ABSTRACT #641 : Strong immune response to therapeutic vaccination EO2401 microbiome-derived vaccine + nivolumab

Interim report of the EOGBM1-18/ROSALIE study

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BACKGROUND

Recurrent glioblastoma (GB) has a poor prognosis and a limited number of treatment options, with an expected median survival of approximately 8-9 months in trials with longer follow-up[1].

EO2401 is a therapeutic vaccine designed to activate commensal specific T cells that are cross-reacting against validated tumor-associated antigens (TAAs). EO2401 includes synthetically-produced HLA-A2 peptides with molecular mimicry to antigens (IL13Rα2, BIRC5 and FOXM1) upregulated in GB, and the CD4-helper peptide UCP2 (**Figure 1**).

Microbiome-mimicry – utilizing non-self-microbiome-derived peptides mimicking TAAs to expand pre-existing commensal memory T cells cross-reacting with the selected TAAs.

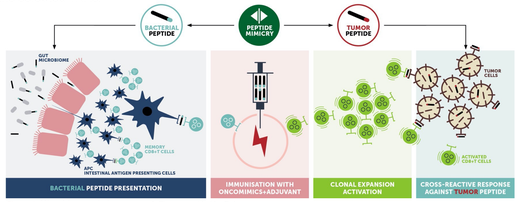
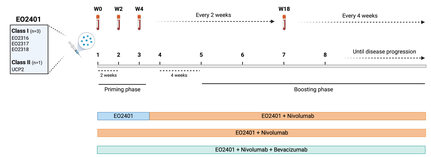


Figure 1. Microbiome-mimicry concept. Figure provided by Enterome.

METHODS

Clinical trial overview – The ongoing phase 1/2 trial (EOGBM1-18, NCT04116658) investigates EO2401 (300 µg/peptide, 3 HLA-class I binding peptides and 1 HLA-class II binding peptide q2w x4 then q4w) with nivolumab (3 mg/kg q2w) or with nivolumab and bevacizumab (10 mg/kg q2w) in patients with GB at first progression/recurrence after surgery and adjuvant radiotherapy/temozolomide. Three patient cohorts are included in the trial (**Figure 2**).



Treatment Cohort	Study part 1	Monitored
Cohort 1 (E→EN) (measurable disease)	3 patients	3 patients
Cohort 2a (EN) (measurable disease)	23 patients	16 patients
Cohort 2b (EN) (non-measurable disease)	3 patients	2 patients
Cohort 3 (ENB) (measurable disease)	11 patients	9 patients

Figure 2. Layout of trial regimens. Left panel shows the three patient cohorts included in the study (Figure created using Biorender.com). Patients from cohorts 1 and 2 receive 4 doses of EO2401 followed by EO2401 + Nivolumab or simultaneous administration of both compounds from the first vaccination on, respectively. Cohort 3 receives in addition Bevacizumab due to its anti-edema properties and for counteraction of VEGF immuno-suppressive activity. Time of blood collection for peripheral blood mononuclear cells (PBMCs) isolation is shown. Table on right panel shows the number of patients included in the different cohorts in the first phase of the study and how many have been monitored so far.

Immuno-monitoring overview – Immune response in cryopreserved PBMCs was investigated either ex vivo or after in vitro antigen-specific stimulation (IVASS) using **tetramer staining**, **IFNγ ELISpot** and **intracellular cytokine staining (ICS)** (**Figure 3**).

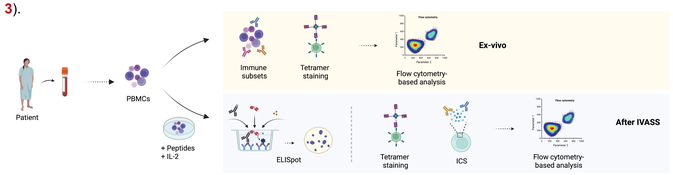


Figure 3. Immuno-monitoring schematics. Isolated and cryopreserved PBMCs are thawed and ex-vivo tetramer staining and immune subset analysis (data not shown) are performed. Rest of PBMCs are cultured in the presence of EO2401 peptides and IL-2 (class I and II separately) for 12 days. Cells are then collected and IFNγ ELIspot, ICS and tetramer staining are performed. Figure created using Biorender.com

RESULTS

Generation of antigen vaccine-specific CD8⁺ T cells that crossreact with TAAs

Tetramer staining demonstrates the ability of microbiome-derived peptides to induce a fast, durable and strong response of vaccine-specific CD8⁺ T cells that cross-react with their specific TAAs.

