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

Alexander Ulges[1], Norbert Hilf[1], Wolfgang Wick[5], Michael Platten[5], Pierre-Yves Dietrich[7], Katrin Frenzel[2], Arie Admon[14], Sjoerd H. van der Burg[8,4], Andreas von Deimling[5], Per thor Straten[9,4], Cecile Gouttefangeas[3,4], Judith R. Kroep[8], Francisco Martínez-Ricarte[10], Hideo Okada[12], Christian H. Ottensmeier[15], Berta Ponsati[11], Hans S Poulsen[13], Stefan Stefanovic[3], Ghazaleh Tabatabai[6], Hans-Georg Rammensee[3], Ugur Sahin[2], Dominik Maurer[1] and Regina Mendrzyk[1]

[1]Immatics biotechnologies GmbH, Tuebingen, Germany; [2] BioNTech AG, Mainz, Germany; [3] Eberhard Karls Universität Tübingen, Tübingen; Germany; [4] CIMT/CIP - Association for Cancer Immunotherapy, working group Cancer Immunoguiding Program, Mainz, Germany; [5] University Hospital Heidelberg, Heidelberg, Germany; [6] University Hospital Tübingen, Tübingen, Germany; [7] Université de Genève, Genève, Switzerland; [8] Leiden University Medical Center, Leiden, The Netherlands; [9] Center for Cancer Immune Therapy (CCIT), Department of Hematology, University Hospital Herlev, Denmark, and Department of Immunology and Microbiology, University of Copenhagen, Denmark; [10] Vall d'Hebron University Hospital, Barcelona, Spain; [11] BCN Peptides S.A., Barcelona, Spain; [12] University of California San Francisco, San Francisco, USA, and Parker Institute for Cancer Immunotherapy, San Francisco, USA; [13] Ringhospitalet, Copenhagen, Denmark; [14] Technion - Israel Institute of Technology, Haifa, Israel; [15] University of Southampton, Southampton, UK

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 **a**ctively **p**ersonalized peptide **vac**cines (**APVAC1** and **APVAC2**), that were selected based on transcriptome, immunopeptidome and mutational analysis of the patient's individual tumors. Here we present comprehensive data on *in vivo* immunogenicity of APVAC1 and APVAC2 peptides. Furthermore we show associations between different immune response variables and pre-treatment biomarkers.

- multicenter phase I clinical trial performed in 5 European countries and at 6 individual clinical sites
- Highly personalized, two track (APVAC1 and APVAC2) vaccination approach, integrated into glioblastoma standard of care treatment (Fig. 1)
- APVAC1 peptides (class I & II): Non-mutated tumor antigens selected on a warehouse-based approach
- APVAC2: 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-ICLC and GM-CSF as adjuvants
- 13 patients were evaluable for APVAC1 and 10 for APVAC2 analysis
- APVAC1 & APVAC2 vaccines showed expected safety profile and outstanding *in vivo* immunogenicity

The GAPVAC concept

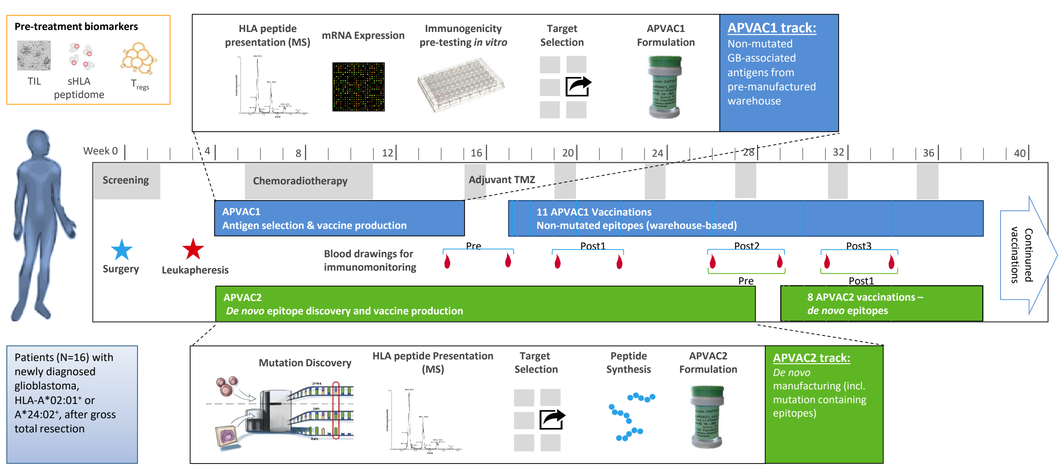

Figure 1: Schedule of events for GAPVAC phase I trial. Schedule of events for GAPVAC phase I trial showing essential events, timelines, vaccinations and blood drawings for immunomonitoring.

