

CORRECTION

Correction: Profiling HPV-16-specific T cell responses reveals broad antigen reactivities in oropharyngeal cancer patients

Kunal H. Bhatt, Michelle A. Neller, Sriganesh Srihari, Pauline Crooks, Lea Lekieffre, Blake T. Aftab, Howard Liu, Corey Smith, Liz Kenny, Sandro Porceddu, and Rajiv Khanna

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The authors regret that in the original version of Fig. S1 B ii, the x-axis labels E5-5, E5-6, E5-7, E5-8, and E5-9 were accidentally written as E5-6, E5-7, E5-8, E5-9, and E5-10. In addition, “45 h” was corrected to “4 h” in the Fig. S1 C i legend, as indicated in bold and underline. The corrected Fig. S1 and its legend are shown here. The errors appear in PDFs downloaded before October 18, 2022.

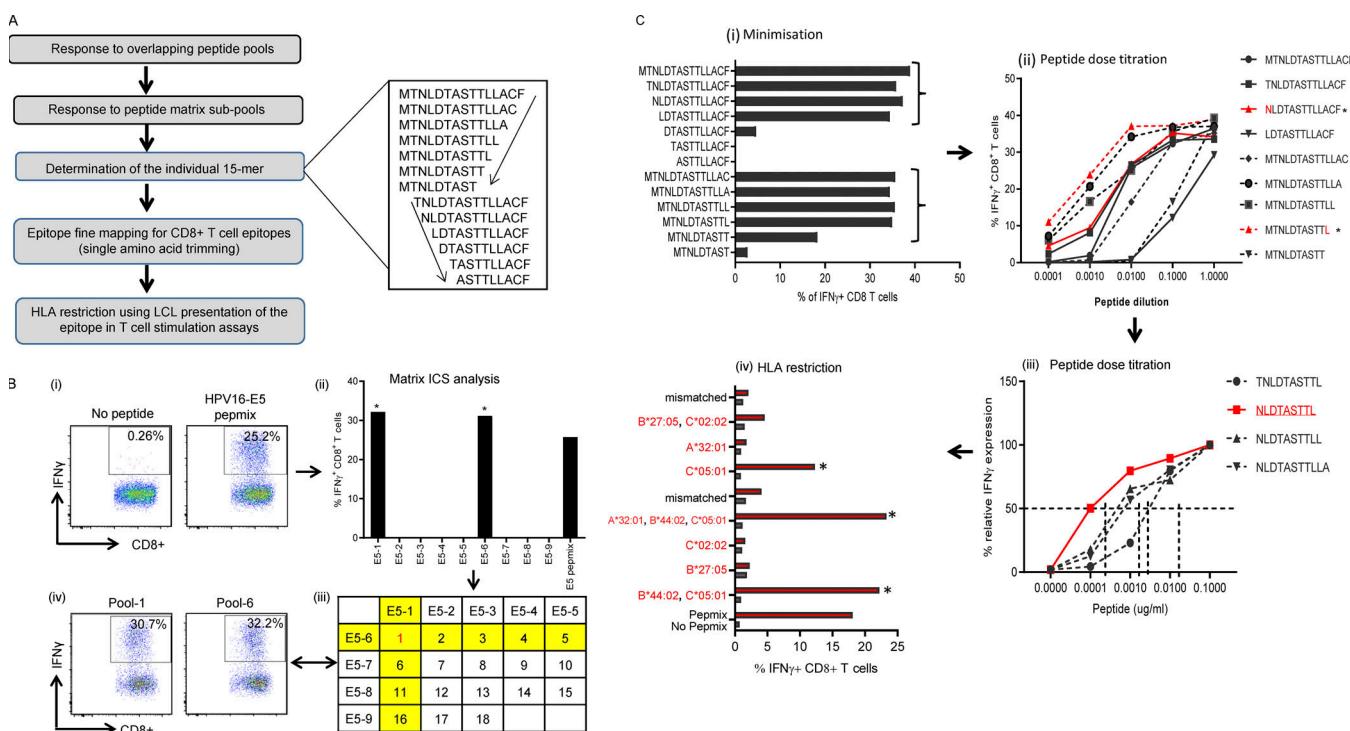


Figure S1. Mapping of HPV-16 epitopes recognized by CD8+ T cells from OPC patients. (A) Overview of the process for mapping HPV epitopes recognized by T cells from OPC patients. An example of the trimming of a 15-amino acid sequence to define the minimal T cell epitope is shown to the right of the flowchart. The amino acid sequence was trimmed from the C-terminal and N-terminal ends, as indicated by the arrows. **(B)** Representative data showing the identification of a T cell determinant. (i) T cell cultures were generated by stimulating PBMC with OPPs from HPV-16 antigens. After 14 d in culture, the T cells were restimulated with OPPs from individual antigens, and intracellular IFN- γ production was analyzed by flow cytometry. Reactive T cells were further analyzed to determine the cognate peptide. (ii) Subpools of peptides were made for each antigen (nine subpools for E5 are pictured) and used in an IFN- γ ICS assay. The T cell response against each subpool is shown in the bar graph. (iii) The responses against each subpool were overlaid on a two-dimensional matrix, which shows the common individual peptides among the pools. The positive responses against pools 1 and 6 are highlighted in yellow, showing the common peptide 1 in red (row and column highlighted in yellow; common peptides in red text). (iv) Flow cytometric plots showing the T cell responses against subpools 1 and 6, assessed by intracellular IFN- γ staining. **(C)** Fine epitope mapping and HLA restriction. (i) Minimization of the active epitope within the 15-amino acid sequences derived from the peptide matrix analysis. T cell cultures responding to peptide 1 from the E5 subpools were tested against a range of shorter peptides within the peptide 1 sequence. The shorter peptides were synthesized by sequentially trimming one amino acid from the N-terminus and C-terminus down to a nine-amino acid peptide. T cell cultures were assayed for the expression of IFN- γ upon stimulation with each of the trimmed peptides at a concentration of 1 μ g/ml for **4 h**. The figure shows the CD8+ T cell response for each trimmed peptide from E5 subpool 1 (MTNLDTASTTLLACF). The peptides that stimulated a T cell response, denoted by brackets, were further analyzed in a dose titration assay. (ii) Peptide dose titration assay. T cell cultures were stimulated with serial decimal dilutions of selected minimized peptide sequences to determine the exact epitope sequence within the longer peptides. A representative example of the titration assay is shown; in this example, NLDTASTTLL is the likely minimal epitope sequence. (iii) Modified peptide sequences were tested in a dose titration assay to determine if the addition of extra amino acids at the N- or C-terminus could enhance peptide specificity. The example pictured demonstrates that NLDTASTTLL is the likely minimal epitope sequence targeted by HPV-16-E5-specific CD8+ T cells from this patient. (iv) Representative HLA class I restriction analysis for epitopes mapped from HPV antigens. A panel of lymphoblastoid cell lines with one HLA allele matched to the patient were loaded with each individual peptide for 1 h before coincubation with T cells for 4 h. Responses were assessed by intracellular IFN- γ staining. The OPC patients whose T cells responded to NLDTASTTLL had a class I HLA type of A*32:01, B*27:05, B*44:02, C*02:02, or C*05:01. In this example, the strongest response was elicited against antigen-presenting cells expressing HLA-C*05:01; therefore, this was determined to be the HLA restriction of NLDTASTTLL. Asterisk indicates positive response.

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