Profiling of the neoantigen-specific T cell response after adjuvant TG4050 individualized therapeutic vaccination in a randomized phase I trial for locally advanced resected HPV-negative HNSCC

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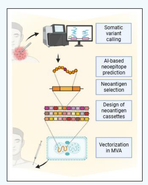
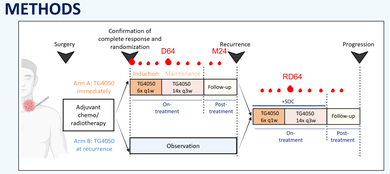
BACKGROUND



- TG4050 is an individualized vaccine including up to 30 patient-specific neoantigens predicted from sequencing of patient's tumor material vectorized in modified vaccinia Ankara (MVA) viral vector
- TG4050 is expected to induce neoantigen-specific CD8 T cell responses which eliminate residual tumor cells and prevent recurrence

- TG4050 was evaluated as adjuvant monotherapy in a randomized phase I clinical trial in locally advanced HNSCC, in patients with a complete response after surgery followed by (chemo-)radiation therapy (NCT04183166)
- Here, we present an analysis of the exploratory objective of the study: in-depth profiling of the neoantigen-specific T cell response to treatment

METHODS



- Apheresis was performed for collection of PBMC at Baseline and after 7 doses of TG4050 (D64). Additional longitudinal blood samples were collected up to one year after end of treatment (M24)
- T cell responses to vaccine neoantigens were quantified by *ex vivo* IFN-γ ELISpot and pMHC class I tetramer analyses using PBMCs
- Neoantigen-specific CD8 T cell phenotype and transcriptional signatures at D64 were analyzed by flow cytometry and scRNAseq of pMHC class I tetramer-sorted cells. MVA, CMV and influenza-specific tetramers were used as comparators for HLA-A*02:01 patients
- Bulk TCR DNA sequencing was performed on tumor at surgery and blood at baseline and at D64

STUDY POPULATION

- 33 patients were randomized: 17 in Arm A were treated with TG4050 immediately and 16 in Arm B were kept on observation until recurrence. One patient in Arm A was not evaluable for efficacy
- After a median follow-up of 30 months, none of the 16 evaluable Arm A patients have relapsed while 3 of the 16 Arm B patients have relapsed, two of which could be treated with TG4050 at recurrence
- The DFS rate at 2 years is 100% in Arm A and 81% in Arm B with a log-rank one-sided p value = 0.0367

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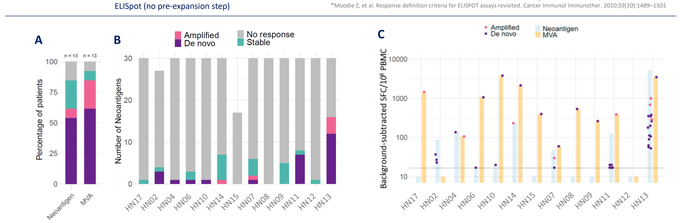
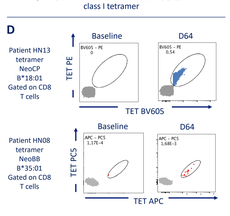
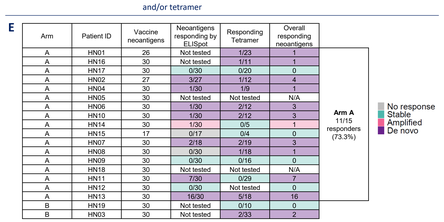
① POLYEPITOPIC RESPONSES TO VACCINE NEOANTIGENS ARE DETECTED IN THE BLOOD OF PATIENTS TREATED WITH TG4050







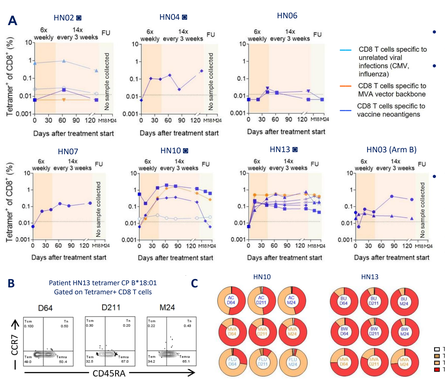
Arm	Patient ID	Vaccine neoantigens	Neoantigens responding by ELISpot	Responding Tetramer	Overall responding neoantigens
A	HN01	26	Not tested	1/23	1
A	HN16	30	Not tested	1/11	1
A	HN17	30	0/30	0/20	0
A	HN02	27	3/27	1/12	4
A	HN04	30	1/30	1/9	1
A	HN05	30	Not tested	Not tested	N/A
A	HN06	30	1/30	2/12	3
A	HN10	30	1/30	2/12	3
A	HN14	30	1/30	0/5	1
A	HN15	17	0/17	0/4	0
A	HN07	30	2/18	2/19	3
A	HN08	30	0/30	1/18	1
A	HN09	30	0/30	0/16	0
A	HN18	30	Not tested	Not tested	N/A
A	HN11	30	7/30	0/29	7
A	HN12	30	0/30	Not tested	0
A	HN13	30	16/30	5/18	16
B	HN19	30	Not tested	0/10	0
B	HN03	30	Not tested	2/33	2

