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Adam S Kibel

Brant A Inman

Russell K Pachynski

Tuyen Vu

Nadeem A Sheikh

*See next page for additional authors*

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**Authors**

Adam S Kibel, Brant A Inman, Russell K Pachynski, Tuyen Vu, Nadeem A Sheikh, and Daniel P Petrylak

## Videos of Sipuleucel-T Programmed T Cells Lysing Cells That Express Prostate Cancer Target Antigens

Adam S. Kibel , MD<sup>1,2,\*</sup> Brant A. Inman , MD<sup>3</sup> Russell K. Pachynski , MD<sup>4</sup> Tuyen Vu, MS<sup>5</sup> Nadeem A. Sheikh, PhD<sup>5</sup>, Daniel P. Petrylak , MD<sup>6</sup>

<sup>1</sup>Affiliations of authors: Dana-Farber Cancer Institute, Boston, MA, USA; <sup>2</sup>Brigham and Women's Hospital, Boston, MA, USA; <sup>3</sup>Duke University, Durham, NC, USA; <sup>4</sup>Washington University in St Louis, St Louis, MO, USA; <sup>5</sup>Dendreon Pharmaceuticals, LLC, Seattle, WA and <sup>6</sup>Yale Cancer Center, Yale School of Medicine, New Haven, CT

\*Correspondence to: Adam S. Kibel, MD, Dana-Farber / Brigham and Women's Cancer Center, 45 Francis St, Boston, MA 02115-6105, USA (e-mail: akibel@bwh.harvard.edu).

### Abstract

Sipuleucel-T, an autologous cellular immunotherapy, was approved to treat metastatic castration-resistant prostate cancer in 2010 in the United States. Treatment with sipuleucel-T primes the immune system to target prostate acid phosphatase, which is expressed by prostate cancer cells, potentially leading to lysis of cancer cells. Expanding on previously reported indirect evidence of cell killing with sipuleucel-T treatment, we sought to provide direct evidence of cell lysis through visualization. We used advanced video technology and available samples of peripheral blood mononuclear cells from subjects enrolled in the STAMP trial (NCT01487863). Isolated CD8+ T cells were used as effector cells and cocultured with autologous monocytes pulsed with control or target antigens. Differentially stained effector and target cells were then video recorded during coculture. Here, we present video recordings and analyses of T cells from sipuleucel-T-treated subjects showing—for the first time—direct lysis of cells that express prostate cancer target antigens, prostate acid phosphatase, or prostate-specific antigen.

In this brief report, we share videos illustrating sipuleucel-T-programmed human T cells recognizing and destroying cells that express the prostate cancer target antigens, either prostatic acid phosphatase (PAP), or prostate-specific antigen (PSA). This report expands on a previous report of indirect evidence of cell killing, based on flow cytometry, in samples from men with metastatic castrate-resistant prostate cancer treated with sipuleucel-T (1).

Sipuleucel-T is an autologous cellular immunotherapy approved in 2010 to treat asymptomatic or minimally symptomatic metastatic castrate-resistant prostate cancer (2). Sipuleucel-T treatment comprises 3 infusions given approximately every 2 weeks. Each infusion involves collecting peripheral blood mononuclear cells (PBMCs) via apheresis, then isolating the PBMCs and culturing them *ex vivo* with PA2024 (a fusion protein, comprising PAP and human granulocyte macrophage colony-stimulating factor), and finally intravenously infusing the resultant product back into the subject.

Cells in this infusion stimulate peripheral immune responses against PAP and PA2024 and increase cytokine production; further, they cause trafficking of T cells to the prostate in the localized setting (2-5). Also, memory cytolytic T-

lymphocyte activity against both PA2024 and PAP, as measured by a flow cytometry assay, is induced (1). These immune responses correlate with overall survival (1,6), along with the breadth of posttreatment humoral response (7). To date, although hypothesized, tumor cell lysis has not been demonstrated directly.

