Novel immunotherapy based on commensal-derived peptides to drive an effective CD8+ T cell response against selected Tumor-Associated Antigens (TAAs).

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BACKGROUND

Peptide-based immunotherapy predominantly employs epitope peptides to stimulate immune cells, targeting tumor-associated antigens (TAAs) or tumor-specific antigens (TSAs), thereby defining a quite robust strategy against several cancers.

The OncoMimics™ approach takes this strategy a step further by using commensal-derived peptide antigens, which are homologous to TAA-derived peptides (TAAps), to elicit even more potent immune response against tumors.

Our findings highlight the critical role of gut microbiota in shaping immune responses, with specific commensal peptides showing significant homology with TAAs. We show that these OncoMimics™ peptides trigger cross-reactive cytotoxic CD8+ T cell responses against TAAs in humans, paving the way for a novel and effective anti-cancer strategy.

General concept : peptide mimicry

OncoMimics™ peptides: non-self-commensal-derived peptides mimicking TAAs to generate potent anti-tumor immune responses through expanding pre-existing cytotoxic T cells.

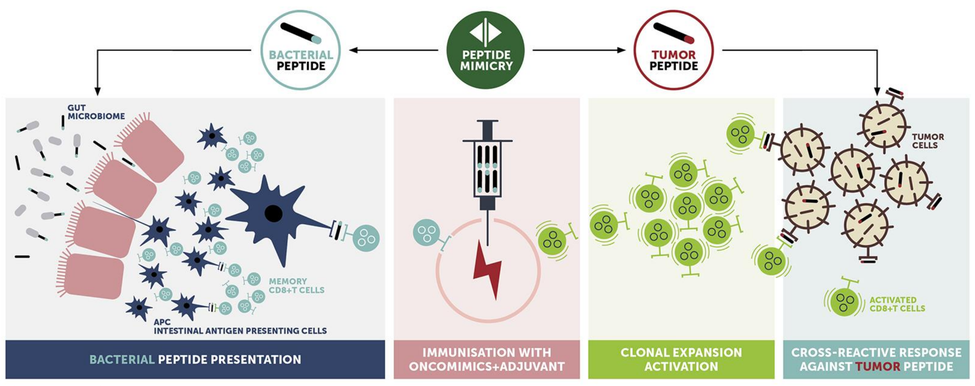
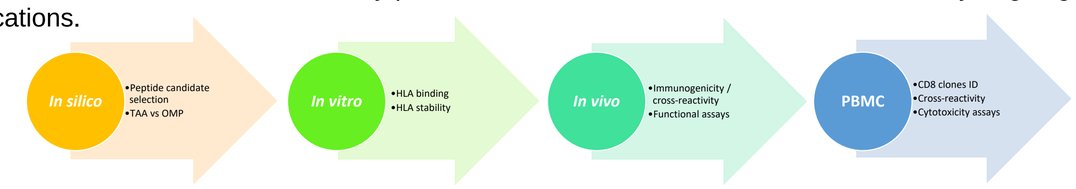


Figure 1. Microbiome-mimicry concept.

General pipeline for discovering OncoMimics™ peptides (OMPs)

OncoMimics™ discovery pipeline overview: To advance OncoMimics™ peptide candidates for clinical trials, we follow a structured discovery process. Five Phase 1/2 clinical trials are currently ongoing in four indications.



Product	Clinical study	Indication	OncoMimics™ (OMP)	Targets (TAA)
EO2401	ROSALIE	Recurrent glioblastoma	OMP16 OMP17 OMP18	IL13RA2(345-353) BIRC5(96-104) FOXM1(694-702)
	SPENCER	Adrenal tumors		
EO2463	SIDNEY	B cell malignancies	OMP64 OMP65 OMP66 OMP72	CD22(24-32) CD37(48-56) BAFF-R(77-85) CD20(118-126)
EO2040	CLAUDE	Colorectal cancer (CRC)	OMP17 OMP18	BIRC5(96-104) FOXM1(694-702)
EO4010	AUDREY	Colorectal cancer (CRC)	OMP17 OMP18 OMP10 OMP11 OMP12	BIRC5(96-104) FOXM1(694-702) UBE2C(124-132) CDC20(170-178) KIF2C(63-71)

Figure 2. General OncoMimics™ discovery pipeline and ongoing phase 1/2 clinical trials with indicated OncoMimics™ peptides. EO2401, EO2463, EO2040 and EO4010 includes synthetically-produced HLA-A*02 peptides with molecular mimicry to antigens indicated in the table above. Targeted indication and name of the study are also indicated.