Figure 1. Circulating T cell responses to vaccine neoantigens at D64. A-C, *Ex vivo* IFN-γ ELISpot for 362 individual neoantigens and the MVA peptide pool in 13 Arm A patients. **A,** Frequency of patients with responses to at least one neoantigen and to the MVA peptide pool. **B,** Number of responding neoantigens for each patient. **C,** Magnitude of the response to TG4050 treatment (de novo or amplified) for neoantigens (blue, sum of all responding neoantigens, each response is one dot) and MVA (orange) as background-subtracted SFC/10^6 PBMC. Values below the cut-off of 17 SFC/10^6 PBMC are displayed as 10 SFC/10^6 PBMC **D,** Representative peptide MHC class I stainings at baseline vs D64, gated on live CD8 T cells. **E,** Overall neoantigen-specific responses detected at D64 by ELISpot and/or tetramer. Color coding as in b, based on best response for each patient.

- Responses to treatment (defined as *de novo* or amplified response from baseline to D64 for at least one neoantigen) detected in 8 / 13 Arm A patients (61.5%), 7/8 of which (87.5%) showed at least one *de novo* response
- In responders, the number of responding neoantigens ranged from 1 to 16 per patient (median = 2)
- Vector immunogenicity did not preclude or blunt responses to treatment, as evidenced by the detection of responses to neoantigens even in patients with pre-existing or ongoing T cell responses to MVA antigens

- Peptide-MHC class I tetramers were used to test selected predicted CD8 T cell neoepitopes (2 to 34 combinations per patient) by flow cytometry on baseline vs D64 PBMCs
- Responses were detected in 9/14 (64.3%) Arm A patients and 1/2 Arm B patients
- Overall, neoantigen-specific T cell responses were found in 11/15 (73.3%) Arm A patients by *ex vivo* IFN-γ ELISpot and/or tetramer, with a median of 3 responding neoantigens in responders

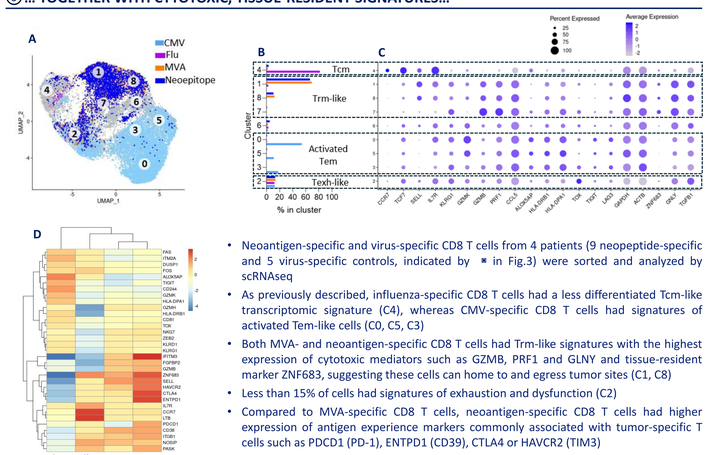
② NEOANTIGEN-SPECIFIC CD8 T CELLS ARE LONG-LASTING AND HAVE AN EFFECTOR PHENOTYPE…



- Neoantigen-specific CD8 T cells persisted over one year after the end of treatment
- Neoantigen-specific CD8 T cells had an effector phenotype (Tem or Temra). The phenotype was comparable to that of MVA-specific CD8 T cells but tended to be more differentiated than that of influenza-specific CD8 T cells in the same patient
- The distribution of Tem and Temra varied between patients and between different epitopes in the same patient. However, for a given neoepitope the phenotype was remarkably conserved throughout treatment and up to one year after the end of treatment (M24)

Figure 2. Dynamics and phenotype of neoantigen-specific CD8 T cells. A, Longitudinal pMHC-I tetramer study. Tetramers for MVA and treatment-unrelated viral epitopes (CMV and flu) were used as controls for HLA-A*02 patients. All patients with reactive neoantigen tetramers > 5 / 40,000 CD8 T cells at at least one time-point are reported. Time-points with frequencies < 5 / 40,000 (0.017% of CD8 T cells) are displayed as half this value.
B-C, Phenotyping of neopeptide-specific Tetramer+ CD8 T cells.
B, Representative gating of Tn, Tcm, Tem and Temra based on CCR7 and CD45RA expression. **C,** Distribution of phenotype in longitudinal samples for representative tetramers from 2 Arm A patients. AC, BU and BW are neoantigen-specific tetramers, MVA and flu are shown for comparison.