We used banked PBMC samples from subjects who displayed week 26 post-sipuleucel-T treatment antibody responses against PA2024, PAP, and PSA during the STAMP study (NCT01487863) (8). The responses of the subjects included in the current assessments are described in Table 1. The study was approved by the institutional review board of each site, and subjects provided informed consent. Samples from 3 timepoints were assessed: baseline (ie, week 0, before sipuleucel-T treatment) and after sipuleucel-T treatment (weeks 6 and 26).

Samples containing cryopreserved PBMCs for each timepoint for each subject were thawed and treated as described previously (1). Next, monocytes were isolated using negative selection techniques (EasySep human monocyte enrichment kit [STEMCELL Technologies, Vancouver, BC, Canada] and CD8+ cell isolation kit [Miltenyl Biotec, Auburn, CA]). Isolated CD8+ T cells were stained with LysoBrite Red (AAT Bioquest, Sunnyvale, CA) to allow

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**Table 1.** Demonstration of long-term responses after sipuleucel-T treatment in the STAMP study in subjects included in the current assessments<sup>a</sup>

Subject	Antibody fold-change from week 0			
	PSA		PAP	
	Week 6	Week 26	Week 6	Week 26
Subject #1	2.40	2.98	4.87	4.40
Subject #2	5.52	4.91	7.86	7.65
Subject #3	2.94	3.58	6.96	6.81

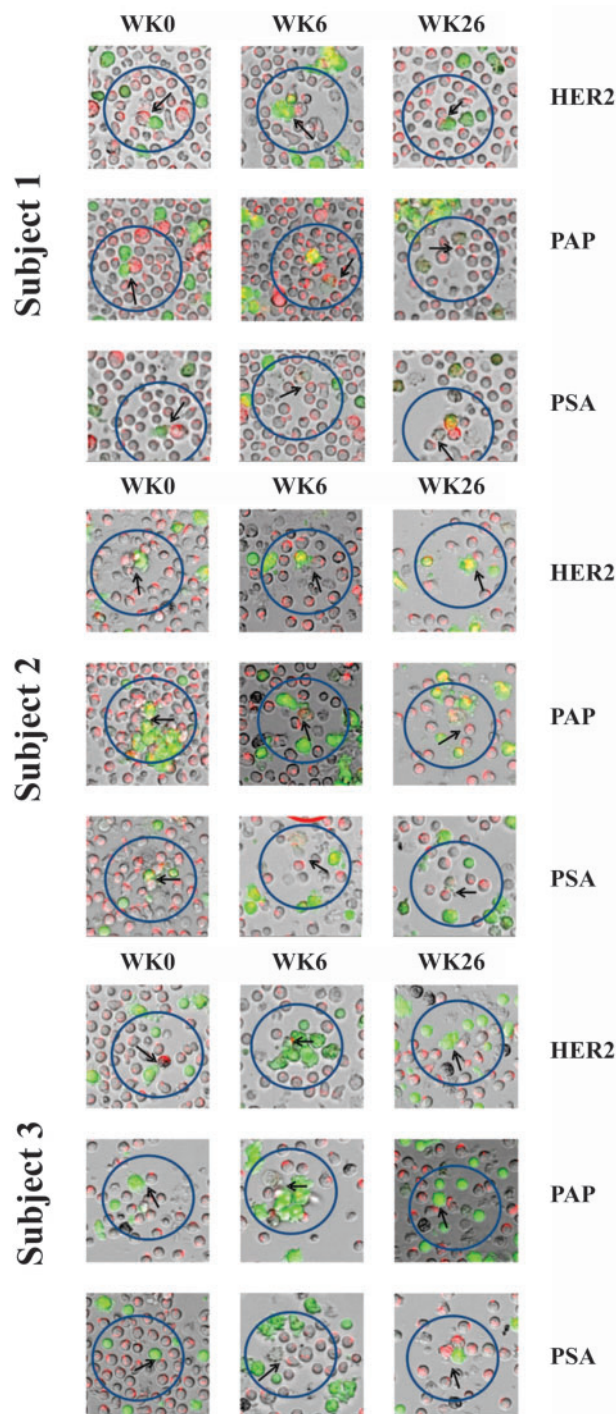
<sup>a</sup>Responses are described by prostate-specific antigen (PSA)- and prostatic acid phosphatase (PAP)-antibody fold-changes from week 0 to week 26. Methodology as described in Small et al. (8).

