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Identification of frequently presented non-mutated tumor-specific immunogens for the development of both off-the-shelf and personalized vaccines without need for tumor biopsy

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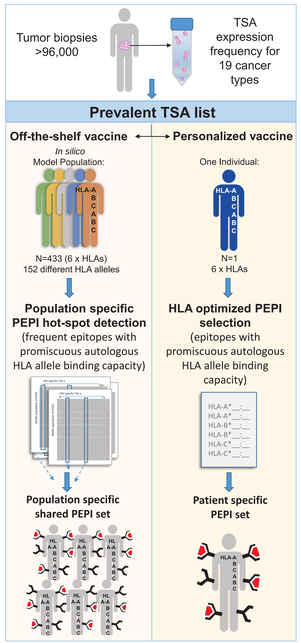
Background
Vaccines have little chance of destroying heterogeneous tumor cells since they rarely induce polyclonal T-cell responses against the tumor. The main challenge is the accurate identification of tumor targets recognizable by T cells. Presently, 6-8% of neoepitopes selected based on the patients' tumor biopsies are confirmed as real T cell targets[1, 2]. To overcome this limitation, we developed a computational platform called Personal Antigen Selection Calculator (PASCal) that identifies frequently presented immunogenic peptide sequences built on HLA-genetics and tumor profile of thousands of real individuals [3]. Here we show the performance of PASCal for the identification of both shared and personalized tumor targets in metastatic colorectal cancer (mCRC) and breast cancer subjects.

Methods
Expression frequency of the tumor-specific antigens (TSAs) ranked in PASCal's database (based on 7,548 CRC specimen) was compared to the RNA-sequencing data of CRC tumors obtained from TCGA. Using PASCal, 12 shared PEPIs (epitopes restricted to at least 3 HLA class I alleles of a subject from an *in silico* cohort) derived from 7 TSAs were selected as frequent targets (calculated probability: average 2.5 [95%CI 2.4-2.8] TSAs/patient). Spontaneous immune responses against each of the twelve 9mer peptides were determined by ELISpot using PBMCs of 10 mCRC subjects who participated in the OBERTO-101 study [4]. PEPIs selected for a breast cancer subject based on her HLA genotype were also tested.

Results
Each of the 106 tumors analyzed expressed at least 13, average 15 of the 20 top-ranked TSAs in PASCal's database confirming their prevalence in CRC. 7/10 subjects had spontaneous CD8⁺ T-cell responses against at least one peptide selected with PASCal. Each peptide (12/12) was recognized by at least one patient. Patients' T-cells reacted with average 3.6/12 (30%) peptides confirming the expression of average 2.8 [95%CI 1.0-4.6] TSAs (n=10). After HLA-matching, among the subjects for whom we could select at least 4 PEPIs (average 5) from the list of 12 peptides (n=6), average 2.5 (50%) peptides were positive. Of the 12 PEPIs selected with PASCal for a breast cancer subject, we detected spontaneous T-cell responses against 9 PEPIs, indicating that at least 75% of the selected peptides were present in the subject's tumor and were recognized by T-cells.

Conclusions
PASCal platform accommodates both tumor- and patient heterogeneity and identifies non-mutated tumor targets that may trigger polyclonal cytotoxic T-cell responses. It is a rapid tool for the design of both off-the-shelf and personalized cancer vaccines negating the need for tumor biopsy.

Personal Antigen Selection Calculator (PASCal) computational platform

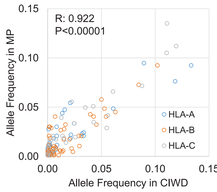


Representativeness of the computational model:

1. *In silico* Model Population (MP) (n=433) assembled of real subjects genetic data (HLA genotype, i.e. all six HLA class I alleles);

covers multiple ethnicities [5-7]: (90 African, 90 European, 45 Chinese, 45 Japanese subjects, and 163 subjects with mixed origin (e.g. United States, Canada, Australia, and New Zealand)).