Ex-vivo analysis – Antigen-specific CD8⁺ T cells against at least one of the 3 microbiome-derived peptides can be detected ex-vivo for 26 out of 28 patients tested (maximum response: EO2316 – 0.24%, EO2317 – 6.3%, EO2318 – 3.6%). CD8⁺ T cells against TAAs BIRC5 and FOXM1 (maximum response of 4% and 0.3%, respectively are seen after vaccination with EO2401 (**Figure 4A**). Most generated vaccine-specific CD8⁺ T cells are either effector memory (EM) or terminally differentiated effector memory (EMRA) based on CCR7 and CD45RA staining (**Figure 4B**).

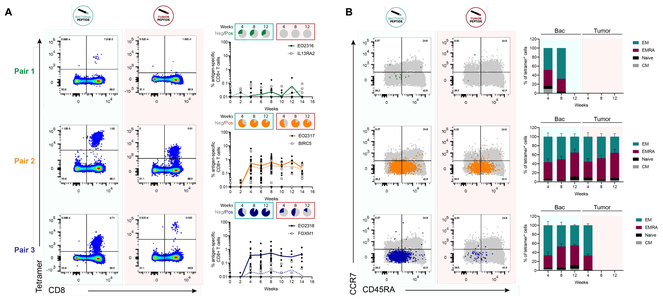


Figure 4. Antigen-specific CD8⁺ T cells detected ex-vivo. A) Representative dot plots for ex-vivo tetramer staining for the bacterial and tumor peptides (left and right, respectively). The three HLA-class I peptides are shown as Pair 1, 2 and 3 which represent EO2316, EO2317 and EO2318 and their TAAs, respectively. Quantification of antigen-specific CD8⁺ T cells is shown on the right. On top of graphs, pie charts showing the % of patients positive for the respective antigen at week 4 (2 vaccinations, n=24), week 8 (4 vaccinations, n=21) and week 12 (5 vaccinations, n=6) are depicted. Values of 0 were transformed to 0.01 to fit log scale. Vaccine-specific CD8⁺ T cells were not detected prior to vaccination, except for one patient in which CD8⁺ T cells against EO2317 were seen (data not shown) **B)** Phenotype of generated vaccine-specific CD8⁺ T cells. Patients with >20 tetramer-positive events were included in analysis. Representative dot plots are shown on the left and quantification is shown on the right.

IVASS analysis – In vitro stimulation with vaccine peptides induces a strong expansion of bacterial-specific CD8⁺ T (maximum responses: EO2316 – 18%, EO2317 – 46%, EO2318 – 40%) that often recognise their homologous TAAs (maximum responses: IL13Rα2 – 0.1%, BIRC5 – 34%, FOXM1 – 6%). Antigen-specific CD8⁺ T cells against at least one of the 3 microbiome-derived peptides can be detected after IVASS for 30 out of 31 patients tested. Strong cross-reactivity, particularly for BIRC5 (approximately 50%) is seen (**Figure 5A**). Detected TAA-specific CD8⁺ T cells bind the respective bacterial peptide (**Figure 5B**).

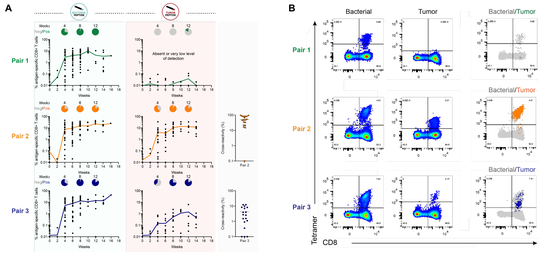


Figure 5. IVASS leads to strong expansion of vaccine-specific CD8⁺ T cells that crossreact with TAAs. A) Quantification of antigen-specific CD8⁺ T cells. Pair 1 (EO2316/ IL13Rα2), pair 2 (EO2317/BIRC5) and pair 3 (EO2318/FOXM1) are shown on the top, middle and lower row, respectively. On top of the graphs, pie charts showing the % of patients responding to the respective antigen at week 4 (n=28 for bacterial and n=16 for tumor), week 8 (n=24 for bacterial and n=12 for tumor) and week 12 (n=6 for both) are depicted. Values of 0 were transformed to 0.01 to fit log scale. Cross-reactivity (ratio of TAA- to bacterial- specific CD8⁺T cells frequencies x 100 (%)) is shown of the right for each pair. **B)** Representative dot plots for combinatorial tetramer staining with both bacterial and tumor peptides together. Tumor-specific CD8⁺ T cells were overlapped with bacterial-specific CD8⁺ T cells to show cross-reactivity. Overlapping human-specific CD8⁺ T cells are shown in color in the dotplots.

Vaccine-specific T cells produce high levels of cytokines and are multifunctional

T cells stimulated with vaccine peptides produce high levels of activation markers, namely CD154, the cytokines IL-2, IFNγ and TNF, and the degranulation marker CD107a. Production of multiple markers is observed upon stimulation indicating a strong response against both HLA-class I and HLA-class II peptides.

