Microbiome derived peptides stimulate strong immune response against tumor associated antigens and trigger in vivo tumor regression after vaccination

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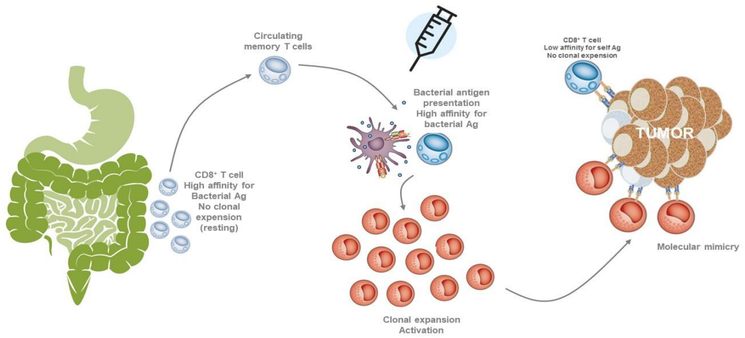
ABSTRACT

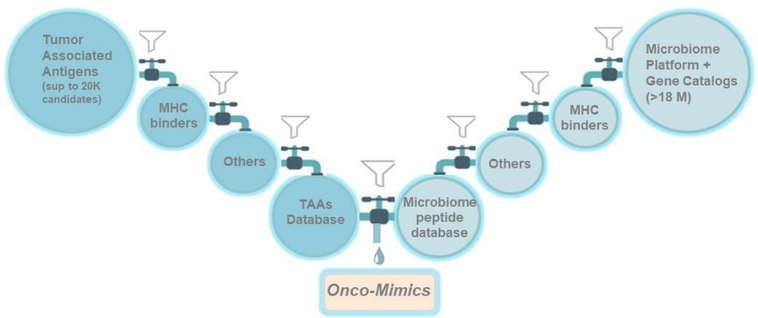
Peptide-based vaccination is an immunotherapeutic approach for the treatment of cancer that aims to deliver immunogenic peptides corresponding to specific tumor associated antigens to patients. The ability of a peptide cancer vaccine to generate a strong immune response depends on several factors including the avidity of the peptide for the MHC complexes, the capacity of the antigen to be recognized by the immune system as self or non-self and the pre-existence of T cell clones (naïve or memory) that are able to recognize those peptides. Gut colonization is one of the factor that drive the development of T cells. The presence of commensal specific memory T-cell in the gut and in the periphery is now currently admitted. Several evidences support that these T cells can be re-activated and can migrate to inflammatory sites where the antigen is expressed. We have designed an innovative, microbiome-based approach for the development of therapeutic peptide cancer vaccines.



By developing a dedicated in silico pipeline, we were able to identify bacterial antigens that could elicit strong immune response in WT mice. These bacterial antigens could be selected to generate T cell reactivity against self-peptides that are by themselves not immunogenic. The same pipeline was used to identify bacterial antigens able to bind human HLA-A2 with high affinity and displaying molecular mimicry with selected tumor associated antigens. While HLA-A2 transgenic mice vaccination with tumor associated antigens doesn't lead to immune response induction, vaccination with bacterial antigens results in a strong immune response against both bacterial peptides and selected tumor associated antigens. Furthermore, adoptive transfer of T cell from mice immunized with bacterial peptides into tumor engrafted nude mice leads to tumor control in the presence of Check Point Inhibitors.

