrolled GAPVAC-101 patients (n=16). SD = standard deviation. Reference values for the standard therapy are provided29. \*Completed treatment assumed with 75 mg/m² for 6-7 weeks calculated with 1.75 m² body surface area. \*\*According to treatment schedule, patient data not published29. **e**, CONSORT-like diagram for patients in the GAPVAC-101 trial. Completion for APVAC1 and APVAC2 vaccinations according to the trial protocol equals to at least 11 and 8 vaccinations, respectively. Three patients left the study before receiving APVAC2: Two patients withdrew their consents; one patient experienced a grade 3 seizure (unrelated to vaccinations) and withdrew from the study. Additionally, no biomarker data for definition of APVAC2 was available for patient 04 because the tumour specimen was necrotic.

**Extended Data Figure 3** ** Functional characterization of APVAC responses. a**, Expression of activation/exhaustion marker PD-1 for three different immune responses pre-vaccination and at different post-vaccination timepoints. Top: Exemplary response to viral marker peptide HBV-001. Middle and bottom: Exemplary APVAC1-specific CD8+ T-cell responses. Numbers in plots show ratio of PD-1 mean fluorescence intensity (MFI) for target-specific cells (blue curve) divided by MFI of total CD8+ T cells (grey curve). **b**, Summary of PD-1 evaluations as shown in a. Maximal MFI ratio for each CD8+ T-cell response to APVAC1 peptides (n=16) and to the viral marker peptide HBV-001 (n=2) from four patients (02, 05, 08, 14). Bars indicate mean. **c**, Flow cytometry data for Vital-FR assay shown in Fig. 2c. PTP-013-specific CD8+ T cells were sorted from post-vaccination T cells from patient 16, *in vitro* expanded in the presence of PTP-013 peptide for three weeks and tested for killing of K562-A2 cells loaded with titrated amounts of PTP-013 peptide (CFSE-labelled) in comparison to K562-A2 cells loaded with an irrelevant peptide (FarRed-labelled). Data at the four lowest peptide concentrations are shown. Due to limited availability of patient PBMC, this experiment was performed only once with n=3 replicates. **d**, BCA-002-reactive T cells from pre-vaccination PBMC from patient 11 were cloned and expanded with PHA/IL-2. Obtained clones were tested in a flow cytometer-based cytotoxicity assay using T2 cells loaded with titrated amounts of peptide. Results for n=10 different clones are shown with calculated EC50 values between 49 pM and 2.8 nM (means of 2-3 replicates per clone). **e**, ‘NLGN4X T cells’ as described for Fig. 2e were able to lyse specifically the A\*02+ GB cell line P3XX that endogenously expresses NLGN4X (LDH release assay). HD T cells = healthy donor derived T cells, not target-specific. Results from n=3 replicates are shown (median and range). **f**, NLGN4X T cells were either co-cultured with GB cell line U87MG transduced with NLGN4X-nLuc (red), irrelevant control NRCAM-nLuc (black) or in the absence of target cells (blue). With increasing E:T ratio, complete eradication of NLGN4X-nLuc expressing target cells is shown (NanoLuc® luciferase-based killing assays, median and range from n=4 replicates shown). RLU = relative luminometer units. For additional functional data of warehouse peptides see also18. **g**, Expression of the source genes PTPRZ1 and NLGN4X in the used GB cell lines as compared to the housekeeping gene β-actin measured by quantitative PCR. Boxes describe range and median from n=4-6 replicates.

