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SUPPLEMENTAL MATERIAL

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1. Genetic profile of the tumor

The genetic profile of the patient's tumor was analyzed by WES (for details concerning WES see point 4 of this document) in September 2015. **Supplementary Table S1** displays an overview of the most relevant somatic variants that were detected. Due to detection of a high tumor mutational burden, variant evaluation was restricted to genes with known potential therapeutic relevance. Therapeutic options, predicted response and according Level of Evidence (LoE) were estimated based on previous work by Amaral *et al.* who adapted LoE from the Oncology Knowledge Base (OncoKB).[1 2] There was no evidence for homologous repair deficiency, any relevant copy number alteration (deletions and/or amplifications) of large genomic segments, or germline variants. Please note that the data presented are adapted to today's knowledge, while all treatment decisions had to be made on basis of what was known in 2015.

Supplementary Table S1:

Gene	Functional category	Variant	NAF	Effect on protein function	Related pathway	Therapeutic option	Predicted response	Level of evidence
MLH1	splice_region	c.883A>T; p.Ser295Cys	0.62	probably inactivating	mismatch repair	Immune checkpoint inhibitor	sensitive	1A
ATM	stop_gained	c.2329A>T; p.Arg777*	0.36	inactivating	cell cycle, homologous recombination	PARP inhibitor	sensitive	2A
						ATR inhibitor	sensitive	3
	stop_gained	c.9139C>T; p.Arg3047*	0.25	inactivating		DNA-PK inhibitor	unclear	5
PIK3CA	missense	c.1030G>A; p.Val344Met	0.25	probably activating	PI3K/AKT/mTOR	PI3K inhibitor	sensitive	2A
						mTOR inhibitor	sensitive	2B
						AKT inhibitor	sensitive	3
						EGFR/HER inhibitor	unclear	R2
RB1	stop_gained	c.2308C>T; p.Gln770*	0.43	inactivating	cell cycle	CHK1 inhibitor	sensitive	3
						Aurora kinase inhibitor	sensitive	4
						CDK4/6 inhibitor	unclear	R2
FBXW7	stop_gained	c.1756G>T; p.Gly586*	0.26	inactivating	Notch	mTOR inhibitor	sensitive	3
						Notch1 inhibitor	unclear	R2

Supplementary Table S1: **Overview of tumor somatic variants with therapeutic relevance.**
NAF: *Novel allele frequency*, the frequency with which the mutated allele occurs in the sequencing data (1 is 100%). The observed frequencies are influenced by the tumor content and do not directly correlate with the variant's frequency in the tumor. The somatic alterations were classified with respect to their functional effect on protein levels in the following categories: inactivating/activating/function altered, likely inactivating/activating/function altered, unknown and benign (details in the methods section). **Predicted response:** represents the predicted response with

consideration to known interferences and pathway crosstalks. Please note that the predicted drug-response is made based on the identified biomarkers only and does not take clinical (or tumor entity specific) features into consideration. **Level of Evidence:** for legend see **Supplementary Table S2**. Due to suspected MMR deficiency, evaluation of variants close to repetitive regions is limited and technical artefacts cannot be excluded. Please note that a predicted sensitive response concerning single variants may not be true for hypermutated tumors (high tumor mutational burden).

Supplementary Table S2:

LoE	
1A	**Approved drug, specific to the biomarker and entity** Drug is approved for the biomarker within the same entity (FDA and/or EMA)
1B	**Approved drug, specific to entity but not specific to the biomarker OR specific to biomarker, but only in organ related entities** Drug is approved independently of the biomarker within the same entity OR drug is approved for the biomarker in an organ related entity, e. g. benign tumor (FDA and/or EMA). The reported biomarker must have significant clinical relevance, despite biomarker-independent approval of the indicated drug.
2A	**Approved drug, specific to the biomarker for a different entity** Drug is approved for the biomarker in a different entity (FDA and/or EMA)
2B	**Approved drug, not specific to the biomarker for a different entity** Drug is approved independently of the biomarker in a different entity (FDA and/or EMA). The reported biomarker must have significant clinical relevance, despite biomarker-independent approval of the indicated drug.
3	**Efficacy of the drug is currently being/was analyzed in clinical trials**
4	**Efficacy of the drug is based on preclinical analyses and/or case reports**
5	**Hypothetical response** The biomarker could hypothetically induce response to the drug
R1	**The variant and/or biomarker is associated with a non-response, decreased response, or resistance to a specific drug or drug class in the same entity. The information is based on high impact guidelines (NCCN and/or ESMO)** The variant and/or biomarker is associated with a non-response, decreased response, or resistance to a specific drug or drug class in the same entity. The information is based on high impact guidelines (NCCN and/or ESMO)

R2	**Biomarker might be associated with a non-response or a resistance** The biomarker might be associated with a non-response, reduced response, or resistance to the stated drug class in this or another tumor entity (based on current literature)

Supplementary Table S2: **Level of Evidence (LoE) existing for each treatment option and used to classify information in table S1.**
Please note the LoE classification reported here is taken from Amaral *et al.*.[1]

2. Determination of TMB and analyzation of MSI

To determine TMB, WES was performed in-house using Agilent All Human Exome (v6) enrichment for both blood and tumor tissue. Somatic variants were called by first calling each sample separately and then comparing resulting callsets with additional verification of potential somatic variants to see whether they remained uncalled in the germline sample due to filtering (i.e., low coverage, strand bias). Tumor mutational burden (TMB) was defined as the number of somatic SNV-, InDel- and essential splice site variants (NAF ≥ 0.1) per megabase of coding DNA. Somatic variants seen in more than 1% of all cases analyzed in our lab are not counted. Tumor mutational burden is classified as high if ≥ 10 Mut/Mb are present in the tumor[3 4].

