A Phase 1 Study of PolyPEPI1018 Vaccine Plus Maintenance Therapy in Patients with Metastatic Colorectal Cancer with a Predictive Biomarker (OBERTO-101)

#3557

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Background
The goal of this study was to evaluate the safety, tolerability and immunogenicity of a single dose of PolyPEPI1018 as an add-on to maintenance therapy in subjects with metastatic colorectal cancer (mCRC). PolyPEPI1018 is a peptide vaccine containing 12 unique epitopes derived from 7 conserved cancer testis antigens (CTAs) frequently expressed in mCRC. These epitopes were designed to be Personal EPItopes (PEPIs), i.e. predicted by our novel PEPI test to bind to at least three autologous HLA alleles and more likely to induce T-cell responses than epitopes presented by a single HLA.

Methods
mCRC patients in the first line setting received the vaccine (dose: 0.2 mg/peptide) just after the transition to maintenance therapy with a fluoropyrimidine and bevacizumab. Vaccine-specific T-cell responses were first predicted by the PEPI test (using the patient's complete HLA genotype and antigen expression rate) and then measured by ELISpot after one cycle of vaccination.

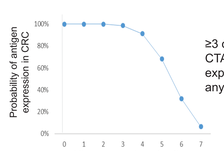
Results
Eleven patients were vaccinated with PolyPEPI1018. The most common adverse events were transient skin reactions (local inflammation at the site of the injections, e.g. erythema, redness and itchiness) and flu-like syndrome. No grade 3 or higher adverse events related to the vaccine occurred. Initial analysis on 4 patients demonstrated that T-cell responses were elicited by 96% of vaccine peptides. The overall percentage agreement between PEPI test-predicted and Elispot-measured CD8+ T cell responses was 71%, consistent with our retrospective analysis on 64 vaccine clinical trials involving 1,790 patients. Two of these 4 patients had unexpected tumor size reduction. Based on these encouraging results, the trial was amended to administer 3 doses of PolyPEPI1018 given 12 weeks apart.

Conclusions
PolyPEPI1018 combined with maintenance therapy was safe and well-tolerated in mCRC patients. Unprecedented immune responses were induced after single dose, with broad CRC-specific T cell responses and high accuracy prediction of CD8+ T cell responses. This promising activity in mCRC patients led to a trial amendment to administer 3 doses of PolyPEPI1018 in combination with systemic therapy.

DESIGN AND PRECLINICAL TESTING OF POLYPEPI1018 VACCINE

PolyPEPI1018 contains six 30aa long peptides each formed by joining two immunogenic 15mer fragments (each including 2 PEPIs) derived from 7 cancer testis antigens (CTAs). These antigens are tumor-specific and frequently expressed in CRC tumors based on analysis of 2,391 biopsies.

CRC Antigens	Expression Rate (2,391 CRC biopsies)
TSP50	89%
EpCAM	88%
Survivin	87%
CAGE1	74%
SPAG9	74%
MAGE-A8	44%
FBXO39	39%



≥3 of these 7 CTAs are expressed in any CRC

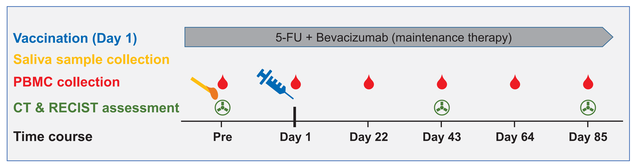
Preclinical immunogenicity testing of the vaccine was performed *in silico* on HLA-genotyped subjects of a random, representative model population and a CRC cohort.

Previously, the model population accurately predicted the measured immune response rates of 64 clinical trials conducted in 1,790 patients using 42 different vaccines (p=0.001).[1]

PolyPEPI1018 vaccine antigens	Predicted immunogenicity: Model population (n=433)	CRC cohort (n=37)
TSP50	53%	62%
EpCAM	69%	41%
Survivin	36%	32%
Cage	68%	81%
SPAG9	28%	3%
FBXO39	90%	100%
Mage-A8	18%	0%
Any antigen	**98%**	**100%**
Any 2 antigens	**92%**	**92%**
Any 3 antigens	**72%**	**65%**

OBERTO-101 TRIAL DESIGN (NCT03391232)

The clinical study was designed to evaluate safety, tolerability and immunogenicity of subcutaneous injection of PolyPEPI1018 Vaccine as an add on immunotherapy to the standard-of-care maintenance therapy in subjects with mCRC. The first part of the study investigated the administration of a single vaccine dose during 12-week follow-up period.



