A Personal Antigen Selection Calculator (PASCal) for the design of personal cancer vaccines

#1181PD

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ABSTRACT

Background: The current challenge in developing effective cancer vaccines is the accurate prediction of epitopes that induce CD8+ cytotoxic T-cell responses. Recent technological advances have enabled development of patient-specific therapeutic vaccines. However, in these vaccines only about 10-20% of the predicted neoepitopes induced CD8+ T-cell responses in patients. To overcome this limitation, we developed PASCal for improved selection of peptides (epitopes) that induce T-cell responses.

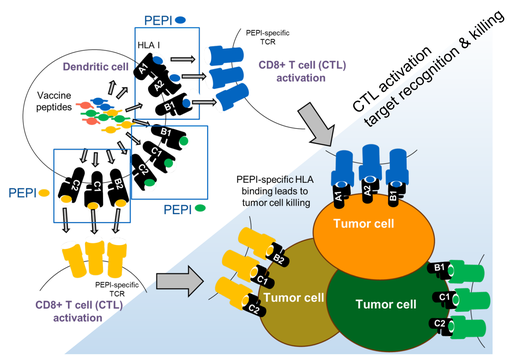
Methods: PASCal operates by 3 moduls: (1) a validated epitope database containing 10^8 true HLA-epitope pairs derived from 1300 tumor antigens and HLA class I and II molecules covering the HLA genotype of 26000 subjects. (2) Expression frequency-based shared tumor antigen database established for 19 indications based on >96000 tumor biopsies. (3) Algorithm for the identification of immunogenic peptides by the selection of personal epitopes (PEPIs) binding to ≥3 autologous HLA alleles. Using PASCal, personal 20mer peptide vaccines were designed for 3 HLA-genotyped cancer patients (with ovarian-, breast- and colorectal cancer). Immunogenicity of the vaccines was tested by ELISPOT and Intracellular Citokine Staining (ICS).

Results: Personalized cancer vaccines contained PEPIs from 12 disease specific tumor-antigens most frequently expressed in the patients disease. T-cell responses were induced by 100% of peptides. An average of 11/12 PEPIs induced CD8+ T-cell responses and 12/12 induced CD4+ T-cell responses in each patient. Pre-existing antigen specific T-cell reactivities were detectable against 25% of vaccine antigens (demonstrating the expression of the target vaccine antigens by the patient's tumor), the others were induced *de novo*. Both CD8+ and CD4+ T-cells were polyfunctional, as evident by secretion of multiple cytokines determined by *ex vivo* ICS.

Conclusion: We used the largest validated database of tumor epitopes reported to-date along with an algorithm successfully selecting immunogenic peptides to develop personalized cancer vaccines. PEPIs outperform reported immunogenicity of personalized neoantigen vaccines and induced unprecedented immune responses in cancer patients.

PERSONAL EPITOPES (PEPIs)

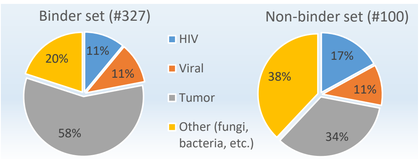
PEPI is an epitope restricted by ≥3 autologous HLA of the individual capable to mount T cell response against the cell expressing the same PEPI.



PEPI VALIDATION

- **Analytical validation – prediction of HLA-epitope binding** was determined with an established Reference Standard containing HLA-peptide pairs determined by experimental methods (direct binding assays)

Reference Standard:
✓ Experimentally proven **Binder** and **Non-binder** HLA-epitope pairs
✓ High affinity epitopes (measured IC50 < 500nM)
✓ Broad antigen spectra
✓ Most frequent alleles



Parameter	Definition	Result
Specificity	Ability to identify true HLA-restricted human epitopes	**93%**
Sensitivity	Ability to exclude false HLA-restricted human epitopes	**93%**

- **Clinical validation – prediction of individuals' antigen-specific immune responses**

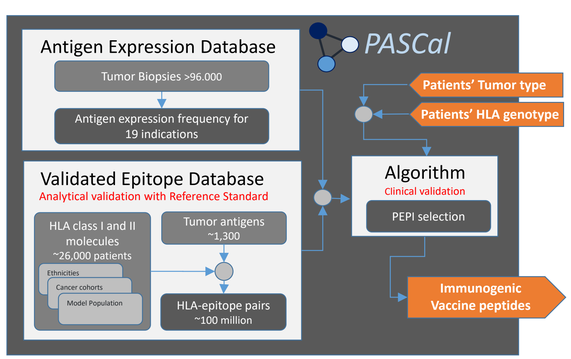
A retrospective study was followed by our Phase I/II OBERTO101 trial: 10 metastatic CRC patients received PolyPEPI1018 vaccine designed by PASCal, optimized for CRC population[3].