Experimental design

The capacity of these OMPs to induce TAAps-specific cross-reactive CD8+ T cell responses in humans is evaluated through peptide-MHC multimer staining and flow cytometry-based cytotoxic assays.

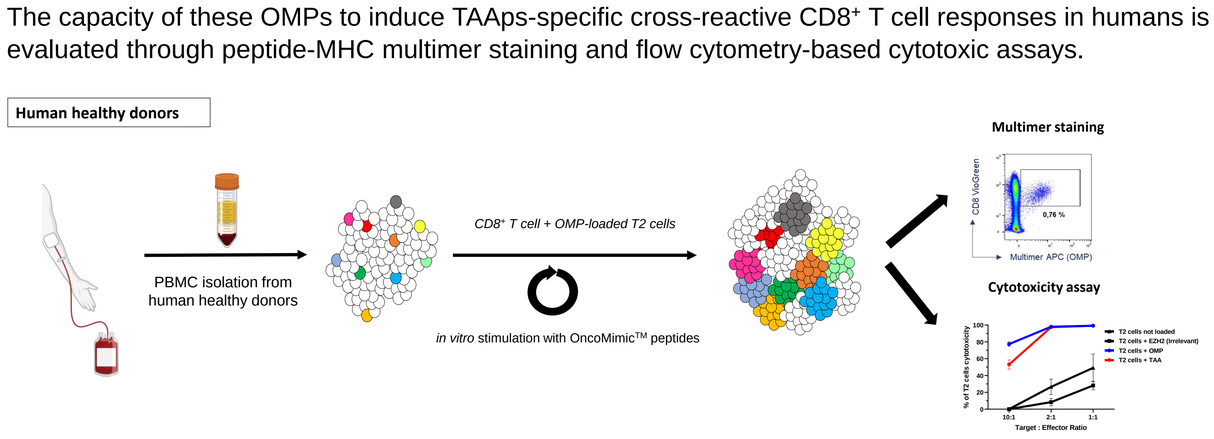


Figure 3. Human PBMCs were isolated from HLA-A*02 healthy donors and expanded with individual OMPs included in EO2401, EO2463 or EO4010 for several rounds of stimulation. For each *In vitro* stimulation (IVS) rounds, OMP-specific CD8+ T cells were co-cultured with OMP-loaded-T2 cells (transporter-deficient HLA-A*02:01 reporter line) at a cell ratio of 10:1 in an ImmunoCult™ medium supplemented with IL-2, IL-7, IL-15 and IL-21 cytokines. IVS rounds were repeated up to four times as described above. The frequency of OMP-specific CD8+ T cells was evaluated by surface staining with anti-CD8 mAb and fluorescently labelled multimers. When OMP-specific CD8+ T cell clone's frequencies and total cell number were suitable, flow cytometry-based cytotoxic assays were performed.

RESULTS

Selected commensal-derived OncoMimics™ peptides induce cross-reactive immune responses against human TAA peptides (TAAps)