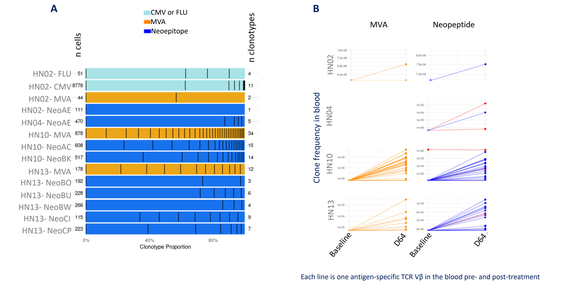
③ … TOGETHER WITH CYTOTOXIC, TISSUE-RESIDENT SIGNATURES…



- Neoantigen-specific and virus-specific CD8 T cells from 4 patients (9 neopeptide-specific and 5 virus-specific controls, indicated by ✖ in Fig.3) were sorted and analyzed by scRNAseq
- As previously described, influenza-specific CD8 T cells had a less differentiated Tcm-like transcriptomic signature (C4), whereas CMV-specific CD8 T cells had signatures of activated Tem-like cells (C0, C5, C3)
- Both MVA- and neoantigen-specific CD8 T cells had Trm-like signatures with the highest expression of cytotoxic mediators such as GZMB, PRF1 and GLNY and tissue-resident marker ZNF683, suggesting these cells can home to and egress tumor sites (C1, C8)
- Less than 15% of cells had signatures of exhaustion and dysfunction (C2)
- Compared to MVA-specific CD8 T cells, neoantigen-specific CD8 T cells had higher expression of antigen experience markers commonly associated with tumor-specific T cells such as PDCD1 (PD-1), ENTPD1 (CD39), CTLA4 or HAVCR2 (TIM3)

Figure 3. Transcriptomic signatures of neoantigen-specific CD8 T cells. A, UMAP and cluster analysis with populations overlayed according to antigen specificity. **B**, Distribution of antigen-specific CD8 T cells in clusters. **C**, Cluster annotation based on expression levels of selected lineage markers.
D, Gene expression levels between CD8 T cells specific for CMV, Flu, MVA and neopeptides. A curated gene list based on association with antigen-experienced T cells is shown.

④ … AND CAN BE EXPANDED FROM PRE-EXISTING TUMOR INFILTRATING T CELL CLONES



- Clonality of Tetramer+ CD8 T cells was analyzed by scRNAseq. All except one neoantigen-specific CD8 T cell responses were polyclonal, with a median of 6 clonotypes identified per neoepitope (1-19)
- Bulk TCR Vβ sequencing confirmed expansion of neoantigen-specific T cell clones in the blood between baseline and D64.
- In 3 of the 4 patients analyzed, at least one of the neoepitope-specific clones expanded after treatment was present in the tumor before treatment.

Figure 4. Clonality of neoantigen-specific CD8 T cells. A, Total number and relative proportion of clonotypes found for each patient and epitope by scRNAseq. **B,** Bulk TCR Vβ DNA sequencing was performed in the blood and in the resected tumor. The evolution of frequency of clones matched to Tetramer+ CD8 T cells Vβ within all T cell clones in the blood, at Baseline and D64, is shown. T cell clones also found in the resected tumor by bulk TCR DNA sequencing are highlighted in red.

KEY MESSAGES

- Polyepitopic responses to vaccine neoantigens were detected by *ex vivo* IFN-γ ELISpot or pMHC class I tetramer analyses in the blood of treated patients.
- These responses were maintained throughout treatment and persisted for over one year after the end of treatment.
- Vaccine neoantigen-specific CD8 T cells had an effector phenotype at all time-points analyzed, even after the end of treatment. At the transcriptomic level, neoantigen-specific CD8 T cells resembled cells specific for the MVA vector. Both cell populations displayed higher expression of cytotoxic markers (GZMB, PRF1, GLNY) and markers associated with tissue-resident phenotype (ZNF683, CX3CR1) than CD8 T cells specific for CMV or influenza.
- Neoantigen-specific CD8 T cell had higher levels of markers associated with chronic antigen stimulation and tumor experience (transcripts encoding for PD-1, CD39, CTLA4, TIM3)
- TCR repertoire analysis showed that vaccine neoantigen-specific CD8 T cell responses were polyclonal and comprised both *de novo* responses and expansion of tumor-infiltrating T cell clones.

CONCLUSION: Together, this translational data is consistent with the model in which TG4050 induces tumor neoantigen-specific cytotoxic T cell responses that prevent tumor recurrence.