Exemplary APVAC1 class I and class II responses

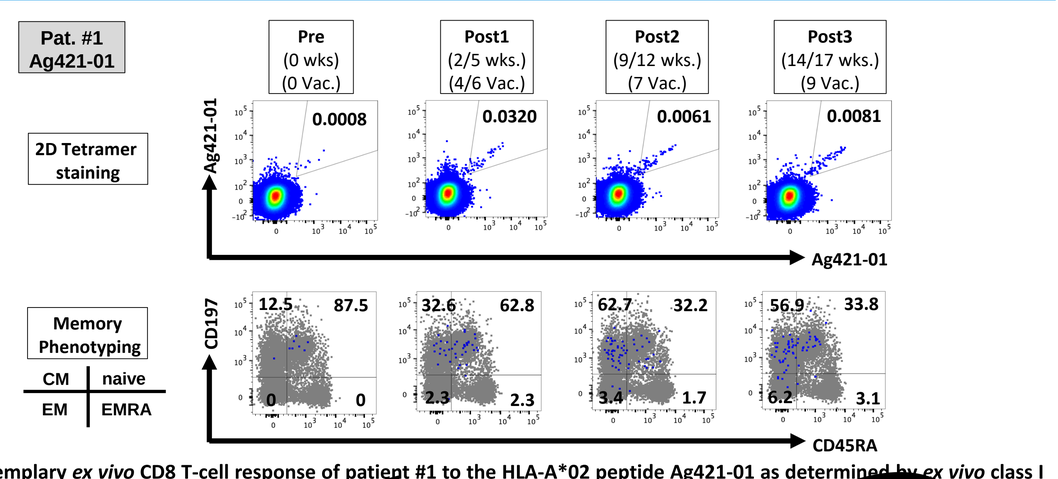

Figure 2: Exemplary *ex vivo* CD8 T-cell response of patient #1 to the HLA-A*02 peptide Ag421-01 as determined by *ex vivo* class I 2DMM assay. CD8 T cell response to Ag421-01 is s[illegible]prior (Pre) and at three different timepoint p[illegible]tion. Top row shows 2D multimer (2DMM) staining pre-gated[illegible]8 T lymphocytes. Bottom row shows mem[illegible]henotypin[illegible]ntigen-specific T cells (blue dots) and total CD8 T cells (gray dots) according to CD197 a[illegible]A expressio[illegible]M Central memory cells; EM effector memory cells; EMRA CD45RA+ effector memory cells).

Exemplary APVAC2 responses

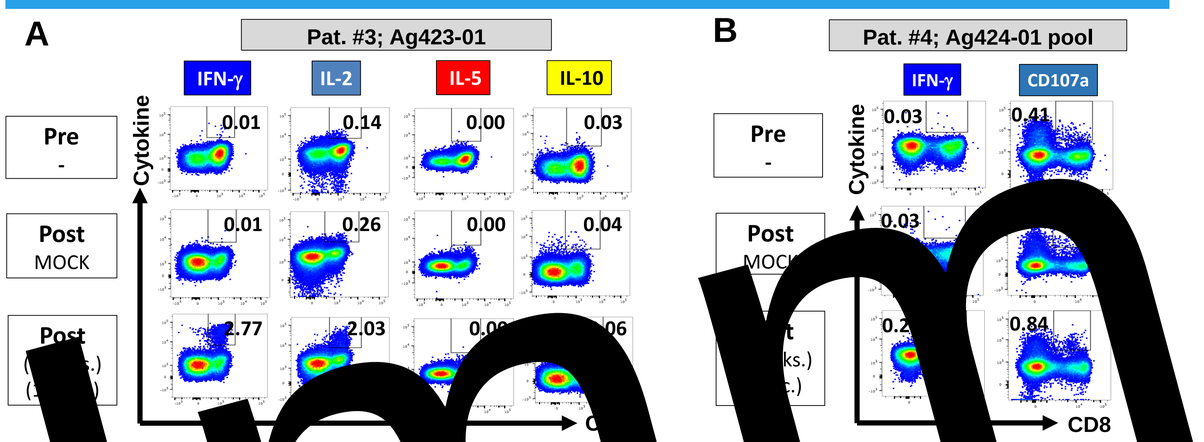

Figure 4: Exemplary CD[illegible] response of [illegible]nt #3 to person[illegible] mutated [illegible]II peptide Ag42[illegible](A) and CD8 T c[illegible] respon[illegible] patient #4 t[illegible]lass I pool of p[illegible]lized mutated p[illegible]e Ag424-0[illegible]easured by par[illegible]ssay. Production indicate[illegible]okines pre-t[illegible]nt (Pre) and at [illegible]ost-treatment tir[illegible]nt is show[illegible]ividual graphs ar[illegible]gated on CD8⁻ (A) or CD4⁻ [illegible]mphocytes. [illegible]ers in plots indi[illegible]equency of cyto[illegible]marker po[illegible]cells among CD4[illegible]r CD8 (B) T cells. PBMCs v[illegible]ultured for [illegible]und of *in vitro* s[illegible]tion in presence [illegible] for 11 da[illegible]g cognate peptide. At day 12, cells are restimulat[illegible]6h using cog[illegible]eptide or mock [illegible] in presence of B[illegible]o. Cells ar[illegible]equently harvested and acquired on BD LSRII at day[illegible]

