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CD4+ T cell responses to HLA-DP5-restricted wild-type sequence p53 peptides in patients with head and neck cancer

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Abstract

Wild-type sequence (wt) p53 peptides are attractive candidates for broadly applicable cancer vaccines. Evidence has been accumulating which indicates that CD4+ Th cells have an important role in generating and maintaining antitumor immune responses. To elucidate the nature of CD4+ Th responses to wt p53 epitopes in patients with squamous cell carcinoma of the head and neck (SCCHN), peripheral blood mononuclear cells (PBMCs) from HLA-DP5+ patients were stimulated with HLA-DP5-restricted wt p53 peptides, p53_{108–122} or p53_{153–166}, and tested for the release of IFN- γ and IL-5 in ELISPOT assays. Immunohistochemistry for p53 accumulation in tumors, and ELISA for serum antibodies to p53 were also performed. Eleven (57.9%) of 19 HLA-

DP5+ patients but none of 5 healthy donors had detectable Th1 and/or Th2 responses to wt p53 peptides by ELISPOT assay. Among these 11 responding patients, 9 (81.8%) and all 11 (100%) patients had a tumor burden and p53 accumulation, respectively. On the other hand, two responding patients were in post-operative condition. Interestingly, among nine patients with a tumor burden, four patients with early disease showed either Th1-polarized or mixed Th1/Th2 responses, while five patients with advanced disease showed either Th2-polarized or mixed Th1/Th2 responses. Our results suggest that wt p53_{108–122} and p53_{153–166} peptides stimulate both Th1- and Th2-type CD4+ T cell responses in patients with SCCHN, and anti-p53 Th responses may persist even after surgical resection of the tumor; however, the presence of a tumor and its progression may affect the nature of immune responses to wt p53 peptides.

Keywords

p53; CD4+ T cells; Th1; Th2; Head and neck cancer

Introduction

The targeting of wild-type sequence (wt) p53 epitopes represents an attractive approach for developing cancer vaccines capable of targeting a wide range of human cancers. Current evidence indicates that CD4+ Th cells as well as CD8+ CTL are required for effective antitumor immunity and that peptide-based vaccines are more effective when they include tumor-specific Th cell- as well as CTL-defined peptides [13, 25]. Recently, we have demonstrated the identification of a naturally presented HLA-DRB1*0401-restricted wt p53 peptide, p53_{110–124}, and the ability of these CD4+ T cells to enhance the ex vivo generation and function of antitumor CD8+ T cell effectors [4]. More recently, the presence of anti-wt p53_{25–35} CD4+ Th cells was shown to enhance the in vitro generation/expansion of HLA-A2- restricted, anti-wt p53_{264–272} CD8+ T cells, which from one donor were initially “nonresponsive” to the wt p53_{264–272} peptide [12]. These anti-wt p53 Th cells produced IFN- γ , but not IL-5, indicating Th1 cytokine profiles; thus, anti-p53 Th cells could be crucial participants in the development of CD8+ CTL responses, including anti-wt p53-specific CTLs. Polarization toward the Th1 immune response in cancer patients is favorable for antitumor immunity; however, it is highly likely that skewing toward a Th2 bias is a common feature of various malignant diseases and this imbalance seems to be associated with the development of cancer. Actually, Fedoseyeva et al. [8] demonstrated that wt p53 peptides induced MHC class II-restricted CD4+ T cell responses dominated by T cells displaying a Th2 phenotype, in BALB/c tumor-bearing mice. In humans, Bueter et al. [3] have also reported that the majority of p53 determinants that are immunogenic in colorectal cancer patients induce the activation of IL-10-producing T cells. In contrast, van der Burg et al. [23] have reported that several proliferative responses in colon cancer patients show a Th1 cytokine profile. These data suggest that the pattern of anti-p53 Th responses in cancer patients might be regulated by various factors, including the amounts and nature of p53 epitopes, HLA class II allele, and host immune status.