subsequent effector cell lysosome visualization (red). Isolated autologous monocytes were either pulsed separately with PAP peptides (20mers with 10-aa overlap from New England Peptide, Gardner, MA) or PSA peptides (15mers with 11-aa overlap from JPT Innovative Peptide Solutions, Berlin, Germany). As control, HER2 peptides (20mers with 10-aa overlap from New England Peptide) or unpulsed monocytes were used to test for specificity or not pulsed (Figure 1). Pulsed and unpulsed monocytes were stained with calcein AM (ThermoFisher Scientific, Waltham, MA) to allow for visualization of target cells (green). Monocytes were incubated with CD8+ T cells at a 1:7 effector to target ratio:  $2 \times 10^5$  green-stained monocytes were loaded on a well of a chamber slide system (ThermoFisher Scientific) and then incubated (37°C, 5% CO<sub>2</sub>) for 1 hour before the addition of  $1.4 \times 10^6$  red-stained CD8+ T cells.

Cell activity at room temperature was then recorded for 8 hours using a Leica CRT6500 confocal microscope with LASAF software (Leica Microsystems, Buffalo Grove, IL) at the largest XY-format size (2048 x 2048 pixels) with a recording speed at 400 Hz. Following acquisition, images were analyzed, processed, and recorded as 41-second videos of time-lapse sequences (30 frames per second) using Adobe Premiere Pro CC 2018 (Adobe Systems).

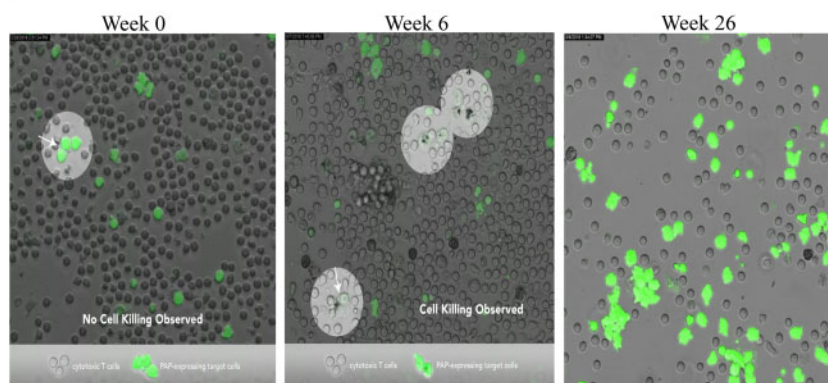
Red-stained CD8+ T cells were tracked from the time they came in contact with the target cells: the green-stained monocytes. T cells in the posttreatment samples exhibited higher motility compared with week 0 (data not shown). We observed that the killing of the target cells, the green-stained PAP- and PSA-pulsed monocytes, was only seen in the presence of CD8+ T cells and only in the weeks 6 and 26 samples (ie, after treatment with sipuleucel-T; Figure 1 and Video; see Supplementary material to view videos A-C, available online). Cell killing was not observed at week 0 (Figure 1), nor was it seen posttreatment in the absence of CD8+ T cells (data not shown). Cell killing was not observed in the following control settings: 1) in the presence of HER2 peptides, 2) unpulsed conditions, or 3) in samples from a normal donor (data not shown). T cells not exposed to sipuleucel-T did not recognize PAP- and PSA-pulsed target cells, because the T cells did not exhibit prolonged contact with target cells and did not exhibit cell killing. After treatment, T cells displayed serial lysis; namely, individual T cells were able to lyse multiple targets sequentially.