HLA allele set of MP covers 97.4 % of the HLA alleles of > 8M subjects*



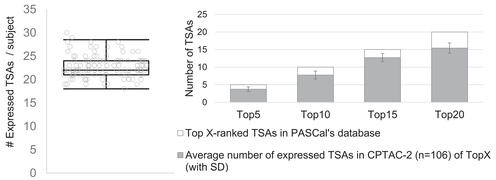
Alleles	#alleles in MP	Coverage in CIWD
HLA-A*	49	98.7%
HLA-B*	71	95.1%
HLA-C*	32	98.5%
All	**152**	**97.4%**

*listed in Catalog of Common, Intermediate and Well-Documented alleles (CIWD) comprising 4,818 alleles [8]:

R: Pearson correlation coefficient

2. Antigen Expression Database: contains protein/mRNA expression data of shared TSAs obtained from >96,000 biopsies for 19 cancer indications (e.g.: **7,548 CRC specimen)**

CRC-associated TSAs (n=35) in the PASCal's Database are prevalent in CRC

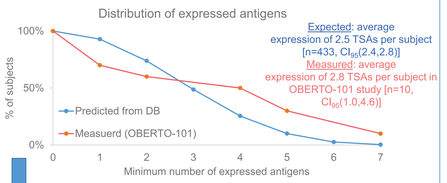


Each circle represent a subject, mRNA expression data of an independent cohort of CRC subjects (n=106; CPTAC-2: Clinical Proteomic Tumor Analysis Consortium) obtained from TCGA database [9].

Study design

TSA selection
7 most frequent CRC specific TSAs were selected.

CRC-associated TSA	Expression frequency based on PASCal
TSP50	89%
EpCAM	88%
Survivin	87%
Cage1	74%
Spag9	74%
Mage-A8	44%
FBXO39	39%



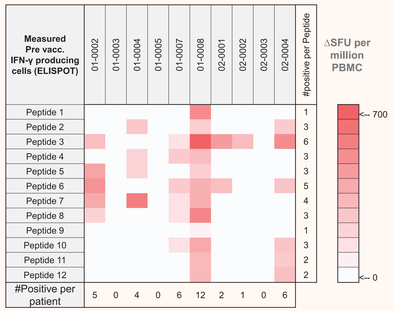
Off-the-shelf vaccine design for a population

Immunogenic fragments of the **7 CRC-associated TSAs** to be included in the **PolyPEPI1018** vaccine were selected:

- **12 shared PEPIs** which were predicted as PEPI hot-spots for subjects in the model population

Peptide (PEPI)	Shared PEPIs	Frequency of PEPI in the model population	Source TSA
Peptide 1	TTMETQFPV	36%	TSP50
Peptide 2	YRAQRFWSW	20%	TSP50
Peptide 3	RTYWIIIEL	51%	EpCAM
Peptide 4	RAIEQLAAM	26%	Survivin
Peptide 5	YVDEKAPEF	28%	EpCAM
Peptide 6	KVAELVRFL	18%	Mage-A8
Peptide 7	KMHSLLALM	42%	Cage1
Peptide 8	STFKNWPFL	15%	Survivin
Peptide 9	KSMTMMPAL	37%	Cage1
Peptide 10	VMSERVSGL	28%	Spag9
Peptide 11	FMNPYNAVL	78%	FBXO39
Peptide 12	FFFERIMKY	46%	FBXO39

Spontaneous CD8⁺ T cell responses of n=10 mCRC subjects from the OBERTO-101 study verified the expression of the predicted PEPIs in subjects' tumors



- Subjects' T cells reacted with average 3.6 / 12 (30%) peptides confirming the expression of average 2.8 [95%CI 1.0-4.6] TSAs (n=10)
- 70% of subjects had spontaneous CD8⁺ T-cell responses against at least one peptide selected with PASCal and each peptide (12/12) was recognized by at least one subject

Personalized vaccine design

Example:
Subject's HLA genotype

02-0004
HLA-A*02:01
HLA-A*02:12
HLA-B*40:02
HLA-B*51:01
HLA-C*02:02
HLA-C*14:02

7 out of 12 shared PEPIs matched with the HLA-genotype of Patient 02-0004, ie were **predicted as PEPIs** for the patient