p53 was originally identified as a transformation-related antigen by IgG antibodies present in the sera of mice immunized against chemically induced tumors [7]. Subsequently, anti-p53 IgG was detected in the sera of patients with various types of cancer [6, 20, 26]. The IgG nature of anti-p53 humoral responses in cancer patients mandates that anti-p53 responses involve CD4+ T cells and suggested that p53-specific CD4+ Th cells are induced in these individuals. Tilkin et al. [22] demonstrated that the T cells of breast cancer patients proliferated in response to wt p53 protein, and these p53-specific responses were only detected in patients displaying high p53-specific IgG titers, whereas van der Burg et al. [23] indicated that patients with colorectal cancer displayed anti-p53 T helper immunity in the

absence of measurable p53-specific antibody levels. Similarly, Bueter et al. [3] reported that the presence of T helper immunity was not necessarily linked with a p53-specific IgG response. Thus, the relation between anti-p53 Th responses, including the type of Th1/Th2 responses and the presence of anti-p53 antibodies, remain unclear.

Better understanding of the nature and specificity of anti-p53 Th responses in cancer patients is necessary to better design and develop more effective p53-based cancer vaccines. In the present study, we investigated CD4⁺ Th responses to HLA-DPB1*0501-restricted wt p53 epitopes in patients with squamous cell carcinoma of the head and neck (SCCHN). An HLA-DPB1*0501 is a dominant HLA class II allele expressed in 60% of Japanese and two wt p53-derived peptides, p53_{108–122} and p53_{153–166}, are known to stimulate CD4⁺ T cell responses when presented in the context of the HLA-DPB1*0501 allele [9]; therefore, we chose these two peptides in this study. Moreover, immunological response data were evaluated relative to the p53 status, the presence of anti-p53 antibodies, and the disease status in each subject with SCCHN.

Materials and methods

Subjects

Peripheral blood was obtained from 27 subjects with pathologically and clinically confirmed SCCHN and 5 healthy subjects. The study was approved by the Institutional Review Board at Gunma University Hospital and written informed consent was obtained from each individual. Patient characteristics are summarized in Table 1. Heparinized venous blood (40 mL) was obtained from all subjects, and peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation over Ficoll-Hypaque gradients (Amersham Biosciences, Uppsala, Sweden), washed, and counted in the presence of a trypan blue dye.

HLA-DPB1*0501 genotyping was performed using a commercial DP5 typing panel of PCR primers according to the manufacturer's instructions (Dynal, Oslo, Norway).

Cell line and peptide

The H0301 cell line, HLA-DPB1*0501 homozygous Epstein-Barr virus (EBV)-transformed B lymphocytes (EBV-B cells), used as the peptide-presenting cells in ELI-SPOT assays, was kindly provided by Dr. Nishimura (Kumamoto University, Kumamoto, Japan). It was maintained in RPMI 1640 (Sigma-Aldrich, St Louis, MO) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 units/mL penicillin, 100 µg/mL streptomycin, 2 mM L-glutamine (all reagents from Invitrogen, Grand Island, NY). HLA-DPB1*0501-restricted wt p53-derived peptides, p53_{108–122} (GFRLGFLHSGTAKSV) and (PGTRVRAMAIYKQS) were synthesized using p53_{153–166} standard *N*-(9-fluorenyl) methoxycarbonyl methodology. The peptide was stored as a lyophilized preparation. The amino acid sequence was confirmed by mass spectrometric analysis.

In vitro sensitisation with peptides

PBMCs were cultured in the presence of wt p53-derived peptide (10 µg/ml) in 24-well tissue culture plates (Becton Dickinson Labware, Franklin Lakes, NJ) in a final volume of 2 mL AIM-V medium (Invitrogen) containing 10% (v/v) human AB serum (DS Pharma Biomedical, Osaka, Japan) (complete medium: CM). After 3 days, half of the medium from each well was replaced with CM-containing peptide (10 µg/ml). Following 4 days of incubation at 37°C, PBMCs were harvested, washed and tested in ELISPOT assays. In some cases, intracellular flow cytometric analysis was also performed.