**Extended Data Figure 4 ** **Additional immune response data.** **a**, APVAC1 CD8+ T-cell response parameters including MCIF (see Online Methods), Treg frequencies at baseline and predominant phenotypes of APVAC-induced CD4+ T-cell responses for APVAC1 immune-evaluable patients (n=13). n.e. = not evaluable. **b**, Frequency of NLGN4X-001-specific T cells of patient 02 by differentiation phenotype summarized from the flow cytometry data shown in Fig. 2b. **c**, TH1 CD4+ T-cell response against the APVAC1 HLA class II peptide PTP-010 in PBMC from patient 08 confirming ELISpot results (Fig. 5d) and reactivity in TIL (Fig. 5c). **d**, MCIF for GAPVAC-101 patients (n=13 evaluable) by APVAC1-specific CD4+ T-cell response type. For response type colour codes see (a). **e**, Pre-treatment Treg for patients with high vs. low MCIF (evaluable for n=12 patients). **f**, Peak frequencies of IFN-γ producing APVAC1 pan-DR peptide-specific CD4+ T-cells in patients with high Treg frequencies (>median: 3.6%) vs. low Treg frequencies. Each dot represents one response (n=24, two for each of the 12 evaluable patients). **d-f**, Median and 95% CI of median are depicted, p values determined by two-sided Mann Whitney U test. **g**, Immune responder rates (with and without memory shift) in GAPVAC-101 and a previous vaccine trial (IMA950-101) with the invariant multi-peptide vaccine IMA95016 for the warehouse antigens BCA-002, NLGN4X-001, PTP-005 and the viral marker HBV-001 shared by both vaccines. Antigen responses were evaluated in both cases by the same *ex vivo* multimer 2D assay. Numbers below bars indicate number of patients analysed for the respective antigen. In IMA950-101 with comparable GB patient population, specific CD8+ T-cell responses were rarely detected *ex vivo*. In the GAPVAC-101 trial*,* measurable and sustained CD8+ memory T-cell responses (with at least duplication of memory cell frequencies) against the same tumour antigens were frequent. The pre-immunogenicity testing in GAPVAC-101 is likely not the main cause of the observed higher response rate, as progenitor T cells for the three compared antigens are abundant in A\*02+ individuals (see Supplementary Table 1). **h**, APVAC2-specific CD4+ T-cell responses for all evaluable patients (n=10). Cell frequencies are only shown for key cytokine-positive T cells. P = prior to APVAC2 vaccination; 1, 2, 3, … = post-treatment measurements. Phenotypes assignment as indicated. Additional CD8+ T-cell responses to APVAC2 peptides are indicated with the detected lead cytokine; \*pre-defined response criteria not met. #Response to 13-M06 was negative for any key cytokine; TNF-α-positive cells are shown. **a-h**, Due to limited availability of patient PBMC, immune response analyses were only be performed once per patient. For assays with limited patient material, see statistics section.

**Extended Data Figure 5** **** ***Ex vivo* ICS** **flow cytometry data for APVAC1-induced CD4+ T-cell response. a**, Exemplary flow cytometry data for response shown in Fig. 2f (CD4+ T-cell response of patient 07 to pan-DR antigen BIR-002).IL-5 was negative at all timepoints (not shown). **b,** MOCK control stimulation for (a). **a,b,** Due to limited availability of PBMC, immune response analyses were only performed once per patient. For assays with limited patient material, see statistics section.

**Extended Data Figure 6** **** **Pan-ICS** **flow cytometry data.** **a**, Pan-ICS flow cytometry data for CD4+ T-cell response of patient 16 to mutated APVAC2 peptide 16-M06 as summarized in Fig. 3a. Production of indicated cytokines pre-treatment (Pre) and at 3 different post-treatment timepoint pools (Post1 to Post3) is shown. Post2 and Post 3 timepoint pools originate from the ‘Continued Vaccination’ phase (see Fig. 1). Dot plots are gated on CD8neg lymphocytes. Note that the pan-ICS cytokine panel differs from the one used for the *ex vivo* ICS assay (see Online Methods). **b**, MOCK control stimulation for (a). **c**, Pan-ICS flow cytometry data for CD8+ T-cell response of patient 15 against the mutated APVAC2 peptide 15-M05 as summarized in Fig. 3b. A pool of all possible nonamers from 15-M05 was used for read-out. Production of indicated cytokines is shown pre-treatment (Pre) and at 2 different post-treatment timepoint pools (Post1 to Post2). Dot plots are gated on CD4neg lymphocytes. Note that the APVAC2 analysis timepoints are not identical to APVAC1 analysis timepoints due to the independent vaccination start (Fig. 1). **d**, MOCK control stimulation for (c). **a-d,** Due to limited availability of PBMC, immune response analyses were only performed once per patient. For assays with limited patient material, see statistics section.

