Praxis für Humangenetik Tübingen
Dr. med. Dr. rer. nat. Saskia Biskup
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Frau
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Paul-Ehrlich-Straße 23
72076 Tübingen

Surname	Banat
Forename	Mohammed Hasan Mousa
Date of birth	27.01.1978
Sex	Male
Patient-ID	81273
Date of first vacc.	23.08.2021
Date of blood draw	23.+24.08.2021 (V1+V2) 11.11.2021 (V6)
Report date	15.12.2021

Immune monitoring report – Banat, Mohammed Hasan Mousa (*27.01.1978)

Indication	**Left temporal astrocytoma, WHO grade II (ID 02/2009)**
Order	**Immune monitoring during patient-individual anti-tumor vaccination**

Dear Dr. Biskup,

Please find below the immune monitoring results after patient-individual anti-tumor peptide vaccination.

SUMMARY

About 2.5 months after the start of vaccination, $CD4^+$ and $CD8^+$ T cell responses towards several of the vaccinated peptides were detected.

RESULTS

Blood from the patient was analyzed for peptide-specific T cell responses in the course of a patient-individual anti-tumor peptide vaccination regime.

Blood was drawn on the day of the first vaccination (23.08.2021), on the day of the second vaccination (24.08.2021) and about 2.5 months after the vaccination regime was started (11.11.2021). At all time points, peripheral blood mononuclear cells from the patient were isolated and cryopreserved until start of the analysis.

For the immune monitoring, PBMC were thawed and T cells were pre-amplified with peptides included in the vaccination cocktail. Due to limited cell counts after thawing, peptides had to be combined into pools for analysis (see page 3 and 4 for details). Additionally, due to limited cell counts after blood draw, cells from the day of the first vaccination and the second vaccination were also pooled. Activation of $CD4^+$ T cells (T helper cells, or Th1 cells) and $CD8^+$ T cells (cytotoxic T cells) was measured by flow cytometry after re-stimulation with the single peptides or DMSO (unstimulated). Both, $CD4^+$ and $CD8^+$ T cells are involved in specific anti-tumor immunity (Braumüller et al., 2013, PMID: 23376950; Tran et al., 2014, PMID: 24812403; Schumacher und Schreiber, 2015, PMID: 25838375; Kreiter et al., 2015, PMID: 25901682; Heemskerk et

al., 2013, PMID: 23258224; Dudley et al., 2010, PMID: 20668005; Ott et al., 2017, PMID: 28678778; Sahin et al., 2017, PMID: 28678784).

An overview of the detected responses can be found on page 3 and 4.

EVALUATION AND RECOMMENDATION

About 2.5 months after the initiation of the vaccination regime, weak to very strong $CD4^+$ T cell responses towards six of the 19 vaccinated peptides were detected. These include responses towards peptides 9 and 10, which are spanning the driver mutations IDH1-p.R132H and KRAS-p.K117N, respectively. Additionally, there were weak to very strong $CD8^+$ T cell responses towards three of the vaccinated peptides present.

No T cell responses were present at the start of the vaccination regime.

These results indicate that the administration of the vaccine successfully induced or at least enhanced specific $CD4^+$ and $CD8^+$ T-cell responses towards at least eight of the 19 vaccinated mutation-spanning peptides, among them responses towards two driver mutation-spanning peptides.

As immune reponses towards the vaccine often increase over time, we recommend analyzing another sample in the further course of the vaccination.

Please do not hesitate to contact us if you have any questions.