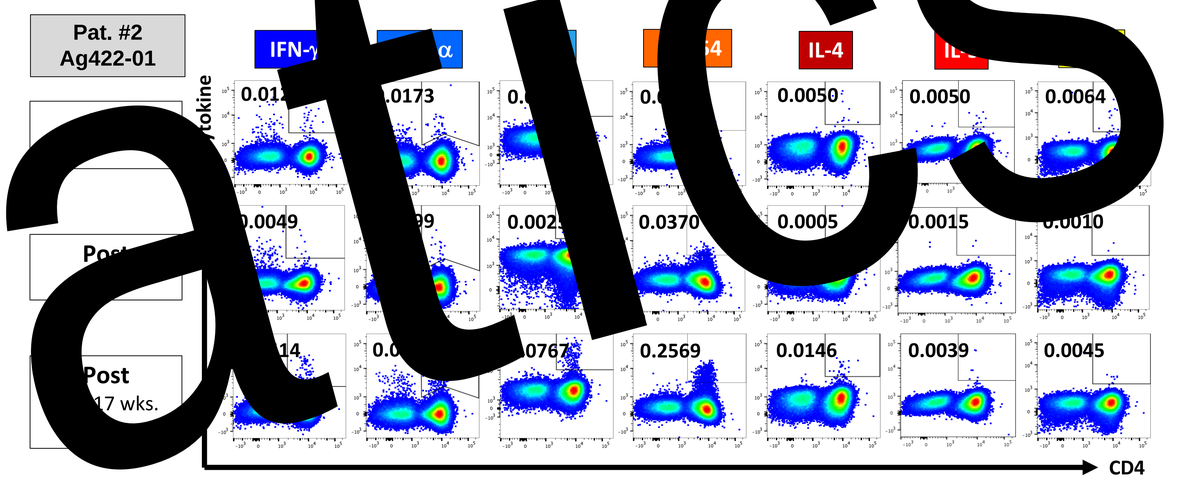

Figure 3: Exemplary *ex vivo* CD4 T-cell response of patient #2 to non-mutated pan-DR binding peptide Ag422-01 analyzed by *ex vivo* class II ICS assay. Production of indicated cytokines prior (Pre) and at one post-vaccination time point pool (9 / 12 weeks; after 7 vaccinations with APVAC peptide Ag422-01) is shown. Individual graphs are gated on CD8⁻ T cells. Numbers in plots indicate frequencies of cytokine/marker positive cells among CD4 T cells. Using boolean gating 2^7 = 128 distinct cytokine patterns can be analyzed as a functional readout on a single cell level.

Summ[illegible]of APVA[illegible]nd APVAC2 *in vivo* immunogenicity and immune responses on patient level