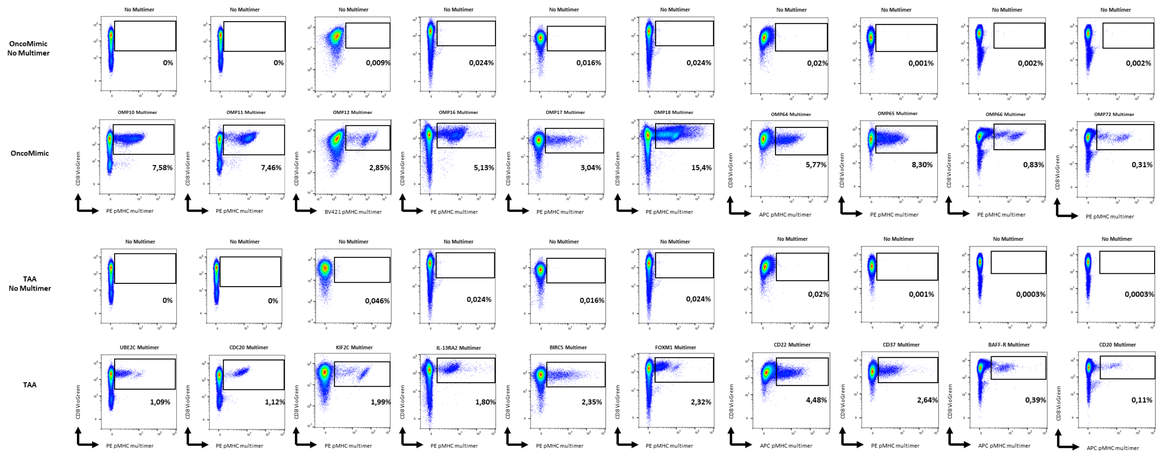


Figure 4. Human CD8+ T cells were stained with multimers loaded with the indicated OMP or TAAp. Frequencies of specific T cells are shown in flow cytometry dot plots. PBMCs were cultured with individual peptides. After several rounds of *in vitro* stimulation, frequencies of OncoMimics™-specific T cell clones and TAAp-cross-reactive clones were assessed by flow cytometry using specific HLA-A*02:01 multimers. A condition without HLA-A*02 multimer was used as negative control to setup the positive HLA-A*02 multimer gate.

OMP-specific CD8+T cells are detected in PBMCs from healthy donors with high prevalence

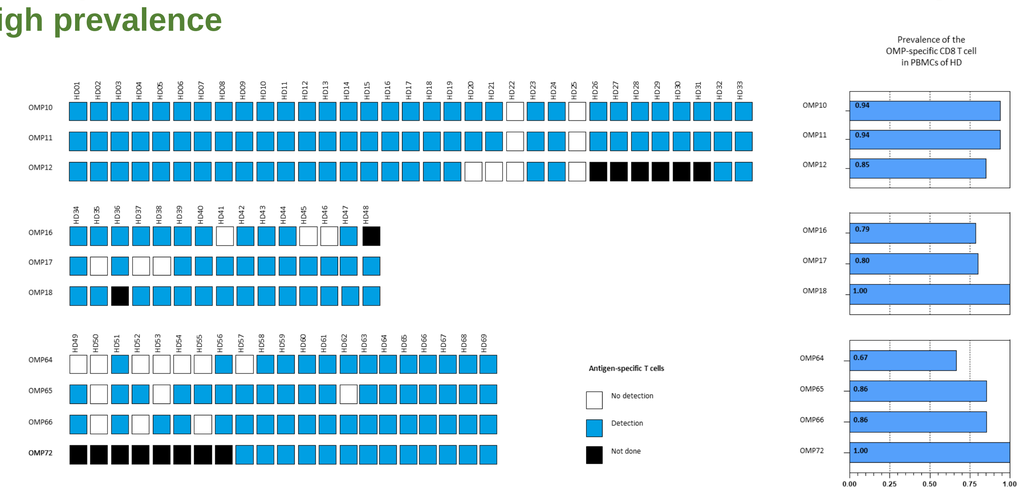


Figure 5. Prevalence of OMP-specific CD8+ T cells within the population. OncoMimic-specific CD8+ T cells were detected in more than 79% of the tested PBMC samples (at least n>14), except for OMP64 with a prevalence at 67%.

Cross-reactive T cells exhibit a cytotoxic response against loaded T2 cells

The capacity of these OMP-specific CD8+ T cells to kill tumor antigen-expressing cells was assessed.