Flow cytometric analysis

Responder cells (1×10^6 cells) were stimulated with 10 $\mu\text{g/mL}$ peptides (wt p53_{108–122} or p53_{153–166}) in the presence of irradiated (30 Gy) PBMCs (1×10^6 cells). After 1 h, 2 μM monensin A (Sigma, St Louis, MO) were added. After an additional 5 h of incubation, responder cells were harvested, washed twice with PBS, and stained with allophycocyanin (APC)-conjugated anti-CD4 monoclonal antibody for 30 min at 4°C. After washing twice, the responder cells were fixed and permeabilised according to the manufacturer's instructions (BD Cytotfix/Cytoper™, Fixation/ Permeabilization Kit, BD Biosciences, San Jose, CA). The cells were stained with PE-conjugated anti-IFN- γ (BD Biosciences) for 30 min at 4°C. As a negative control, cells were stained with mouse IgG isotype control (BD Biosciences). Cells were washed twice, and then analyzed by flow cytometry. Dead cells were eliminated using the viability dye 7-aminoactinomycin (7-AAD; BD Biosciences).

IFN- γ and IL-5 ELISPOT assays

ELISPOT assays were performed in 96-well flat-bottomed plates with nitrocellulose membrane inserts (Millipore, Bedford, MA), as previously described [1, 4]. Briefly, the plates were coated overnight at 4°C with 10 $\mu\text{g/mL}$ anti-human IFN- γ mAb (1-D1 K: Mabtech, Nacka Strand, Sweden), or 5 $\mu\text{g/mL}$ anti-human IL-5 mAb (TRFK5: BD Pharmingen, San Diego, CA) in PBS. H0301 cells used as APCs were pulsed with a wt p53_{108–122} or p53_{153–166} (10 $\mu\text{g/mL}$) and plated in triplicate wells at 1×10^5 cells/well. Responder cells (1×10^5 cells/well) were added to AIM-V medium at a final volume of 200 μL . PBMCs stimulated with 12.5 ng/mL PMA and 1 $\mu\text{g/mL}$ ionomycin were used as a positive control. The plates were incubated at 37°C for 20 h for IFN- γ assessment, and 40 h for IL-5 assessment. After incubation, the plates were washed with PBS/0.05% Tween 20, and supplemented with biotinylated anti-IFN- γ mAb (7-B6-1: Mabtech) or biotinylated anti-IL-5 mAb (JES1-5A10: BD Pharmingen). After 2 h incubation, plates were washed with PBS/0.05% Tween 20, and developed with the avidin–peroxidase complex (Vectastain Elite kit; Vector) for 1 h. 3-amino-9-ethyl-carbozole (Sigma, St Louis, MO) was added and incubated for 5 min for the IFN- γ ELISPOT assay and the TMB substrate for peroxidase (3,3',5,5'-tetramethylbenzidine; Vector Laboratories) was added and incubated for 10 min for the IL-5 ELISPOT assay. Spot numbers were automatically determined using a computer-assisted video image analyzer (Zeiss-Kontron, Jena, Germany). The mean number of spots in control wells (no peptide) was subtracted from the mean number of spots in experimental wells. A T cell response to a given wt p53 peptide was considered to be positive if at least 10 cells per 1×10^5 responder cells secreted IFN- γ or IL-5. In these cases, Student's two-tailed *t* test was performed to determine whether there was a significant difference between the number of cytokine-secreting T cells in peptide-stimulated and unstimulated wells, as described by Nagorsen et al. [16].

Immunohistochemistry for p53 and detection of p53 antibody in SCCHN

Immunohistochemical detection of p53 in tumor specimens was performed as previously described [19]. Briefly, formalin-fixed, paraffin-embedded tissue blocks were sectioned (2 μm thick) and deparaffinized. The slides were autoclaved in 0.01 M citrate buffer for 15 min (w/v) for antigen retrieval. After washing, sections were incubated with 1% (w/v) bovine serum albumin (Sigma-Aldrich, St Louis, MO) and 5% (w/v) normal horse serum (IBL, Gunma, Japan) for 30 min and subsequently treated overnight with anti-p53 mAb (DO-7; DAKO Cytomation, Inc., Carpinteria, CA). Next, sections were incubated with biotinylated goat anti-mouse antibodies (DAKO, Copenhagen, Denmark). The avidin-biotin complex technique (Vectastain Elite Kit; Vector Inc., Burlingame, CA) was performed according to the manufacturer's instructions, and 3,3'-diaminobenzidine tetrahydrochloride (DAB; Wako, Osaka, Japan) was used for color development. Sections were counterstained with hematoxylin. To avoid a scoring bias, p53 expression was evaluated by two independent

observers. Staining was scored as either positive (>10% of tumor cell nuclei were stained) or negative.