MSI was analyzed based on tumor and normal WES data using MANTIS.[5]

3. Peptide Prediction and Vaccine Design

In general, the selected peptides are predicted to activate not only cytotoxic T cells but also T helper cells. Therefore, in addition to short peptides with a length of 8-11 amino acids predicted to bind to HLA class I molecules, also long peptides with a length of 17 amino acids potentially binding to HLA class II molecules were chosen.

3.1 Prediction of neoepitopes

Somatic missense variants only present in the tumor but absent in the normal tissue of the patient were identified by exome analysis. Identified somatic missense variants (single nucleotide variants)

are sent to the Centre for Bioinformatics Tuebingen, Dept. of Computer Science, University Tuebingen for epitope prediction and HLA typing.[6] MHC class I epitopes are predicted using SYFPEITHI, netMHC-3.0 and netMHCpan-2.4.[7-9] Peptides containing somatic variants that are classified as binder by at least one prediction method are further evaluated. The respective thresholds for classification as binder are defined as <500 nM for netMHC and netMHCpan as well as >50% of maximal score for SYFPEITHI. Peptides resembling a wildtype sequence in the human proteome (based on UniProtKB/Swiss-Prot, human, 9/7/14) are excluded.

3.2 Selection of peptides

An in-house developed and proprietary neoepitope selection algorithm was used to filter and prioritize putative neoepitopes according to the following features: Putative HLA class I epitopes with high HLA class I binding prediction scores derived from variants with high allele frequencies were selected. Peptides predicted to bind to different HLA class I molecules of the patient were prioritized. Peptides classified as binder by > 1 prediction method were prioritized. Predicted neoepitopes with superior predicted binding affinity compared to wildtype peptide were prioritized. Putative HLA class II epitopes with a length of +/-17 amino acids were designed to contain variants (SNVs/small insertions or deletions) with high allele frequencies. Peptides spanning variants in possible tumor drivers were prioritized. Peptides with a high percentage of hydrophobic amino acids, peptides with a high probability for gelation or dimerization were excluded to avoid solubility problems in an aqueous solution and problems during synthesis. Peptides derived from genes most probably not expressed in the patient's tumor entity were excluded. For this purpose, protein expression information for the patient's tumor entity were manually checked in the Human Protein Atlas database (https://www.proteinatlas.org/) and integrated in the peptide selection process. The bioinformatically identified somatic variants corresponding to all selected peptides were manually reviewed in the sequencing data and filtered for false positives.

3.3 Statistics

Estimated tumor content: 70%

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The HLA genotype was calculated based on the sequencing analysis as:

HLA-A*03:01, HLA-A*26:01, HLA-B*15:01, HLA-B*35:01, HLA-C*04:01

Number of bioinformatically identified somatic variants (before manual review): 993

Short peptides (MHC I)

Number of Peptides generated: 88876

Number of Peptides after Filtering: 87738

Number of Predictions: 1036965

Number of Predicted Binders: 17128

Number of Predicted Non-Binders: 1019837

Number of Binding Peptides: 3631

Number of Non-Binding Peptides: 32041

Short peptides selected for vaccine: 7

Long peptides (MHC II)

Long Peptides generated from data: 1062

Long Peptides removed by self-identity filter: 31

Long Peptides selected for vaccine: 3

4. Performance of WES, ddPCR and immune monitoring

4.1 WES and ddPCR

Whole Exome Sequencing and data analysis as well as ddPCRs were accomplished as previously described by Forschner *et al*..[10]

4.2 Immune monitoring

Isolation of peripheral blood mononuclear cells (PBMC) from whole blood: Peripheral blood mononuclear cells (PBMC) were isolated by Biocoll Separation Solution (Biochrom) and cryopreserved for later use.

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Cell culture and analysis of specific T cells: PBMC were thawed and cells were cultured in TexMACS medium (Miltenyi Biotec) overnight to recover, stimulated with patient-individual mutated peptides (1 µg/ml) and cultured 11 days in the presence of low dose IL-2 (10U/ml; Miltenyi Biotec) and IL-7 (10 ng/ml; Miltenyi Biotec). For analysis, cells were restimulated with peptides or incubated with DMSO (unstimulated negative control; VWR), SEB (Staphylococcal Enterotoxin B as unspecific positive control; Sigma-Aldrich) or viral peptides (stimulation control; Miltenyi Biotec). Activated cells were measured after intracellular cytokine staining by flow cytometry as described previously.[11] Analyzed markers included: Live/dead-Staining (Zombie Aqua Dye; BioLegend), CD8 (clone SK1; BioLegend) and CD4 (clone RPA-T4; BioLegend) to identify T cell populations, as well as IFN-γ (clone 4S.B3; BioLegend), TNF (clone MAb11; BioLegend), IL-2 (clone MQ1-17H12; BioLegend) and CD154 (clone 24-31; BioLegend) 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 frequency of reactive T cells was calculated subtracting % of positive T cells within the corresponding unstimulated negative control sample.

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