Yours sincerely,

Saskia Biskup	Julia Steinhilber	Simone Kayser	Tabea Riedlinger
MD, PhD	PhD	PhD	PhD
Consultant for Human Genetics	Cancer diagnostics	Cancer diagnostics	Cancer diagnostics

Cocktail	Peptide-specific immune responses						Pool V1+V2	23.+24.08.2021 (V1+V2)		Pool V6	11.11.2021 (V6)	
	No	Peptide	Gene and Coding info	NAF (DNA)	NAF (RNA)	HLA		CD4	CD8		CD4	CD8
A	1	PIIIGHHAY	IDH1:NM_005896.3:c.395G>A: p.R132H	0.38	0.35	A*29:02	1	-	-	1	-	-
A	2	FIYLSSNCF	CPE:NM_001873.4:c.989A>T: p.N330I	0.4	0.48	A*29:02, B*15:03, C*12:03	2	-	-	2	-	+ SI: 2.5 (0.4%)
A	3	ALDPANGYMY	LRP5:NM_002335.4:c.484C>A: p.H162N	0.21	0.26	A*01:01, A*29:02				3	-	-
A	4	AGVLNAGSY	CLASP2:NM_015097.3:c.1898C>T: p.A633V	0.17	0.23	B*15:03						
A	6	VAVETMHKM	TOGARAM1:NM_015091.4: c.4651C>G:p.L1551V	0.15	0.43	B*15:03, C*12:03						
A	8	PEHLKDESA	MAP6:NM_033063.1:c.1940C>T: p.P647L	0.22	0.2	B*45:01						
A	10	VPMVLVGNNCDLPSRTV	KRAS:NM_004985.5:c.351A>T: p.K117N	0.12	0.13	class II	3	-	-	4	++ SI: 4.0 (1.2%)	-
A	12	RSGRLGAGVLNAGSYAS	CLASP2:NM_015097.3:c.1898C>T: p.A633V	0.17	0.23	class II	4	-	-	5	-	-
A	14	AVNSDLSSSLEERMQSP	NOL4:NM_003787.4:c.722A>G: p.N241S	0.19	0.33	class II	5	-	-	6	-	-
A	16	RAIALDPANGYMYWTDW	LRP5:NM_002335.4:c.484C>A: p.H162N	0.21	0.26	class II				7	++ SI: 3.1 (0.5%)	+ SI: 2.7 (0.2%)

Vn: day of/after vaccination n. **HLA:** HLA which was predicted to bind the peptide. **NAF:** Novel allele frequency, frequency with which the mutated allele was occurring in the tumor sequencing (1 is 100%). The observed frequencies are influenced by the tumor content of the analyzed sample and hence do not correlate directly to the mutation frequency in the tumor. **SI:** Stimulation index, ratio of polyfunctional activated $CD4^+$ or $CD8^+$ T cells (positive for at least two activation markers of CD154, IFN-γ, TNF and/or IL-2) in the peptide-stimulated sample compared to the unstimulated control. Additionally, the percentage of activated $CD4^+$ or $CD8^+$ T cells (positive for at least one activation marker of CD154, IFN-γ, TNF and /or IL-2) above background and after *in vitro* amplification is given. The percentage does not directly reflect the frequencies *in vivo*. Please note that SI and % values should be considered only in combination and not independently from each other. SI ≥2: weak response (+), SI ≥3: positive response (++), SI ≥5: strong response (+++), SI ≥10: very strong response (++++).

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Cocktail	Peptide-specific immune responses						Pool V1+ V2	23.+24.08.2021 (V1+V2)		Pool V6	11.11.2021 (V6)	
	No	Peptide	Gene and Coding info	NAF (DNA)	NAF (RNA)	HLA		CD4	CD8		CD4	CD8
B	5	SGYVRPIPV	SMCHD1:NM_015295.3:c.664G>A: p.V222I	0.16	0.2	C*12:03	6	-	-	8	-	-
B	7	EKAWNVYPY	PITPNB:NM_012399.5:c.269C>T: p.A90V	0.08	0.03	B*15:03						
B	9	WVKPIIIGHHAYGDQYR	IDH1:NM_005896.3:c.395G>A: p.R132H	0.38	0.35	class II	7	-	-	9	+++ SI: 7.1 (2.6%)	-
B	11	YSVPGGMQDFIYLSSNCFE	CPE:NM_001873.4:c.989A>T: p.N330I	0.4	0.48	class II	8	-	-	10	**++++ SI: 10.5 (2.2%)**	-
B	13	DHSGYVRPIPVPRSLNS	SMCHD1:NM_015295.3:c.664G>A: p.V222I	0.16	0.2	class II	9	-	-	11	++ SI: 3.0 (1.9%)	-
B	15	DPMVPEHLKDESAMAT	MAP6:NM_033063.1:c.1940C>T: p.P647L	0.22	0.2	class II	10	-	-	12	-	-
B	17	DVNQHGSDPESEETRKL	IWS1:NM_017969.3:c.358T>C: p.S120P	0.08	0.08	class II	11	-	-	13	-	-
B	18	VFHEKAWNVYPYCRTIV	PITPNB:NM_012399.5:c.269C>T: p.A90V	0.08	0.03	class II	12	-	-	14	-	**++++ SI: 73.9 (9.5%)**
B	19	NSKVNLVAVETMHKMIP	TOGARAM1:NM_015091.4: c.4651C>G:p.L1551V	0.15	0.43	class II	13	-	-	15	++ SI: 4.7 (0.9%)	-