Ab to p53 in the patient's sera was detected by ELISA purchased from PharmaCell Immunotech Coulter (Miami, FL) using microtiter plates coated with recombinant human wt p53 protein.

Statistical analysis

Student's two-tailed *t* test and Fisher's exact probability test were used for statistical analysis of data. All *p* values <0.05 were considered significant. Analyses were performed using Statcel2 (OMS Publishing, Tokorozawa, Japan).

Results

wt p53 peptide-specific IFN- γ and IL-5 production by PBMCs obtained from healthy donors

Two wt p53 peptides (p53_{108–122} and p53_{153–166}) were tested for their ability to induce wt p53 peptide-specific Th responses. Separate cultures of PBMCs were set up with each peptide. Following 2 $\times$ IVS, responder cells were evaluated for peptide-specific CD4⁺ T cell responses in IFN- γ (Th1-type) and IL-5 (Th2-type) ELISPOT assays. We first tested PBMCs from five healthy donors; however, none of the healthy donors showed IFN- γ and/or IL-5 production significantly above the background for any of the two peptides in this protocol (data not shown).

wt p53 peptide-specific IFN- γ production by PBMCs obtained from patients with SCCHN

PBMCs obtained from 27 patients with SCCHN (19 HLA-DP5⁺ and 8 HLA-DP5⁻ patients) were used in these experiments. Data in Table 2 indicate that PBMCs obtained from 7 (36.8%) of 19 HLA-DP5⁺ patients specifically produced IFN- γ to either of these two peptides tested, and 4 (21.1%) and 3 (15.8%) yielded a response to wt p53_{108–122} and wt p53_{153–166} peptides, respectively. As expected, no PBMCs obtained from eight HLA-DP5⁻ patients responded to wt p53 peptides. In order to confirm the presence of IFN- γ -secreting CD4⁺ T cells within the PBMCs tested, in some cases (SCCHN-10, 11, 12, 17, 19), intracellular IFN- γ flow cytometric analysis was performed. As can be seen from the representative results shown in Fig. 1, both wt p53_{108–122} and p53_{153–166} peptide-specific CD4⁺ T cells could be detected in PBMCs of patients.

wt p53 peptide-specific IL-5 production by PBMCs obtained from patients with SCCHN

In order to evaluate Th2 CD4⁺ T cell responses to wt p53 peptides, IL-5 ELISPOT assays were performed. Overall, 8/19 HLA-DP5⁺ cancer patients specifically demonstrated the production of IL-5 in response to wt p53 peptides, and 3 (15.8%) and 5 (26.3%) responded to wt p53_{108–122} and wt p53_{153–166} peptides, respectively (Table 2). Unexpectedly, PBMCs obtained from SCCHN-25, an HLA-DP5⁻ patient, produced IL-5 in response to two wt p53 peptides tested, suggesting that these peptides may be naturally present in the context of multiple HLA class II molecules.

Correlation of Th responses to wt p53 peptides with disease status, p53 accumulation, and anti-p53 Ab

