Warehouse approach for the development of personalized cancer vaccines by using Personal Antigen Selection Calculator (PASCal) without need for tumor biopsy

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ABSTRACT

Background: Analysis of current data with cancer vaccines suggests that the lack of efficacy is likely due to their two primary design challenges: 1. Vaccines have little chance of destroying heterogeneous tumor cells since they rarely induce polyclonal T-cell responses; 2. Even when polyclonal T-cell responses have been successfully induced, they are often directed against tumors where the target is absent (not expressed). Recent mutated neoantigen-based vaccines (MNeoV) aim to solve this latter issue, however only about 10-20% of selected epitopes proved to induce CD8+ T-cell responses in patients. In addition, development of MNeoV for commercial use is challenging. To overcome these limitations, we developed PASCal for improved selection of peptides (epitopes) that induce T-cell responses targeted against heterogeneous tumor cells.

Methods: PASCal operates by 3 moduls: (1) a validated epitope database containing 10^8 true HLA-epitope pairs (2) Expression frequency-based shared tumor antigen database established for 19 indications based on >96,000 tumor biopsies. (3) Validated algorithm for the identification of immunogenic peptides by the selection of personal epitopes (PEPIs) binding to multiple autologous HLA alleles[1,2]. Using PASCal, a library of 3,286 immunogenic 20mer peptides derived from 184 antigens associated with 19 cancer indications - based on 16,000 subjects' HLA genotype (both class I&II alleles) was compiled. Personal vaccines were selected and tested for 3 HLA-genotyped metastatic cancer patients (with ovarian-, breast- and colorectal cancer). Immunogenicity of the vaccines was tested by IFN-γ ELISPOT.

Results: Personal cancer vaccines were selected to fulfill the following criteria: 12 immunogenic peptides derived from 12 different tumor-specific antigens frequently expressed in the patient's disease type, with the expected number of expressed antigens on the patient's tumor cells of at least 3 (by statistical estimation). CD8+ T-cell responses were induced by 97%, CD4+ T-cell responses by 85% of peptides, confirming aimed polyclonal T-cell responses. Long lasting CD8+ T-cell responses were detected *ex vivo* 4.5 months, *in vitro* 14 months after last vaccination. Pre-existing T-cell reactivities were detected against at least 25% of vaccine antigens demonstrating their presence in the patients' tumor, confirming the success of vaccine design strategy aiming to induce polyclonal T-cell responses against at least 3 antigens expressed by the tumor.

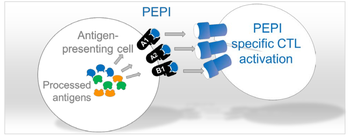
Conclusion: PEPIs outperform reported immunogenicity of mutated neoantigen-based personal vaccines and induced unprecedented immune responses in cancer patients. The „off-the-shelf" personalized approach with PASCal enables commercially scalable vaccine development, without need for tumor biopsy and on-demand manufacturing.

PERSONAL EPITOPES (PEPIs)

PEPI is an epitope restricted by ≥3 autologous HLA of the individual capable to mount T cell response against the cell expressing the same PEPI.

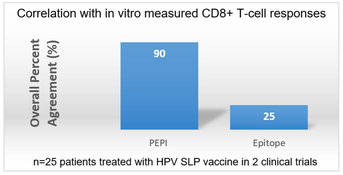
Hypothesis:

- Patient-specific PEPIs activate the T-cells and drive an effective response against the target cells expressing the same PEPIs



Finding:

- No correlation between single HLA-binding epitopes and HPV-specific T-cell responses of patients
- 90% agreement between PEPIs and CD8+ T-cell responses ($p < 0.001$)[2]