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

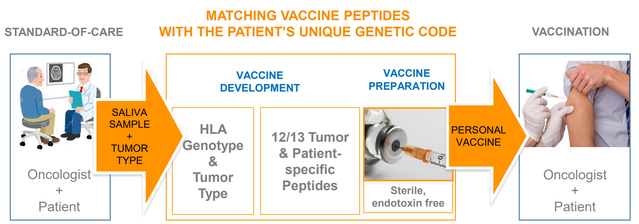
*80 patients; 6 clinical trials; 157 dataset **10 patients; Treos phase I/II clinical trial; 70 dataset; PEPI Test CE-mark device

PASCAL TECHNOLOGY

Key step: selection of validated Personal EPItopes (PEPIs) specific to the patient's HLA genotype, not only to individual alleles[1,2]



PROOF OF CONCEPT STUDIES USING PASCal



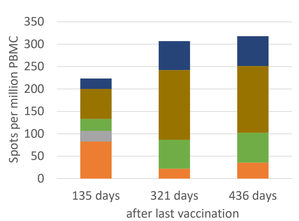
Vaccine: 12/13 long peptides (PEPIs) derived from 12/13 shared cancer testis antigens frequently expressed in the patient's tumor type
Adjuvant: Montanide ISA 51VG
Administration: subcutaneous injection into 2 arms and 2 tights
Doses received: multiple doses (≥3 doses/patient)
Patients were clinically monitored conform their standard-of-care and 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 (PEPIs) generating any T cell response :		100%	

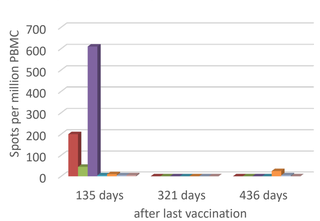
*Flu like syndrome, Fatigue, palpitations and low fever, redness, itchiness at the site of the injections.

LONG LASTING POLYFUNCTIONAL IMMUNE RESPONSES

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

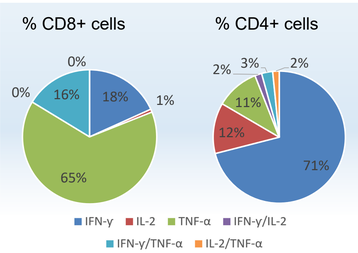


Effector (*Ex vivo*) CD8+ T cell responses were detected 135 days (**4.5 months**) after last vaccination

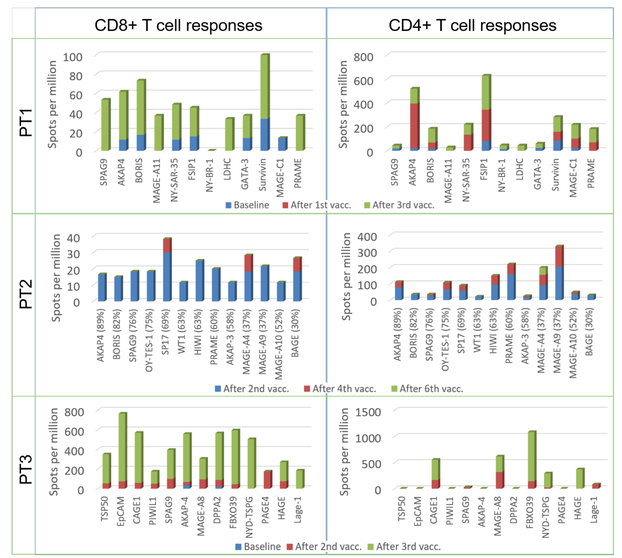


DETAILED IMMUNOLOGICAL ANALYSIS

Ex vivo detected polyfunctional T cell responses for PT1 measured by Intracellular staining (ICS)



Pre-existing and de-novo induced immune responses against multiple antigens



SUMMARY

- PASCal is a new platform for
  - the design of true immunogenic peptides - clinically validated
  - target selection without the need for tumor biopsy - confirmed by detected pre-existing immune responses
  - 19 cancer indications – including the ones with low mutational burden
- PEPIs outperform reported immunogenicity of personalized mutation-based neoantigen vaccines and induced unprecedented immune responses in cancer patients.

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

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