To evaluate the impact of the tumor burden, p53 accumulation, and the presence of anti-p53 Ab on anti-p53 Th responses, further analysis was performed in 19 HLA-DP5⁺ patients tested (Table 3). Overall, 11 (57.9%) of 19 HLA-DP5⁺ patients had detectable Th1 and/or Th2 responses to wt p53 peptides by ELISPOT assay. These included 3/11 (27.3%) patients with only Th1 responses, 4/11 (36.4%) patients with only Th2 responses, and 4/11 (36.4%)

with mixed Th1/Th2 responses. Among 11 patients with Th responses to wt p53 peptides, 9/11 (81.8%) and all 11 (100%) patients had a tumor burden and p53 accumulation, respectively. On the other hand, two patients with Th responses to wt p53 peptides were in a post-operative condition. Thus, anti-p53 Th responses were found more frequently in patients with a tumor burden ($p = 0.022$) or p53 accumulation ($p < 0.001$) than in those with NED or no p53 accumulation significantly (Table 4). Meanwhile, there was no relation between anti-p53 Th responses and the presence of anti-p53 Ab ($p = 0.337$). Interestingly, among 9 patients with a tumor burden, four patients with early disease (stage I and II) showed either Th1-polarized or mixed Th1/Th2 responses to wt p53 peptides, while five patients with advanced disease (stage III and IV) showed either Th2-polarized or mixed Th1/Th2 responses. Although these data derive from relatively few patients, they suggest that disease progression may shift toward a Th2 skewed immune status.

Discussion

The primary objective of this study was to determine whether CD4+ T cells specific to wt p53-derived peptides could be induced from PBMCs obtained from patients with SCCHN. To date, several HLA class II-restricted, CD4+ T cell-defined wt p53 epitopes have been identified and potentially could be used in vaccines against malignancies [4, 9, 12, 17]. Among these epitopes, p53_{108–122} and p53_{153–166} were defined as HLA-DP5-restricted peptides [9]. In this study, we analyzed wt p53-specific Th immunity in more detail using these two wt p53-derived-peptides. Our results indicated that the two peptides tested were comparable and could induce IFN- γ production as well as IL-5, suggesting that they stimulate Th1 and Th2 precursors in a subset of patients. Moreover, CD4+ T cell responses were confirmed by intracellular IFN- γ flow cytometric analysis. In contrast, in five healthy donors, no significant responses were detected, suggesting the absence of wt p53-specific T cell precursors in these individuals. PBMCs obtained from one HLA-DP5- patient were able to produce IL-5 in response to p53_{108–122} and p53_{153–166}. The length of the HLA class II peptide and the ability of the peptide to bind in the groove of HLA molecules can result in the HLA class II peptide being a promiscuous binder; therefore, several epitopes derived from tumor antigens have been shown to elicit Th responses across multiple HLA class II alleles [5, 10, 14, 15]. Indeed, p53_{108–122} has also been identified as an epitope presented by HLA-DRB1*0101 and -DRB1*0401 alleles [17].

Genetic alteration in p53 is perhaps the most frequently encountered genetic event associated with human cancer, and provides the basis for enhanced presentation by tumors of p53 proteins for immune recognition. In this study, 7 of 19 HLA-DP5+ patients tested had anti-p53 Ab in their sera, and this frequency of anti-p53 Ab is similar to the results from previous reports [6, 20, 26]. Moreover, six of these seven patients had p53 accumulation in their tumor, indicating a good correlation between the presence of anti-p53 Ab and p53 accumulation. As expected, all 11 patients with anti-p53 Th responses showed p53 accumulation in their tumor, suggesting that p53 accumulation may be an important component in the development of cellular response as well as humoral responses. Notably, anti-p53 Th responses were elicited in two of eight patients with NED, suggesting that anti-p53 Th responses may persist for one or more years, even after the antigen source has been removed by surgical resection of the tumor. Such Th responses in patients might strongly influence the prevention or promotion of disease relapse. Van der Burg et al. [23] also demonstrated that anti-p53 Th responses were long-lasting in patients with resected primary colorectal cancer; however, the significant higher detection of anti-p53 Th responses in patients with a tumor burden compared to those with NED suggest that sufficient amounts of p53 epitopes may be required to maintain anti-p53 Th effector cells. Meanwhile, the level of anti-p53 Ab varies depending on the tumor burden rather than anti-p53 Th responses. Indeed, several reports have shown that anti-p53 Ab can decrease in treated patients [11,

27]; thus, the detection of humoral and cellular immune responses to wt p53 epitopes is not necessarily linked.

