

**Complete regression of metastatic cervical cancer following treatment with HPV-targeted tumor-infiltrating T cells**

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## **DATA SUPPLEMENT**

### **Patients and Methods**

#### **Study Design**

The timeline for cell preparation and patient treatment is depicted in Data Supplement, Fig. 1A. Following tumor excision and cell product generation, patients received lymphocyte-depleting chemotherapy for seven days. HPV-TIL were generated as described below and administered in a single infusion over 20 to 30 minutes. High-dose bolus aldesleukin was given with the number of doses determined by patient tolerance or a maximum of 15 doses. Patients were discharged from the hospital when their blood counts recovered to safe levels and they were otherwise medically stable. Follow up assessments of tumor response and adverse events were conducted as depicted in Data Supplement, Fig. 1A. Follow-up evaluations included a medical history, physical examination, tumor imaging, and laboratory tests. Baseline imaging studies were obtained within four weeks before initiation of the conditioning regimen. Follow-up imaging was obtained at predetermined intervals (Data Supplement Fig. 1A). Long term follow-up was at the discretion of the Principal Investigator. Patients were removed from the study upon disease progression.

#### **Generation of HPV-TIL cell products**

TIL cultures were generated from excisional biopsies of tumors that were at least 1 cm<sup>3</sup> in volume. All excisional biopsies were obtained by surgical resection. The schema for generation of the HPV-TIL infusion product is depicted in Data Supplement, Figure 1B.

T-cell cultures were initiated from tumor fragments and expanded using IL-2 (6000 IU/ml) containing culture media.<sup>1</sup> Cultures with lymphocyte outgrowth were tested for reactivity against HPV-16 or HPV-18 E6 and E7 as described below. The HPV-type of the target oncoproteins was matched to that of the tumor, which was determined by reverse transcription polymerase chain reaction with primers and probes specific for HPV-16 or HPV-18 E6 and E7 (Life Technologies). Primer and probe sequences are provided in Data Supplement, Table 3. Flow cytometric analysis of each culture was performed using antibodies specific for CD3, CD4, CD8, and CD56 (BD Biosciences). Cultures were selected for additional expansion<sup>1, 2</sup> for patient administration based on HPV-oncoprotein reactivity, rapid growth, high T-cell purity, and high frequency of CD8+ T-cells. Two patients had no detectable HPV-reactivity from their initial TIL cultures (Patient 7 and Patient 8). For these patients, cultures for administration were selected based on the other criteria above. In preclinical and clinical studies, we have generated TIL from 29 of 29 resected cervical cancer specimens.

### **Immunological assays**

HPV-reactivity of TIL cultures, infused TIL and peripheral blood T-cells was determined by coculture of T-cells (30,000 to 80,000 cells all assays; 10,000 to 20,000 for ELISPOT) with autologous dendritic cells (30,000 to 40,000 cells) loaded with peptide pools spanning patient's tumor HPV-type specific E6 and E7, and gp100 (negative control). Target peptide pools consisted of 1  $\mu$ M of 15-mer peptides overlapping by 11 amino acids (Miltenyi Biotec). For peripheral blood T-cell assays, CD3+ T-cells were isolated by magnetic bead separation according to manufacturer's instructions (Miltenyi

Biotech) prior to coculture. Dendritic cells were generated from the adherent fraction of peripheral blood mononuclear cells (PBMC) or from CD14<sup>+</sup> cells isolated from PBMC with magnetic beads according to manufacturer's instructions (Miltenyi Biotec). Isolated cells were cultured in DMEM (Gibco) supplemented with 10% human serum and 1000 IU/ml GM-CSF and 500 IU/ml IL-4 (PeproTech) for five to six days. IFN- $\gamma$  production was analyzed by enzyme-linked immunosorbent assay (ELISA) (R&D Systems or Thermo Fisher Scientific and/or enzyme-linked immunospot (ELISPOT) (Mabtech) assays following overnight coculture. CD137 upregulation assays were performed by flow cytometric analysis using antibodies specific for CD3, CD4, CD8, and CD137 (BD Biosciences) and counterstaining with propidium iodide (BD Pharmingen) after 20 to 24 hour coculture.<sup>3</sup>

Intracellular cytokine production was assessed by flow cytometry. GolgiStop and GolgiPlug (BD Biosciences) were added when cocultures were initiated. T-cells were stimulated with phorbol myristate acetate (0.05  $\mu$ g/ml) and ionomycin (0.5  $\mu$ g/ml) as a positive control. After 6 hours of stimulation, cells were processed using the Cytotfix/Cytoperm kit (BD Biosciences) according to the manufacturer's instructions. Briefly, cells were pelleted, washed, and stained for cell surface markers with fluorescently labeled CD3, CD4 and CD8 (all BD Biosciences) for 30 minutes, at 4°C. Subsequently, cells were washed, fixed and permeabilized, washed in Perm/Wash buffer and stained with fluorescently labeled antibodies against of IFN- $\gamma$ , TNF- $\alpha$  and IL-2 (all eBioscience) for 30 minutes, at 4°C. Finally, cells were resuspended in phosphate buffered saline containing 1% fetal calf serum (Lonza) prior to acquisition on a flow

cytometer. All flow cytometry data were acquired with a BD FACSCanto II cell analyzer (BD Biosciences) and analyzed with FlowJo software, Mac version 10 (TreeStar).

### **Analysis of multiple cytokine levels**

Levels of 17 cytokines (IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-17, granulocyte-colony stimulating factor (G-CSF), granulocyte monocyte-colony stimulating factor (GM-CSF), IFN- $\gamma$ , monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1 $\beta$ , and tumor necrosis factor (TNF)- $\alpha$ ) were measured in sera from patients and in the supernatants of overnight cocultures using Bio-Plex Pro Human Cytokine 17-plex Assay (Bio-Rad Laboratories) according to the manufacturer's instructions. Specific levels of IL-4 and GM-CSF in the supernatants are not reported as residual levels of these cytokines were measured in media alone. Cytokine measurements were acquired using the Bio-Plex 200 system (Bio-Rad).

## Data Supplement References

1. Dudley ME, Wunderlich JR, Shelton TE, et al: Generation of tumor-infiltrating lymphocyte cultures for use in adoptive transfer therapy for melanoma patients. *J Immunother* July 26:332–342, 2003
2. Jin J, Sabatino M, Somerville R, et al: Simplified method of the growth of human tumor infiltrating lymphocytes in gas-permeable flasks to numbers needed for patient treatment. *J Immunother* 35:283–292, 2012
3. Wolf M, Kuball J, Ho WY, et al: Activation-induced expression of CD137 permits detection, isolation, and expansion of the full repertoire of CD8+ T cells responding to antigen without requiring knowledge of epitope specificities. *Blood* 110:201–210, 2007