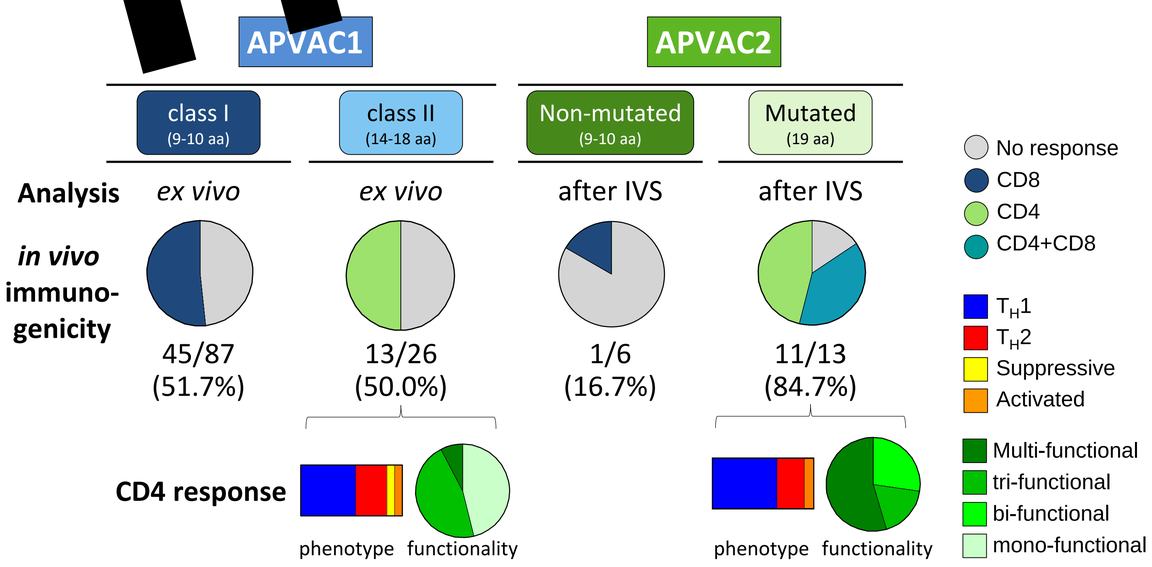

Figure 5: *In vivo* immunogenicity of APVAC1 and APVAC2 peptides. Summary of the *in vivo* immunogenicity for non-mutated APVAC1 peptides, as well as APVAC2 peptides, subdivided into non-mutated 9-10aa and mutated 19aa peptides. Notably, while selection of APVAC1 peptides included *in vitro* pre-immunogenicity testing, non-mutated APVAC2 peptides were identified by mass-spectrometry and only selected, if no mutated 19aa peptides could be generated (see also Fig.1). Furthermore, immunogenicity of APVAC1 peptides was assessed *ex vivo*, while determination of immune responses to APVAC2 peptides by panICS assay included one round of *in vitro* stimulation. Besides *in vivo* immunogenicity of APVAC1 and APVAC2 peptides the predominant T-helpercell phenotype as well as polyfunctionality of CD4 T cell responses is shown. Numbers below *in vivo* immunogenicity pie charts show immunogenic peptides/peptides vaccinated and (immunogenicity [%]).

Patient	APVAC1-induced CD8+ T-cell responses			T_{reg} pre-vaccination [% of CD4]	Predominant phenotype of CD4+ T-cell responses		APVAC2-induced CD8+ T-cell response
	Number of responses	Memory cells [% of total CD8]	MCIF		APVAC1	APVAC2	
16	5	0,013%	116,6	2,8	no response	T_H1	no
8	5	0,024%	59,6	3,9	T_H1	T_H1	yes
1	4	0,006%	19,1	2,1	T_H1	T_H1	no
13	7	0,017%	15,7	n,t,	no response	T_H2	yes
2	3	0,008%	12,7	2,6	T_H1	T_H1	no
7	3	0,013%	7,1	3,3	T_H1	T_H1	yes
14	5	0,007%	6,6	1,5	no response	T_H2	yes
10	5	0,013%	4,1	3,9	no response	n.e.	n.e.
5	3	0,020%	2,0	4,3	T_H1	no response	no
12	1	0,005%	1,9	6,9	T_H2	no response	no
4	3	0,066%	1,5	3,8	T_H2	n.e.	n.e.
15	1	0,005%	0,8	4,2	T_H2	T_H1	yes
6	0	0,001%	0,7	3,1	suppressive	n.e.	n.e.
Median	3	0,013%	6,6	3,6			

Table 1: Summary of key parameters of immune response for evaluable patients. Table shows keyparameters of immune responses to APVAC1 and APVAC2 peptides like: Number of responses for APVAC1 peptides; % APVAC1 specific cells 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 '**m**emory **c**ell **i**nduction **f**actor' (MCIF), baseline frequency of T_{reg} cells, predominant phenotype of CD4 T cell responses to APVAC1 and APVAC2 peptides, as well as APVAC2 specific CD8 T cell responses. Furthermore, patients showing a higher induction of vaccine-induced memory T cells (as indicated by the MCIF) had lower pre-treatment T_{reg} frequencies, a predominant Th1 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 MCIF might be a reliable factor to identify patients with higher immunogenicity.

Summary and Conclusion of APVAC1 and APVAC2 *in vivo* immunogenicity

APVAC1 (non-mutated)

- High immunogenicitiy of APVAC1 class I peptides *in vivo*, concurrend with persistent CD8 T cells responses mainly of 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 reponses predominantly of type 1 T-helper cell (T_H1) phenotype (Fig.3; Table 1) and measured *ex vivo*.
- Induction of APVAC1-specific CD8 memory cells reversely correlated with baseline regulatory T cell (T_{reg}) frequency (Table 3)

APVAC2 (mainly Neo-epitopes

- High immunogenicity of APVAC2 peptides *in vivo* with potent and polyfunctional CD4 T cell responses, mostly of T_H1 phenotype (Fig.4; Fig5; Table 1)
- CD4 T cell responses often concurred with nested CTL responses (Fig. 5; Table 1)

Acknowledgement: We thank Ines Groskopf, Melanie Adis and Katy McCann for the excellent technical conduction of the GAPVAC immunomonitoring assays.

Immatics Biotechnologies GmbH
Immatics US, Inc.

www.immatics.com
info@immatics.com

Follow us on