Several recent reports have demonstrated that cellular responses to p53 are associated with clinicopathologic parameters, including the accumulation of T cells at the tumor site, tumor differentiation, and disease stage [2, 24]. Our previous study showed that CD8+ T cell precursors responsive to wt p53 epitopes were detected in the circulation of most subjects with early disease relative to subjects with advanced disease who were largely in the non-responder category [18]. Interestingly, in patients with a tumor burden, our data indicated that patients with early disease showed either Th1-polarized or mixed Th1/Th2 responses, while those with advanced disease had either Th2-polarized or Th1/Th2 responses. In tumor-bearing mice, Fedoseyeva et al. [8] showed an anti-p53 CD4+ T cell response biased toward the Th2 type during the course of tumorigenesis. Similarly, in patients with renal cell carcinoma, Tatsumi et al. [21] also demonstrated that Th1-type biased CD4+ T cell reactivity to EphA2 peptides could only be observed in a subset of stage I patients, and Th2- or T regulatory-type biased CD4+ T cell reactivity was almost always observed in stage IV patients. To clarify any potential disease-associated Th1/Th2 bias in response to wt p53 peptides in patients with SCCHN, more extensive studies will be required; however, our data and those of others suggest that tumor development may shift toward a Th2 skewed immune status and play facilitating roles in disease progression; therefore, vaccine strategies able to shift the balance toward Th1 polarization may be essential for more effective immunotherapy in patients with advanced disease.

Taken together, our study demonstrates that wt p53_{108–122} and p53_{153–166} peptides stimulate both Th1 and Th2-type CD4+ T cell responses in patients with SCCHN, and that Th responses to wt p53 epitopes may persist even after surgical resection of a tumor. Moreover, the nature of immune responses to wt p53 peptides may be skewed toward Th2-type responses during tumor progression. The value of using Th-defined epitopes for vaccination is that, in addition to augmenting CTLs, they could augment other elements of host defense, including non-MHC-restricted inflammatory cells and natural killer cells, which can also function in tumor eradication; however, inadequate performance of an anti-cancer vaccine may lead to tolerance and/ or tumor escape; therefore, it will be necessary to employ much more robust vaccination strategies, possibly combining strong Th1-polarizing adjuvants and different therapeutic modalities.

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Fig. 1.

Flow cytometric analysis for the detection of IFN- γ -producing CD4+ T cells in PBMCs stimulated with wt p53 peptides in a patient with SCCHN (Table 2: SCCHN-10 for p53₁₀₈₋₁₂₂; SCCHN-11 for p53₁₅₃₋₁₆₆). *Numbers* indicate the percentage of IFN- γ -producing CD4+ T cells

Table 1

Characteristics of patients with SCCHN tested in this study

Patient	Age/sex	HLA-DPS	Tumor site	Stage	Disease status at time of evaluation (month)
SCCHN-1	37 M	+	Oral cavity	III	NED (45)
SCCHN-2	81 M	+	Hypopharynx	IVA	NED (25)
SCCHN-3	72 M	+	Hypopharynx	III	NED (12)
SCCHN-4	77 F	+	Hypopharynx	IVA	NED (21)
SCCHN-5	79 M	+	Maxillary	IVA	NED (29)
SCCHN-6	89 M	+	Larynx	IVA	Local Dis. + LN mets.
SCCHN-7	48 F	+	Maxillary	III	Local Dis.
SCCHN-8	85 F	+	Oral cavity	IVA	Local Dis. + LN mets.
SCCHN-9	78 M	+	Maxillary	IVA	Local Dis.
SCCHN-10	77 M	+	Oral cavity	I	Local Dis.
SCCHN-11	82 M	+	Hypopharynx	IVA	NED (22)
SCCHN-12	79 M	+	Maxillary	IVA	Local Dis.
SCCHN-13	55 M	+	Hypopharynx	IVB	Local Dis. + LN mets.
SCCHN-14	76 M	+	Hypopharynx	IVA	NED (6)
SCCHN-15	64 M	+	Oral cavity	II	Local Dis.
SCCHN-16	70 M	+	Larynx	II	Local Dis.
SCCHN-17	64 M	+	Oral cavity	II	Local Dis.
SCCHN-18	65 M	+	Hypopharynx	IVA	NED (36)
SCCHN-19	82 F	+	Oral cavity	I	Local Dis.
SCCHN-20	71 F	-	Maxillary	II	Local Dis.
SCCHN-21	56 F	-	Oral cavity	III	NED (18)
SCCHN-22	55 M	-	Oral cavity	IVA	Local Dis. + LN mets.
SCCHN-23	79 M	-	Oral cavity	II	Local Dis.
SCCHN-24	50 M	-	Hypopharynx	IVA	Local Dis. + LN mets.
SCCHN-25	76 M	-	Oral cavity	I	Local Dis.
SCCHN-26	60 M	-	Oral cavity	III	NED (16)
SCCHN-27	37 F	-	Nasopharynx	IVA	NED (18)