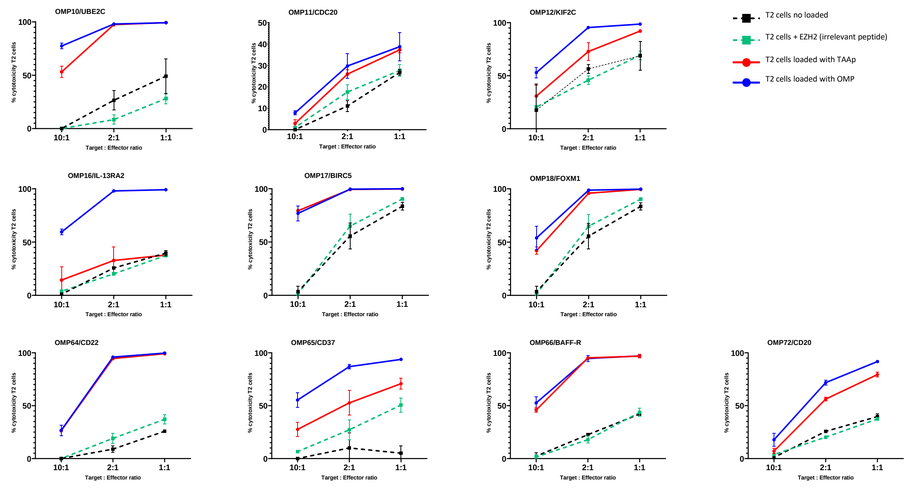


Figure 6. Cytotoxicity assays against T2 cells pulsed with the indicated OMP or TAAp, assessed by flow cytometry. Media and EZH2-B2 irrelevant peptide were used as negative controls. Ag-loaded target cells were co-cultured with Ag-specific CD8+ T cells for 24 hours. Target:effector cell ratio is indicated on graphs. Cytotoxic activity against both OncoMimic and TAA peptide-pulsed T2 cells was observed for all peptides,

CD8+ T cells from a patient treated with EO2463 immunotherapy are cytotoxic and cross-reactive toward TAAp and tumor cell lines

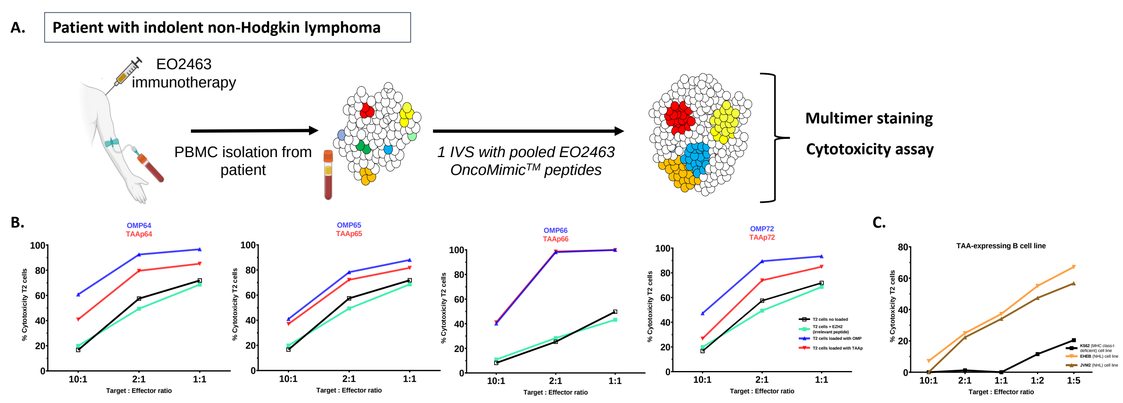


Figure 7. A. HLA-A*02+ patient with indolent non-Hodgkin lymphoma (NHL) received multiple doses of EO2463 therapy in the context of SIDNEY study. PBMCs were isolated from blood drawn from this patient at visit 8 and stimulated with a pool of OMP64,OMP65,OMP66 and OMP72 peptides followed by their expansion with IL-2 for several weeks. OncoMimics™-specific T cells were assessed in a cytotoxicity assay. **B-C.** CD8+ T cells expanded from **A**. were assessed for their cytotoxic function and cross-reactivity against T2 cells pulsed with the indicated peptides or left unpulsed (**B**) or against two full TAA protein-expressing tumor cell lines or an MHC class I deficient cell line (K562 cells) (**C**).

CONCLUSIONS

- ❖ **Validation of the OncoMimics™ proof of concept: commensal-derived peptides sharing a high degree of homology with human tumor-associated antigens can effectively stimulate an anti-tumor immune response.**
- ❖ **OMPs are able to elicit cross-reactive cytotoxic T cell responses against human TAA peptides.**
- ❖ **OMP-specific CD8+ T cells could be detected in the blood of healthy donors (>80% in the population) and show cytotoxic functions against TAA when activated *ex vivo* with OMPs.**
- ❖ **CD8+ T cells obtained from a patient treated with OMP64, OMP65, OMP66 and OMP72 peptides (EONHL1-20/SIDNEY study) showed potent cytotoxic activity against T2 cells loaded with OMPs or TAA peptides and lymphoblastic cell lines expressing full TAA proteins.**

For immune-monitoring data on patients from the EOGBM1-18/ROSALIE study (recurrent Glioblastoma) and the EOADR1-19/SPENCER study (Adrenal tumors), please refer to abstract #638 and abstract #630, respectively.