Cytokine production to vaccine peptides – ICS and IFNγ ELISpot were used to investigate the capacity of T cells to produce cytokines upon stimulation with vaccine peptides – HLA-class I (**Figure 6**) and HLA-class II (**Figure 7**) – after IVASS. Reactivity against pairs 2 and 3 is frequently detected. Strong responses against TAAs, particularly BIRC5, are observed. Pre-existing reactivity against vaccine peptides is seen in some patients (**Figure 6** and **7**).

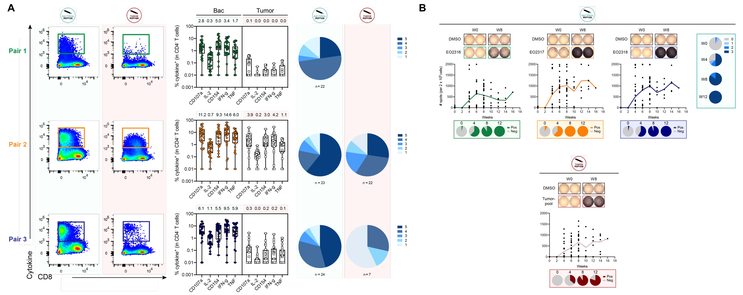


Figure 6. Antigen-specific CD8⁺ T cells produce high levels of activation markers. A) Representative dot plots for one cytokine (IFNγ) are shown on the left panel after o/n stimulation with bacterial or human peptide for all three pairs. Quantification of the % of marker positive CD8⁺ T cells is shown in the middle panel for CD107a, IL-2, CD154, IFNγ and TNF (n=25), with mean values being shown on the top of each marker. Values of 0 were transformed to 0.01 to fit log scale. Pie charts on the right panel show the fraction of patients producing 1, 2, 3, 4 or 5 markers upon stimulation. **B)** Representative IFNγ ELISpot wells are shown on top of respective graphs at week 0 and 8 for negative control (DMSO) and peptide. Tumor peptides are tested as a pool (tumor-pool). Graphs depic the number of spots obtained per 2 x 10^5 cells seeded for EO2316 (left), EO2317 (middle), EO2318 (right) and tumor-pool (lower row). Pie charts below the graphs show the % of responding and non-responding patients prior to vaccination (0) (n=25) and after vaccination (weeks 4 (n=24), 8 (n=20), 12 (n=5)). For bacterial peptides, % of patients that react to 0, 1, 2 or 3 peptides at different timepoints (week 0, 4, 8, 12) is quantified on pie charts on the right.

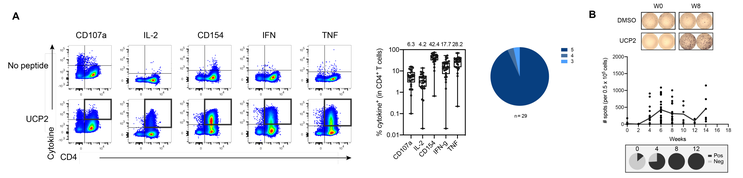


Figure 7. Strong CD4⁺ T cell reactivity is induced against HLA-class II peptide (UCP2) by vaccination. A) Representative dot plots for all activation markers are shown on the left panel after incubation with negative control (No peptide, DMSO) or with HLA-class II peptide (UCP2). Quantification of the % of marker positive CD4⁺ T cells (at peak) is shown in the middle panel for CD107a, IL-2, CD154, IFNγ and TNF (n=30), with mean values being shown on the top of each marker. Pie chart on the right panel shows the fraction of patients producing 1, 2, 3, 4 or 5 markers upon stimulation. **B)** Representative IFNγ ELISpot wells are shown on top of graph at week 0 and 8 for negative control (DMSO) and peptide. Graphs depic quantification of number of spots obtained per 0.5 x 10^5 cells seeded. Pie charts below the graphs show % of responding and non-responding patients prior to vaccination (0) (n=29) and after vaccination (weeks 4 (n=27), 8 (n=24), 12 (n=9)).

CONCLUSIONS

- **EO2401 generates fast (earliest responses after only 1 vaccination) and durable immune responses in patients from the EOGBM1-18/ROSALIE study**
- **Pre-existing bacterial-specific T cells that cross-react with TAA can be seen in few patients after IVASS**
- **Very strong responses against all three EO2401 bacterial peptides (strongest response against EO2317 and 18) and HLA-class II peptide (UCP2)**
- **Crossreactivity against BIRC5 and FOXM1 seen in the majority of patients. Low detection against IL13Rα2 using current in vitro setting**
- **For clinical course please check abstract #642 (Oral presentation)**

References: Wick W, Gorlia T, Bendszus *et al*., Lomustine and Bevacizumab in Progressive Glioblastoma. N Engl J Med. 2017 Nov 16;377(20):1954-1963

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