NED no evidence of disease, *LN mets* lymph node metastasis

Table 2

Th response to wild-type p53 derived peptide in patients with SCCHN

Patient	p108–122		p153–166	
	IFN- γ	IL-5	IFN- γ	IL-5
HLA-DP5 ⁺				
SCCHN-1	0	1	2	7
SCCHN-2	9	16	0	3
SCCHN-3	1	0	0	0
SCCHN-4	0	0	0	0
SCCHN-5	2	4	0	4
SCCHN-6	0	0	0	18
SCCHN-7	11	0	0	69
SCCHN-8	0	0	7	24
SCCHN-9	0	25	51	0
SCCHN-10	13	21	0	0
SCCHN-11	0	7	25	0
SCCHN-12	65	0	7	16
SCCHN-13	0	2	0	0
SCCHN-14	0	0	0	0
SCCHN-15	10	10	0	29
SCCHN-16	0	0	0	8
SCCHN-17	10	0	0	3
SCCHN-18	6	0	5	0
SCCHN-19	0	5	11	3
HLA-DP5 ⁻				
SCCHN-20	0	0	0	0
SCCHN-21	0	0	0	3
SCCHN-22	0	0	0	1
SCCHN-23	3	0	2	0
SCCHN-24	0	7	0	0
SCCHN-25	0	12	3	28
SCCHN-26	3	6	0	3
SCCHN-27	0	0	0	0

The data are mean numbers of spots/ 10^5 T cells/well in IFN- γ or IL-5 ELISPOT assays. Bold number indicates positive for CD4⁺ T cell responses to wild-type p53 derived peptide

Table 3

Summary of Th responses and the p53 status in HLA-DP5+ patients with SCCHN

Patient	Th response		p53 accumulation	Anti-p53 Ab	Disease status
	p108-122	p153-166			
SCCHN-1	-	-	-	-	NED
SCCHN-2	Th2	-	+	-	NED
SCCHN-3	-	-	+	+	NED
SCCHN-4	-	-	-	-	NED
SCCHN-5	-	-	-	+	NED
SCCHN-6	-	Th2	+	+	Tumor burden
SCCHN-7	-	Th2	+	+	Tumor burden
SCCHN-8	-	Th2	+	-	Tumor burden
SCCHN-9	Th2	Th1	+	-	Tumor burden
SCCHN-10	Th1/Th2	-	+	-	Tumor burden
SCCHN-11	-	Th1	+	-	NED
SCCHN-12	Th1	Th2	+	+	Tumor burden
SCCHN-13	-	-	-	-	Tumor burden
SCCHN-14	-	-	-	-	NED
SCCHN-15	Th1	Th2	+	+	Tumor burden
SCCHN-16	-	-	-	-	Tumor burden
SCCHN-17	Th1	-	+	-	Tumor burden
SCCHN-18	-	-	-	-	NED
SCCHN-19	-	Th1	+	+	Tumor burden

Correlation of Th response to wt p53 peptides with disease status, p53 accumulation, and anti-p53 Ab in patients with SCCHN

Table 4

Th responses	Disease status		p53 accumulation		Anti-p53 Ab	
	NED	Tumor burden	p	Positive	Negative	p
Positive	2	9	0.022	11	0	<0.001
Negative	6	2		1	7	0.337
Total	8	11		12	7	6
						12