Poster #1155

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

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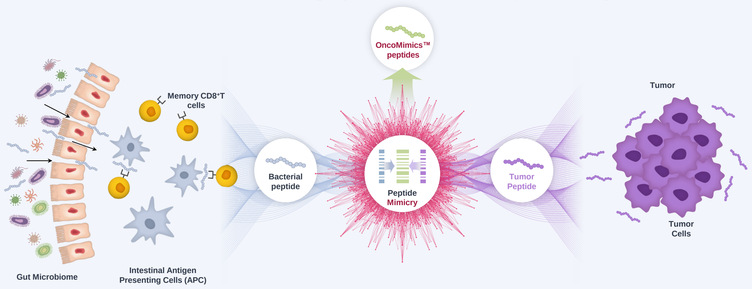
Introduction

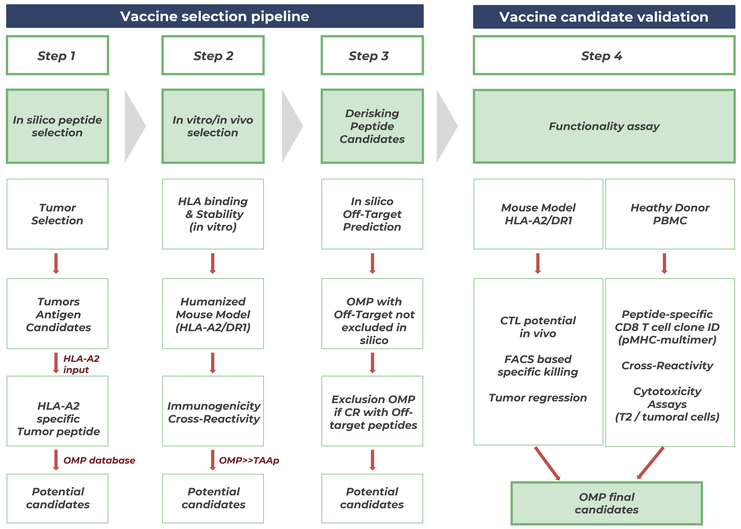
- Peptide-based immunotherapy offers significant potential against cancer by leveraging the body's immune system to eliminate cancer cells, targeting tumor-associated antigens or tumor-specific antigens.
- We present a novel peptide-based immunotherapy relying on the concept of molecular mimicry and cross-reactivity between commensal-derived peptides called **OncoMimics™ peptides (OMPs)** and **tumor-associated antigens-derived peptides (TAAps).**
  - ▸ The aim is to induce strong cytotoxic T cell responses against tumors.



Bacterial Peptide Presentation and T cell Memory Induction → **Proprietary matching algorithm** → **Tumor Peptide Presentation**

- Immune responses induced by OMPs were evaluated in patients from the **EOGBM1-18 ROSALIE clinical trial**, a first-in-human phase Ib/IIa trial in patients with recurrent glioblastoma (NCT04116658).
  - ▸ Patients received an immunotherapy, designated EO2401, comprising three OMPs (OMP16, OMP17 and OMP18) and UCP2.

OMPs discovery: A multi-step process



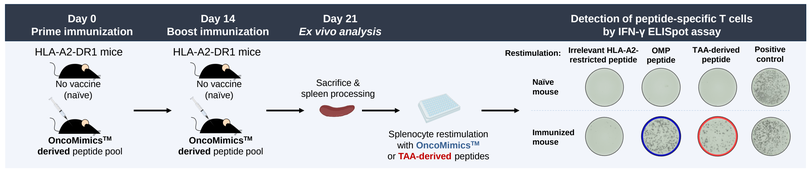
CR: cross-reactivity
CTL: cytotoxic T lymphocyte

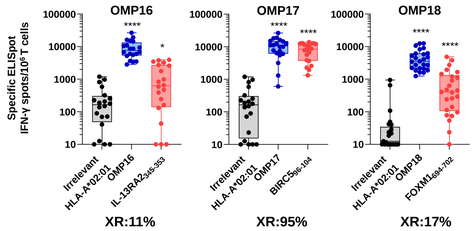
Some illustrations were created with BioRender.com

Results

Selected OMPs are capable of eliciting potent cross-reactive responses in HLA-A2/DR1 mice

OMPs immunogenicity and CD8+ T cell-dependent cross-reactive response against TAAps



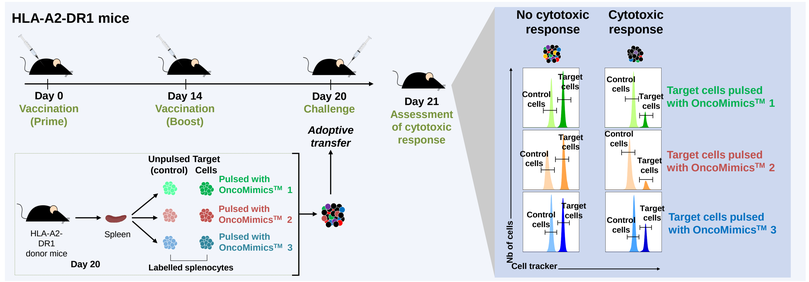


Frequency of peptide-specific T cells that produce IFN-γ was determined using ELISpot analysis of splenocytes from mice vaccinated with the indicated peptides.