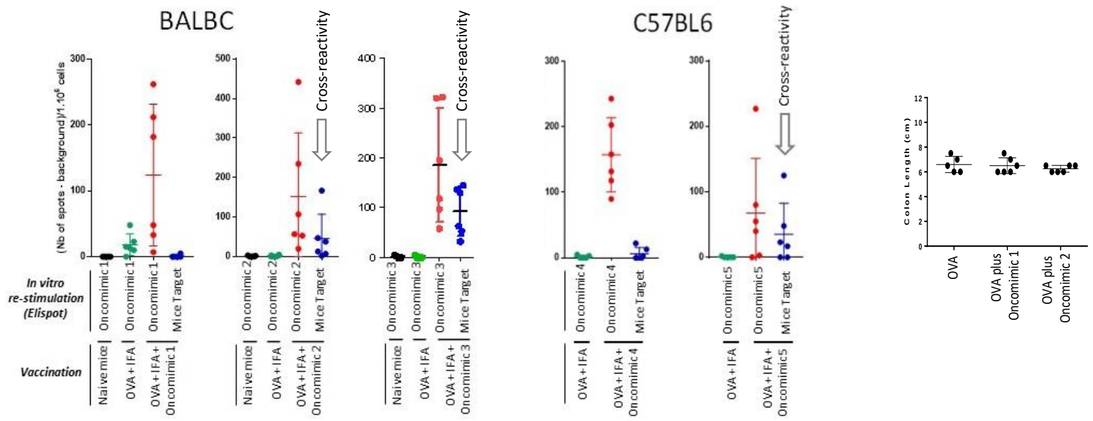
METHODS

Our approach is based on the notion of molecular mimicry, by which a microbial derived peptide holding molecular similarity with a tumor-associated epitope would trigger a tumor-specific cytotoxic T cell immune response. By using a dedicated in silico pipeline, we are able to identify bacterial antigens that could elicit strong immune response in mice.



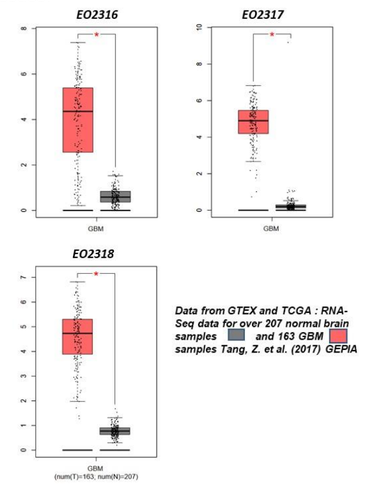
RESULTS

- The pipeline used for identification of Oncomimics allows selection of qualitatively strong immunogenic mimics and generation of immunity against WT mice self epitopes (cross reactivity)
- Immunization of WT mice with commensal antigens is not associated with gut inflammation (either when used in combination with CPI)
- Efficient immunisation lead to CD8 T cell infiltration in tumor and reduce tumor size in a syngenic CT26 tumor model



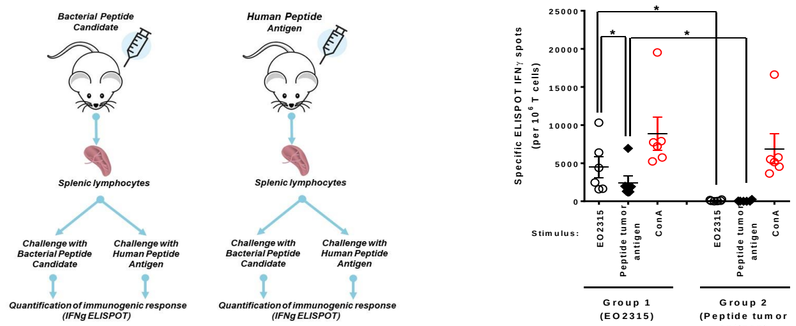
A / BALB/c or C57Bl6 mice were immunized with ovalbumin (OVA) helper peptide and the mouse bacterial peptides (Oncomimics). The animals were immunized twice on day 0 and on day 14 with by s.c. administration at tail base. Seven days after the last injection the animals were euthanized and the spleens were harvested. Splenocytes were prepared and stimulated *in vitro* with the different bacterial peptides and mice homologs to assess their capacity to secrete IFN-γ (ELISpot data). B / Colon were collected for analysis of weight, length and colitis score.

- EO2316, EO2317 and EO2318 are Oncomimics designed to elicit cross reactivity against 3 different driver antigens overexpressed in Glioblastoma and not expressed in normal tissues.



Data from GTEX and TCGA : RNA-Seq data for over 207 normal brain samples and 163 GBM samples Tang, Z. et al. (2017) GEPIA

- Oncomimics designed to target human TAAs induce strong immunogenic response in HLA-A.02 humanized mice and generate immunity against human epitopes