**Extended Data Figure 7** **** **Additional data on APVAC2-induced, neo-epitope specific immune responses.** **a**, Cross-reactivity of two neo-epitope-directed, APVAC2-induced CD4+ T-cell responses with the corresponding wildtype peptides was assessed by ICS. The response shown in the upper panel (08-M01) was specific for the mutated peptide, while the response in the lower panel (16-M06) showed considerable cross-reactivity with the wildtype peptide. Due to limited availability of patient PBMC, this experiment was performed only once. **b**, *Ex vivo* 2D multimer data for CD8+ T-cell response of patient 14 against the predicted neo-epitope HMKVSVYLL contained in the vaccinated peptide 14-M09 as summarized in Fig. 3c. Note that this epitope did not show the highest ranking during APVAC2 selection (underlined in Suppl. Table 4). Pre-treatment and one post-treatment timepoint pool were evaluated. Top row shows 2D multimer staining. Numbers in plots indicate frequency of specific cells among total CD8+ T cells. Middle row shows differentiation phenotyping of specific T cells (blue dots) and total CD8+ T cells (grey dots). Numbers in plots indicate percentage of specific CD8+ T cells with indicated phenotype (colour code as in Fig. 2a, b). Lower row shows PD-1 expression (blue curve = specific CD8+ T cells, grey dots = all CD8+ T cells).

**Extended Data Figure 8  TIL isolation and expansion in GB.** A novel harmonized TIL isolation and expansion protocol for GB specimens was developed in the preclinical phase of the GAPVAC project (see Online Methods). **a**,Cells were expanded in the presence of recombinant human (rh) IL-2 at three concentrations. One centre also performed expansion of melanoma and oropharyngeal TIL for comparison. The number of independent tumours compared across laboratories (preclinical samples) are shown in brackets. Mean and SEM are indicated for each cell subset and individual values are overlaid. **b**, Expansion of glioma TIL in the presence or absence of anti-CD3 antibody OKT3.Indicated number of pieces of tumour obtained from 3 GB patients (outside the GAPVAC-101 trial) were cut in fragments and transferred in 24-well plates with 1 fragment per well. Fragments were cultured in the absence or presence of 30 ng/mL anti-CD3 antibody OKT3. Expanded cells were harvested at the days indicated and subjected to phenotyping by flow cytometry. Percentages of total CD3+, CD4+ and CD8+ T cells, and total cell yield are indicated. **c**, Compositions of TIL cultures derived at one centre from 4 different patients (06, 07, 08, 09) included in the GAPVAC-101 trial. Each dot represents one stain performed either on independent culture wells or after pooling several wells (numbers of independent staining per patient are indicated). The GB-derived TIL generally comprised a mixture of CD4+ and CD8+ T cells that varied between individual cultures. Bars indicate the median over all cultures. **d**, Example of TCR clonotype mapping used for a comparative analysis of clonotypes detected *ex vivo* in the fresh tumour relapse tissue (FT) and in n=3 independent TIL cultures of patient 08. Identical DNA sequences resolve at identical positions in the gel. One prominent BV14 clonotype present in the fresh tissue (arrow) is not amplified in the TIL cultures (left), while two of several BV21 clonotypes in the fresh tumour (\*) are also detected in the TIL (right). At least n=8 clonotypes per matched set of samples were compared.

**Extended Data Figure 9** **** **Survival data for GAPVAC-101 patients.** **a**,**b**, Overall survival (OS, a) and progression-free survival (PFS, b) from diagnosis for all patients that received at least one APVAC vaccination (n=15). Kaplan-Meier estimates of median survival are provided. **c**, Regulatory pathway for active personalized vaccines for GAPVAC-101 as compared to conventional immunotherapies. The pathway for clinical trial application (CTA) for GAPVAC-101 was pre-discussed in scientific advice and pre-IND meetings with the German Paul-Ehrlich-Institute (PEI) and the US Food and Drug Administration (FDA), respectively. Key characteristics in the trial approval process were: 1. Standardization of a drug composition process based on highly variable, patient-individual biomarker data instead of a fixed drug composition; 2. Development of a GMP-compliant core process for manufacturing of variable, personalized multi-peptide compositions instead of an invariable manufacturing process; 3. Clinical trial application based on exemplary data from representative preclinical drug substance batches; 4. Provision of certificates of analysis for all APVACs to the authorities on an ongoing basis during the trial (twice yearly).

**Extended Data Figure 10 ** **Gating strategies for flow cytometry assays.** **a**, Gating strategy and Boolean gating for *ex vivo* 2D multimer assay (CD8+ T-cell immune response analysis, APVAC1). **b**, Gating strategy for *ex vivo* APVAC2 class II ICS assay (CD4+ T-cell immune response analysis, APVAC1). **c**, Gating strategy for HLA class I and class II pan-ICS assay (CD4+ and CD8+ T-cell immune response analysis, APVAC2). **d**, Gating strategy for Treg assay.