Immunomonitoring for actively personalized peptide vaccines (APVACs) during immunotherapeutic treatment of glioblastoma

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Introduction

To improve therapy of Glioma, the Glioma Actively Personalized Vaccine Consortium (GAPVAC) integrated a highly personalized peptide vaccine approach into glioblastoma standard of care treatment. In this phase I clinical trial fifteen patients received two different types of actively personalized peptide vaccines (APVAC1 and APVAC2), that were selected based on transcriptome, immunopeptide and mutational analysis of the patient’s individual tumors. Here we present comprehensive data on in vivo Immunomonitoring of APVAC1 and APVAC2 peptides.

APVAC2 peptides: Primarily mutated neo-epitope 19aa peptides and to some extent non-mutated 9-10aa peptides

15 patients received individualized peptide vaccines in combination with poly-ILC and GM-CSF as adjuvants

13 patients were evaluated for APVAC1 and 10 for APVAC2 analysis

APVAC1 & APVAC2 vaccines showed expected safety profile and outstanding in vivo immunogenicity

The GAPVAC concept

Exemplary APVAC1 class I and class II responses

Exemplary APVAC2 responses

APVAC1 (non-mutated)

High immunogenicity of APVAC1 class I peptides in vivo, concurred with persistent CD8 T cell responses

Mainly central memory [CM] phenotype (Fig. 2; Table 1) analyzed ex vivo

High in vivo immunogenicity of APVAC1 class II peptides with polyfunctional CD4 T cell responses predominantly of type 1 Th1-cell (Th1) phenotype (Fig. 3; Table 3) and measured ex vivo

Induction of APVAC-specific CD8 memory cells reversely correlated with baseline regulatory T cell (Treg) frequency (Table 3)

APVAC2 (mainly Neo-epitope)

High immunogenicity of APVAC2 peptides in vivo with potent and polyfunctional CD4 T cell responses, mostly of Th1 phenotype (Fig 4; Fig 5). Table 1)

CD4 T cell responses often concurred with restored CTL responses (Fig 5; Table 1)

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Figure 1: Schedule of events for GAPVAC phase I trial: Schedule of events for GAPVAC phase I trial showing essential events, timelines, vaccinations and blood drawings for immunomonitoring.

Figure 2: Exemplary ex vivo CD8 T-cell response of patient #1 to the HLA-A*0201 peptide Ag212-21 (as determined by ex vivo class I 28/29 week response). T-cell response in a 25 week post-vaccination time point (25/26 week) is shown. CD8 T cell response in patient #1 to the peptide Ag212-21 is shown. Top row shows 2D mFACS (2DMACS) staining pre- and post-vaccination. Bottom row shows memory phenotyping of antigen-specific T cells (blue dots) and total T cells (black dots). Both are shown to CD3 and CD8 expression (CD3: green, mAbs: CD8). Cells are gated on live leukocytes.

Figure 4: Exemplary CD4 T cell response of patient #3 to personalized mutated class I peptide Ag421-43 (A) and CD4 T cell response of patient #4 to the class I pool of personalized mutated peptide Ag242-43 (B) measured by poly-ILC assay. Patient's individual peptide recipients pre-treatment (Pre) and at one post-treatment time point is shown. Individual graphs are pre-plotted on CD8 (A) or CD4 (B) expression. Frequency is shown in percent of total T cell or the CD8 or CD4 T cell subset. PBMCs were cultured for one round of in vitro stimulation in presence of 0.1 for 11 days using cognate peptide. At day 11, cells are restimulated ex vivo using cognate peptide or mock stimulation of 90% viability. Cells are gated on live leukocytes and acquired on BD LSR II at day 11.

Figure 5: In vivo immunogenicity of APVAC1 and APVAC2 peptides. Summary of in vivo immunogenicity for non-mutated APVAC1 peptides, as well as mutated APVAC2 peptides, evaluated by in vivo neo-epitope induced CD8 T cell responses. Note, the wide selection of APVAC1 peptides evaluated in vivo by immunogenicity testing, non-mutated APVAC2 peptides were identified by mutagenesis. If no minimal 10aa peptides could be generated, see also Fig. 4). Furthermore, immunogenicity of APVAC1 peptides was assessed ex vivo, while determination of immune response to APVAC2 peptides by poly-ILC assay included one round of in vitro stimulation. Besides the frequency of T cells, also polyfunctionality of CD8 T cells were evaluated. FACS analysis shows the predominant T helper-1 phenotype as well as polyfunctionality of CD4 T cell responses is shown. Numbers below in vivo immunogenicity pie charts show immunopeptide/peptides vaccinated and immunogenicity as measured is indicated for each antigen.

Table 1: Summary of key parameters of immune response for evaluable patients. Table shows key parameters of immune responses to APVAC1 and APVAC2 peptides. Note, all patients were vaccinated with any memory phenotype among total CD8 T cells at peak of response, maximum fold induction of APVAC1 specific cells with any memory phenotype as ‘memory cell induction factor’ (MCF). Lower frequency of T cells, phenotypic frequency of CD4 T cell responses to APVAC1 and APVAC2 peptides, as well as APVAC2 specific CD4 T cell responses. Furthermore, patients showed a higher induction of polyfunctional CD4 T cells, indicated by the MCF) to the lower fold increase MCF) frequency. This phenotype of the CD4 T cell responses to APVAC1 peptides, as well as APVAC2-induced CD8 T cell responses. These comprehensive data indicate, that the MCF might be a reliable factor to identify patients with higher immunogenicity.