Peptide 1
Peptide 3
Peptide 6
Peptide 7
Peptide 9
Peptide 10
Peptide 11

Spontaneous T cell response for predicted PEPIs detected by ELISPOT
Among the subjects for whom at least 4 PEPIs could be selected (n=6), average 2.5 (50%) peptides were positive

01-0002	
Peptide 3	+
Peptide 4	-
Peptide 7	+
Peptide 9	-
Peptide 11	-

01-0004	
Peptide 3	-
Peptide 4	+
Peptide 7	+
Peptide 9	-

01-0008	
Peptide 1	+
Peptide 3	+
Peptide 6	+
Peptide 9	+
Peptide 10	+
Peptide 11	+

02-0001	
Peptide 1	-
Peptide 3	+
Peptide 7	-
Peptide 9	-

02-0002	
Peptide 7	-
Peptide 10	-
Peptide 11	-
Peptide 12	-

02-0004	
Peptide 1	-
Peptide 3	+
Peptide 6	+
Peptide 7	-
Peptide 9	-
Peptide 10	+
Peptide 11	+

Summary

PASCal computational tool has been designed to address the heterogeneity of both the subjects and the tumors, major challenges in the development of immunotherapies:

- efficiently approximates the HLA-genetics and tumor antigen expression profile of cancer subjects
- demonstrates that shared TSAs are real immunogenic targets patients have spontaneous immune responses against (mainly believed only for mutated neoantigens)
- together with the promiscuous autologous HLA allele binding epitope (PEPI) concept demonstrated its applicability for both Off-the-shelf and Personalized cancer vaccine design

OFF-THE-SHELF VACCINE design features

- Identified frequently expressed shared immunogens for the population (12 peptide targets derived from 7 TSAs)
- Large fraction of the selected shared immunogens were confirmed in the individual subjects' tumor (expected: 2.5 TSA/subject *vs* measured 2.8 TSA/subject)

PERSONALIZED VACCINE design features

- 24 of 42 (57%) of PEPIs selected based on HLA background reacted spontaneously with T cells of 7 subjects (6 mCRC and 1 breast cancer) – compares favorably to the 6-8% validation rate reported to date for mutated neoepitopes [1, 2]
- Eliminates the need for single tumor biopsies (and the related under-sampling bias)

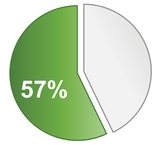
Performance of PASCal for the Identification of Real T Cell-Recognized Tumor Targets

OFF-THE-SHELF APPROACH WITHOUT HLA-preselection

Immunogenic targets confirmed

PERSONALIZED APPROACH WITH HLA-preselection

Immunogenic targets confirmed



Personalized vaccine for a patient with Breast Cancer

For an advanced metastatic breast cancer subject the 12 most frequently expressed breast cancer associated TSAs were selected, then:

Patient 13
HLA-A*02:01
HLA-A*29:01
HLA-B*07:02
HLA-B*35:02
HLA-C*04:01
HLA-C*07:02

- For each 12 TSA, a 9mer was determined using PASCal, which is able to bind at least 3 HLA class I alleles of the subject (PEPI),
- Using the subjects pre-vaccination PBMC, spontaneous T cell response was determined by ELISPOT assay for all 12 peptides individually

Breast cancer associated TSA	Expression frequency in PASCal (based on 4,511 tumor biopsies)
SPAG9	88%
AKAP4	85%
BORIS	71%
Survivin	71%
MAGE-A11	59%
PRAME	55%
NY-SAR-35	49%
FSIP1	49%
NY-BR-1	47%
LDHC	35%
GATA-3	31%
MAGE-C1	12%

Patient 13	
BCPeptide 1	+
BCPeptide 2	+
BCPeptide 3	+
BCPeptide 4	+
BCPeptide 5	-
BCPeptide 6	-
BCPeptide 7	+
BCPeptide 8	+
BCPeptide 9	+
BCPeptide 10	-
BCPeptide 11	+
BCPeptide 12	+

75% (9/12) of the selected targets were present in the subject's tumor

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