RESULTS: SAFETY AND IMMUNOGENICITY OF POLYPEPI1018 VACCINE

Eleven patients were vaccinated with PolyPEPI1018 just after their transition to maintenance therapy with fluoropyrimidine and bevacizumab. Treatment was safe and well tolerated. Transient local erythema and edema at the site of vaccination were observed as expected, as well as a flu-like syndrome with minor fever and fatigue. One SAE "possibly related" to the vaccine was recorded.

Safety

Patient	SAE	Relatedness
10001	Death due to disease progression	Unrelated
10004	Embolism	Unlikely Related
10004	Abdominal pain	Unrelated
10007	Bowel Obstruction	Unrelated
20004	Non-Infectious Acute Encephalitis	Possibly Related

Immunogenicity

Patients with immunological responses	Percentage (n)
CD4+ T cell responses	100% (10/10)
CD8+ T cell responses	90% (9/10)
Both CD8+ and CD4+ T cell resp.	90% (9/10)
Ex vivo detected CD8+ T cell resp.	71% (5/7)
Ex vivo detected CD4+ T cell resp.	86% (6/7)

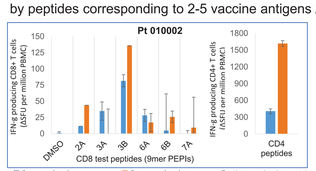
Responses were assessed by ex vivo and enriched IFN-g ELISPOT

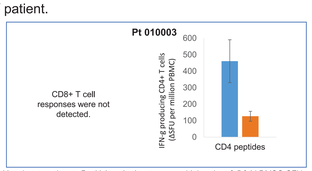
- Pre-existing immune responses against all of the vaccine antigens were detected
- Pre-existing immune responses were boosted
- Antigen-specific immune responses were also induced *de novo*
- Both CD8+ and CD4+ T cell responses were detectable *ex vivo*

DETAILED ANALYSIS OF 4 PATIENTS IMMUNOLOGICAL DATA

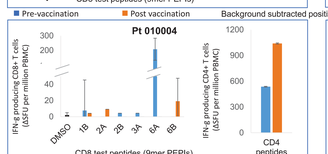
Broad, CRC-specific T cell responses of effector-memory phenotype

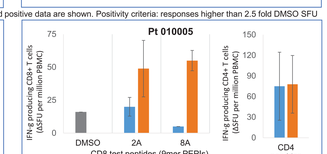
Three of the 4 patients had CD8+ T cell responses against multiple vaccine peptides. All 4 patients had CD4+ T cell response measured by ELISPOT assay after a single round of *in vitro* stimulation. Responses were both *de novo* induced and boosted upon vaccination. T-cell responses were elicited by peptides corresponding to 2-5 vaccine antigens / patient.





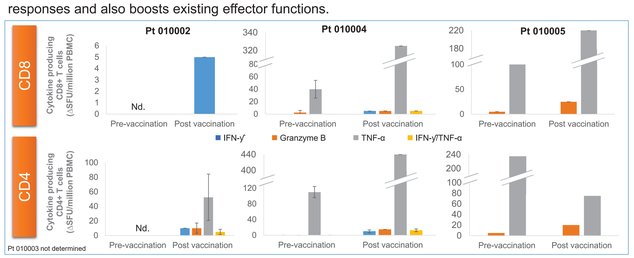
Pre-vaccination Post vaccination Background subtracted positive data are shown. Positivity criteria: responses higher than 2.5 fold DMSO SFU





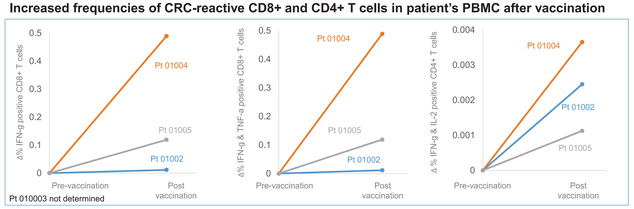
Ex vivo responses of effector phenotype

The 3 patients who had memory responses were further analyzed by *ex vivo* ELISPOT and *ex vivo* intracellular cytokine staining (ICS) assays. Both CD8+ and CD4+ vaccine-specific immune responses were detected. Results confirm that PolyPEPI1018 vaccine generates polyfunctional immune responses and also boosts existing effector functions.