PEPI VALIDATION

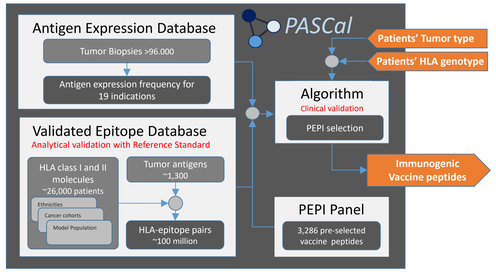
Retrospective study: 6 clinical trials; 80 patients; 157 dataset

Prospective study: Treos' on-going phase I/II clinical trial; 10 patients; 70 dataset[1]

Parameter	Definition	Retrospective validation n = 157*	Clinical validation n = 70**
PPV Positive Predictive Value	The likelihood that an individual with a positive PEPI Test* result has antigen-specific T cell responses	84%	79%
NPV Negative Predictive Value	The likelihood that an individual with a negative PEPI Test result does not have antigen-specific T cell responses	42%	51%
OPA Overall Percent Agreement	The percentage of results that are true results, whether positive or negative	70%	64%
Fisher's exact probability test	p-value of the hypothesis testing	0.01	0.01

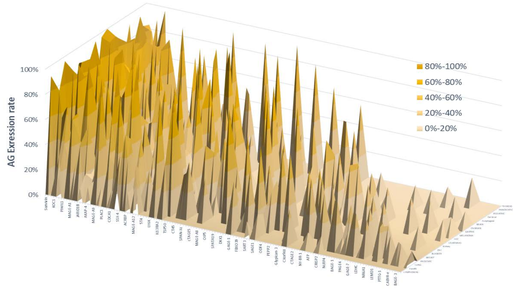
PASCAL TECHNOLOGY ADDRESSES BOTH PATIENT AND TUMOR HETEROGENEITY

Key step: selection of validated Personal EPItopes (PEPIs) specific to the patient's HLA genotype, not only to individual alleles[2,3]



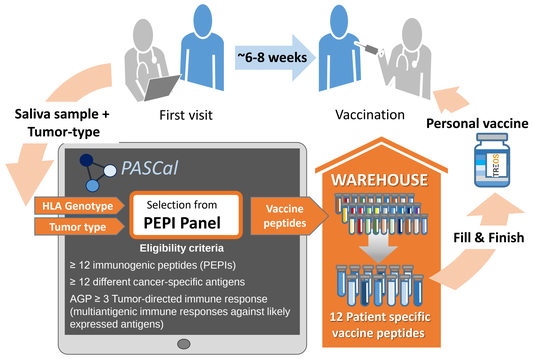
PEPI PANEL

Library of immunogenic peptides for 19 cancer indications



3,286 immunogenic 20mer peptides were derived from 184 shared antigens associated with 19 tumor-types - based on 16,000 subjects' HLA genotype (both class I&II alleles) using PASCal

WAREHOUSE APPROACH FOR PERSONAL VACCINE DESIGN



PEPI VACCINATION INDUCED BROAD T-CELL RESPONSES IN EACH PATIENT

Adjuvant: Montanide ISA 51VG

Administration: subcutaneous injection into 2 arms and 2 thighs

Doses received: multiple doses (≥3 doses/patient)

Patients were vaccinated under the "individuelle Heilversuche" regime in Germany as add-on to patient's standard-of-care

Pts.	Tumor	Safety	#Patient-specific peptides included in the vaccine (#PEPIs)	Tumor specific T cell responses (IFN-γ ELISpot)	
				CD8+	CD4+
PT1	Metastatic Breast Cancer	Safe and well tolerated*	12	11	12
PT2	Metastatic Ovarian Cancer	Safe and well tolerated*	13	13	13
PT3	Metastatic Colorectal Cancer	Safe and well tolerated*	13	13	7
Immunogenic peptides per patient:				12	
Peptides generating any T cell response:				100%	

*Flu like syndrome, Fatigue, palpitations and low fever, redness, itchiness at the site of the injections.