Grey Negative control
Blue OMP
Red TAAp
XR Cross-reactivity between the OMP and its TAA counterpart.

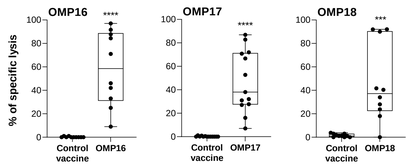
OMPs-based vaccines elicit functional cytotoxic T cells in mice that are cross-reactive against human TAA-derived peptides in vivo

In vivo cytolytic activity of OMP-induced T cells against OMP-pulsed target T cells



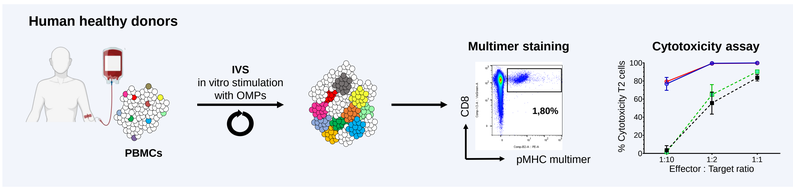
- HLA-A2-DR1 mice were vaccinated with OMPs and challenged post-vaccination with syngeneic splenocytes labeled with cell tracking dye.
- Target T cells were pulsed with OMPs-derived peptides and mixed at equal ratio with unpulsed control cells before being adoptively transferred into immunized mice.
- Frequency of in vivo specific lysis of splenocytes pulsed with the indicated OMPs-derived peptides (challenging peptides) after immunization with the indicated peptide (immunizing peptides).

*** p < 0.001 | **** p < 0.0001



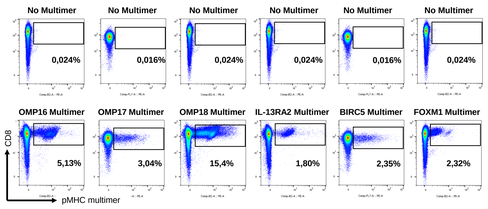
OMP-specific human T cells recognize TAAs and exert specific cytolytic activity

In vitro antigen-specific CD8+ T cell detection in PBMCs from HLA-A2 healthy volunteers



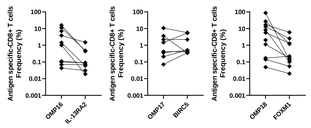
- PBMCs were expanded with OMPs through several rounds of in vitro stimulation (IVS). OMP-specific CD8+ T cells were co-cultured with OMP-loaded-T2 cells (transporter-deficient HLA-A2 reporter line) at a cell ratio of 10:1 in an ImmunoCult™ medium supplemented with IL-2, IL-7, IL-15 and IL-21 cytokines.
- OMP-specific CD8+ T cell frequency was evaluated using OMP-MHC multimer staining, gated on CD8+ T lymphocytes.
- When OMP-specific CD8+ T cell clone's frequencies were suitable, flow cytometry-based cytotoxic assays were performed.

Detection of antigen-specific CD8+ T cell for OMPs and TAAp-derived peptides



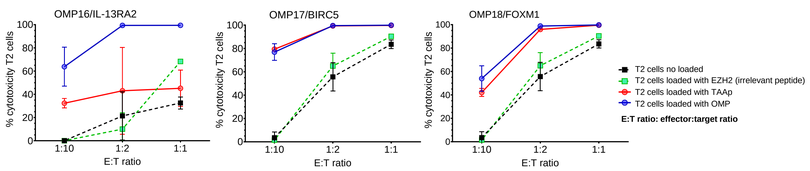
- pMHC multimer stainings were performed with the indicated conjugated HLA-A2 multimer for each OMP and TAAp.
- Conditions without pMHC multimer were used as negative control to define the positive pMHC multimer gate and named no multimer.

Induction of OMP-specific cross-reactive CD8+ T cells against TAAps



- Frequency of antigen-specific CD8+ T cell clones for OMP and TAAp were evaluated after three rounds of IVS with OMPs.
- Each dot represents the frequency obtained for each individual donor (n=7 to 11).

Cytolytic activity of the OMP-specific human CD8+ T cell



- CTL killing activity assessed against T2 cells loaded with the OMP and the TAAp after a 24h incubation.
- Each graph displays representative data from individual healthy donors.
- **Solid lines:** T2 cells loaded with bacterial peptides (OMPs in **blue**) and human derived peptides (TAAp in **red**).
- **Dashed lines:** controls (unloaded in **black**, EZH2-loaded in **green**).

Conclusion

- **Validation of the OncoMimics™ proof of concept:** commensal-derived short peptides mimicking TAAps and eliciting potent CTL responses.
- OMP candidates were validated for their immunogenicity and **ability to elicit cross-reacting TAA-specific CTL responses in HLA-A2-humanized mice.**
- PBMC experiments revealed **efficient cross-reactive OMP-/TAAp-specific CD8+ T cells**, which triggered **cytolytic activity** against target cells presenting homologous TAAs.
- By overcoming current vaccine limitations, OncoMimics™ presents a promising strategy for enhancing cancer immunity and improving patient outcomes.

Presented at EACR 2024

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