Pt 010003 not determined

Increased frequencies of CRC-reactive CD8+ and CD4+ T cells in patient's PBMC after vaccination



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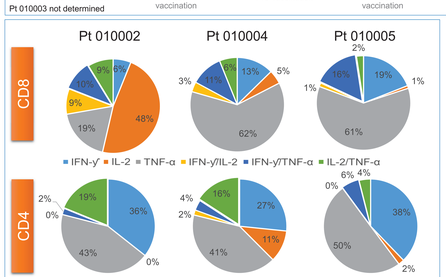


Functionality of CD8+ and CD4+ T cells

The fractions of total CD8+ T cells show high polyfunctionality as measured by *ex vivo* ICS. Fully functional IFN-g/TNF-a positive CD8+ T cells were present in all 3 patients tested.

For CD4+ T cells IFN-g positive, TNF-a positive and IL-2/TNF-a cells dominate.

PEPI Test prediction: Agreement between predicted and measured immune responses

Patient's HLA class I genotype was determined from their saliva sample and served as input data for the prediction of PEPI-specific immune responses for each patient. PEPI Test predicted and ELISPOT measured immune responses (4 x 12 datapoints) were in high agreement.

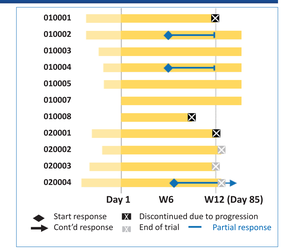
Vaccine peptide	Subpart test peptides (PEPIs)	010002 Measured	010002 Predicted	010003 Measured	010003 Predicted	010004 Measured	010004 Predicted	010005 Measured	010005 Predicted
CRC_P1	1A	-	-	-	-	-	-	-	-
	1B	-	-	-	-	+	-	-	-
CRC_P2	2A	+	+	-	-	+	+	+	+
	2B	-	+	-	-	+	+	-	-
CRC_P3	3A	+	-	-	-	+	-	-	-
	3B	+	-	-	-	-	-	-	-
CRC_P6	6A	+	+	-	-	+	+	-	+
	6B	+	-	-	-	+	-	-	-
CRC_P7	7A	+	+	-	-	-	+	-	-
	7B	-	-	-	+	-	-	-	-
CRC_P8	8A	-	+	-	+	-	-	+	-
	8B	-	-	-	+	-	-	-	-
Overall Percent Agreement [(True Positives + True Negatives) / All] OPA = 71 %									

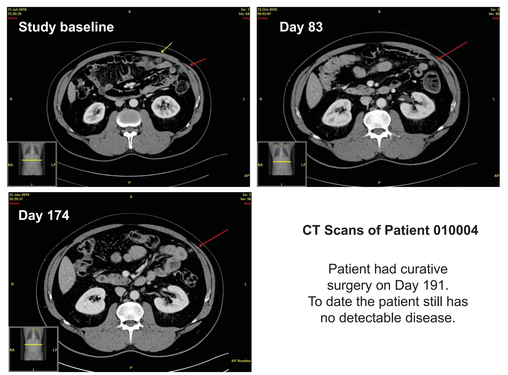
CLINICAL RESPONSES DETECTED DURING 12 WEEK FOLLOW-UP

Of the eleven vaccinated patients on maintenance therapy, 3 had progressed disease (PD), five of them had stable disease (SD) and three of them experienced unexpected partial tumor remission (PR) according to RECIST 1.1 criteria.

Patients active at 12 Week follow up at one clinical site (n=5), were further vaccinated with multiple vaccine doses in the second part of the study. Patients experience durable treatment effect.

One patient was elected for curative surgery.





CT Scans of Patient 010004

Patient had curative surgery on Day 191. To date the patient still has no detectable disease.

SUMMARY

- ✓ **Clinical results in line with preclinical testing**
  - Pre-existing immune responses against multiple CTAs demonstrate their presence in the patient's tumor - supporting our approach for target antigen selection
  - T cell responses against multiple antigens in a large subset of CRC patient population – validate our preclinical immunogenicity testing approach
- ✓ **Primary endpoint: Vaccination was safe and well-tolerated**
- ✓ **Secondary endpoints: Unprecedented immune responses**
  - both effector and memory phenotype after a single dose of PolyPEPI1018 vaccine
  - broad and polyfunctional, conquering the ones observed with personalized neoantigen vaccines[2,3]
  - correctly predicted by PEPI Test based - confirming previous findings that PEPI Test is able to predict subjects' antigen-specific immune responses[4]
- ✓ **Exploratory endpoints: Unexpected tumor responses were obtained**

  Patients experiencing tumor shrinkage responded to higher number of vaccine antigens than patients with stable disease

Based on these encouraging results, the trial was amended to administer 3 doses of PolyPEPI1018 in combination with systemic therapy.

Acknowledgements
We would like to thank the contribution of patients who participated in the study.

References

[1] Lőrincz et al. J Clin Oncol 37, 2019 (suppl; abstr e14298).
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