SUMMARY

- **PASCal** is a new platform for
  - the selection of true immunogenic peptides for patients
  - without the need for tumor biopsy – only saliva sample
- **PEPIs** outperform reported immunogenicity of personalized mutation-based neoantigen vaccines and induced unprecedented immune responses in cancer patients.
- **PEPI PANEL** „off-the-shelf" personalized vaccine approach has the opportunity to enable
  - faster
  - better
  - cheaper and more affordable personal cancer vaccine treatment

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Conflict of interest: ZC, LM, PP, JT, OL, KP, ES, MM and ET are employees of Treos Bio Zrt and hold shares of Treos Bio Ltd.

Case study: Selection of 12 personalized peptide vaccine for a patient with metastatic breast cancer (PT1)

HLA genotype from saliva sample: HLA-A*02:01, 29:01; HLA-B*07:02, 35:02; HLA-C*04:01, 07:02; DRB1*04:03, 11:01; DRB3*02:02, DRB4*01:01; DPB1*04:01, 04:02, DQB1*03:01, 03:05

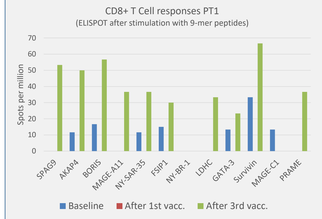
Predicted Immunogenicity: PEPI = 10 ($CI_{95\%}$ 8,12)

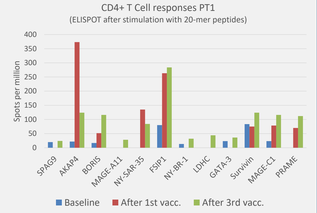
Predicted Tumor-directed immune response: AGP = 6.5

Vaccine TRS-BC-01 peptides	Breast Cancer-specific Antigens	Expression frequency in 17,337 breast cancer tissues	Number of peptide-binding autologous HLA alleles	
			Class I	Class II
BC-01-P1	SPAG9	88%	3	1
BC-01-P2	AKAP4	85%	4	4
BC-01-P3	BORIS	71%	3	2
BC-01-P4	Survivin	59%	3	2
BC-01-P5	MAGE-A11	49%	3	1
BC-01-P6	PRAME	49%	3	5
BC-01-P7	NY-SAR-35	47%	3	5
BC-01-P8	FSIP1	35%	3	6
BC-01-P9	NY-BR-1	31%	3	1
BC-01-P10	LDHC	71%	3	5
BC-01-P11	GATA-3	12%	3	1
BC-01-P12	MAGE-C1	55%	3	8

PEPI VACCINATION RESTORED PRE-EXISTING IMMUNITY AS WELL AS INDUCED DE NOVO T-CELL RESPONSES

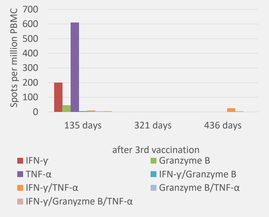
Pre-existing immune responses were detected against 9/12 vaccine antigens confirming the presence of tumor-directed immune responses (and supporting the target selection strategy)



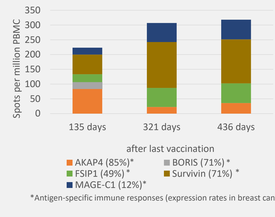


LONG-LASTING IMMUNE RESPONSES

Effector (*Ex vivo*) CD8+ T cell responses were detected 135 days (**4.5 months**) after last vaccination.



Memory CD8+ T cell responses were detected **14 months** (436 days) after last vaccination against 4 tumor antigens.



*Antigen-specific immune responses (expression rates in breast cancer)

TREOS CANCER VACCINE TECHNOLOGY

www.treosbio.com

References: [1]Hubbard JM et al. JCO, 37, 2019 (suppl; abstr 3557), [2]Toke ER et al, JCO, 37, 2019 (suppl; abstr e14295), [3]Lorincz O et al, JCO, 37, 2019 (suppl; abstr e14298)