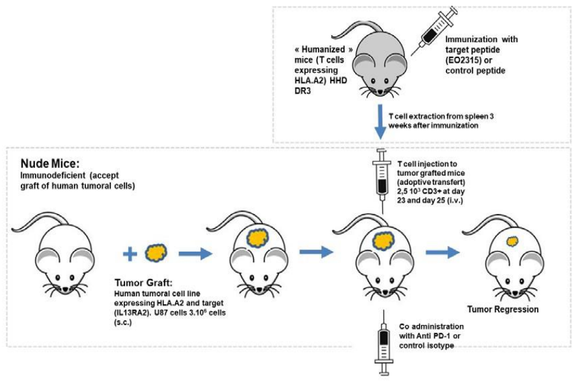


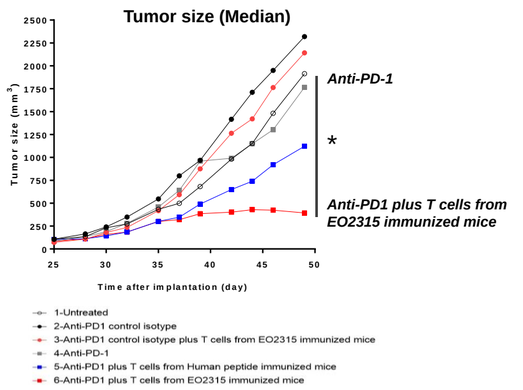
HHD-DR3 Mice were immunized with either the candidate Oncomimic peptide or the corresponding human peptide displaying lower affinity for HLA-A2 than the candidate peptide. The peptides were combined with a common helper peptide and were adjuvanted with incomplete Freund's adjuvant (IFA). The animals were vaccinated twice at 2-week intervals, starting with a prime injection on day 0, followed by a boost injection on day 14. On day 21, the mice were euthanized and the spleens were harvested. Splenocytes were prepared and stimulated in vitro with either the candidate Oncomimics peptide or the human peptide to assess their capacity to secrete IFN-γ as assessed by ELISpot.
Note: HHD-DR3 were developped at Pasteur Institute by F Lemonier team (Koller et al., 1990; Cosgrove et al., 1991 ; Pascolo et al., 1997 ; Kong et al., 1996)

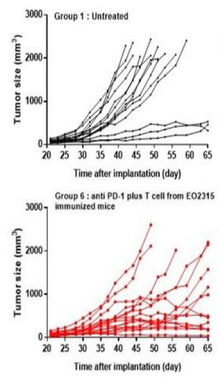
EO2316, EO2317 and EO2318 Oncomimics demonstrate strong immunogenic response in HLA-A.02 humanized mice and cross reactivity against 3 different driver antigens

HHD-DR1 Mice were immunized with the candidate Oncomimic plus a common helper peptide adjuvanted with IFA. The animals were vaccinated twice at 2-week intervals, starting with a prime injection on day 0, followed by a boost injection on day 14. On day 21, the mice were euthanized and the spleens were harvested. Splenocytes were prepared and stimulated in vitro with either the candidate Oncomimics peptide or the human TAA to assess their capacity to secrete IFN-γ as assessed by ELISpot.
Note: HHD-DR1 were developped at Pasteur Institute by F Lemonier team ((Koller et al., 1990; Cosgrove et al., 1991; Pascolo et al., 1997 ; Altmann et al., 1995)

- When used in combination with CPI, Oncomimic controls tumor growth significantly better than CPI alone







To demonstrate the in vivo anti-tumor effect of T cells from bacterial peptide immunized mice, adoptive transfer of T cells in nude mice engrafted with U-87MG cells was used. Immunocompromised nude mice were injected subcutaneously with U-87MG. T cells for adoptive transfer were obtained from HHD DR3 immunized mice. Mice were injected on days 0 and 14 with EO2315, helper peptide (DR3) and IFA. On day 21, the mice were euthanized, spleen were harvested and CD3+ T cells from the spleen were collected for adoptive transfer in tumor engrafted mice (2 transfers of approximately 2.5x10e6 cells were done by intravenous injection at 48 hours apart).

CONCLUSIONS

Many studies are supporting the link between microbiome, clinical response and inhibition of cancer progression in cancer patients treated with targeted immunotherapies or with specific chemotherapeutic agents. Proteins/antigens produced by commensal bacteria could mimic tumoral antigens, and drive expansion of a significant pool of reactive cytotoxic and helper T-cells directed against tumoral cells. Enterome is developing an innovative, microbiome-based approach for the development of therapeutic multipeptide cancer vaccine. Our method has demonstrated the possibility to identify commensal antigen eliciting strong immune response and to generate immunity against human epitopes that are by themselves poorly immunogenic. Our method allows to identify first clinical candidates for Glioblastoma (GBM). A first vaccine candidate targeting three different tumor drivers overexpressed in Glioblastoma and other solid tumors will enter the clinic this year. This multicenter, open-label, FIH in patients with progressive or recurrent GBM will primary evaluate safety and tolerability of our first vaccine candiate as monotherapy and in combination with a CPI. Secondary objective will include tumor progression by MRI assessment and immunogenicity.