Vn: day of/after vaccination n. **HLA:** HLA which was predicted to bind the peptide. **NAF:** Novel allele frequency, frequency with which the mutated allele was occurring in the tumor sequencing (1 is 100%). The observed frequencies are influenced by the tumor content of the analyzed sample and hence do not correlate directly to the mutation frequency in the tumor. **SI:** Stimulation index, ratio of polyfunctional activated $CD4^+$ or $CD8^+$ T cells (positive for at least two activation markers of CD154, IFN-γ, TNF and/or IL-2) in the peptide-stimulated sample compared to the unstimulated control. Additionally, the percentage of activated $CD4^+$ or $CD8^+$ T cells (positive for at least one activation marker of CD154, IFN-γ, TNF and /or IL-2) above background and after *in vitro* amplification is given. The percentage does not directly reflect the frequencies *in vivo*. Please note that SI and % values should be considered only in combination and not independently from each other. SI ≥2: weak response (+), SI ≥3: positive response (++), SI ≥5: strong response (+++), SI ≥10: very strong response (++++).

ADDITIONAL INFORMATION

Methods

Isolation of peripheral blood mononuclear cells (PBMC) from whole blood: Peripheral blood mononuclear cells (PBMC) including T cells were isolated by Ficoll Hypaque and cryopreserved for later use.

Cell culture and analysis of specific T cells: PBMC were thawed and cells were cultured overnight to recover, stimulated with patient-individual mutated peptides and cultured 11 days in the presence of low dose IL-2 and IL-7. This leads to an amplification of specific T cell responses and to a higher sensitivity of the analysis. For analysis, cells were briefly restimulated with peptides or incubated with DMSO (unstimulated negative control), SEB (Staphylococcal Enterotoxin B as unspecific positive control) or viral peptides (stimulation control). Activated cells were measured after intracellular cytokine staining by flow cytometry. Analyzed markers included: Live/dead-Staining, CD8 and CD4 to identify T cell populations, as well as IFN-γ, TNF, IL-2 and CD154 as specific T cell activation markers.

Evaluation of specific responses: Peptide-specific responses were evaluated using the stimulation index (SI). The stimulation index is the calculated ratio of polyfunctional activated $CD4^+$ or $CD8^+$ T cells (positive for at least 2 markers of CD154, IFN-γ, TNF, and /or IL-2) in the peptide-stimulated sample to the negative control sample (DMSO). Additionally, a minimal frequency of 0.1% of reactive T cells positive for at least one activation marker including CD154, IFN-γ, TNF and/or IL-2 had to be reached among a minimum of 10 000 measured $CD4^+$ or $CD8^+$ events.

The evaluation of the calculated stimulation indices is as follows:
SI ≥2: weak response (+)
SI ≥3: positive response (++)
SI ≥5: strong response (+++)
SI ≥10: very strong response (++++)

The stimulation indices and frequencies provide information about the strength of the analyzed immune response but do not directly reflect the responses *in vivo.* Detected responses are amplified and influenced by *in vitro* culturing.

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