Together, these observations suggest that exposure to sipuleucel-T programs T cells to recognize and lyse cells that express the primary target antigen for sipuleucel-T (eg, PAP) or a



**Figure 1.** Still images of cell-cell interactions organized by both applied antigen and timepoint. CD8+ T cells (red) interact with target cells (green). The cells are visualized to reflect these colors. Arrowheads indicate effector - target cell interactions. Interactions suggest antigen-specificity and antitumor activity. The arrow tips indicate the contact point of the target and effector cells of interest. During week 0, more red-stained cells are observed. Target cells pulsed with irrelevant antigen HER2 and tested antigens at week 0 retain their green color after interaction with baseline CD8+ T cells. The green color of target cells pulsed with tested antigens disappears after they are killed by sipuleucel-T stimulated CD8+ T cells at week 6 and week 26. PAP = prostatic acid phosphatase; PSA = prostate-specific antigen.

### Video of sipuleucel-T stimulated T cells killing target cells



**Video.** Three time-lapse videos of cell-cell interactions by timepoint: A) week 0; B) week 6; C) week 26. CD8+ T cells interact with target cells. The green-stained target cells are visualized as white. Effector cells are not highlighted. Arrowheads indicate effector - target cell interactions in sipuleucel-T-treated subjects demonstrating antigen specificity and antitumor activity. The arrow tips indicate the contact point of the target and effector cells of interest. Cell interaction and killing at room temperature were captured using a Leica CRT6500 confocal microscope with LASAF software (Leica Microsystems, Buffalo Grove, IL). The final products were 41-second videos where the sipuleucel-T-stimulated CD8+ T cells were tracked from the time they come in close contact with the green-stained target cells. For purposes of presentation, the videos have been shortened to fit in the file size limitations. Time-lapse recording was performed using the confocal microscope set up as follows: the "XYT" scanning of acquisition mode was activated; 10X objective was set with digital zoom factor at 6; the XY field was set at the largest size format (2048 x 2048 pixels) with a recording speed at 400 Hz; and the time field was set for a duration of 8 hours. After acquisition, images were analyzed, processed, and recorded as time-lapse video sequences using Adobe Premier Pro CC 2018 at a speed of 30 frames per second.

secondary prostate cancer antigen (eg, PSA), because lytic activity was only observed in the samples collected after sipuleucel-T treatment. These results also suggest involvement of antigen spread in the response (5,9). Finally, we were able to detect PAP- and PSA-specific lytic activity out to the week 26 mark, which is in agreement with the vast array of immune response data generated so far, suggesting induction of immunological memory.

Other methods can demonstrate the direct killing of target cells by effector cells, albeit with certain technical limitations. Chromium release or similar cytotoxicity assays require large numbers of both effector and target cells (10), limiting their use when assessing cytolytic T-lymphocyte activity in human samples, given both blood draw restrictions and associated costs. Lack of accessibility to major histocompatibility complex-matched tumor cells, either derived from subjects being assessed or cell lines, further limits this type of research. We addressed these limitations by using autologous, peptide-pulsed monocytes as target cells, allowing us to reduce the requisite number of effector cells and eliminate the need for major histocompatibility complex-matched target cell lines. Direct visualization by time-lapse videography allowed us to closely study direct cell-cell interactions, thus providing direct evidence of induced target cell lysis after sipuleucel-T treatment.

In summary, supporting previous results (1), using an ex vivo human-cell model, our results provide evidence that treatment with sipuleucel-T results in immune activation leading to the destruction of cells expressing the prostate cancer target antigens PAP and PSA and support an additional in vivo mechanism of action in subjects receiving sipuleucel-T.

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**Author contributions:** Conceptualization, NS, TV, AK, and BI; Methodology, NS and TV; Investigation, NS and TV; Writing—Original Draft, NS; Writing—Review & Editing, AK, BI, RP, TV, NS, and DP; Funding Acquisition, NS; Resources, NS; Supervision, NS. All authors reviewed the results and approved the final version of the manuscript.

**Prior presentations:** Inman B, Vu T, Yu Evan Y, et al. PD14-07 Real-time imaging demonstrating T-cell mediated destruction of prostatic acid phosphatase (PAP)-expressing cells in patients (PTS) treated with sipuleucel-T (SIP-T). *J Urology*. 2018; 199(4S):Abstract e307. <https://doi.org/10.1016/j.juro.2018.02.793>.

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## Data Availability

The data underlying this article will be shared on reasonable request to Dendreon Pharmaceuticals, LLC ([mac@dendreon.com](mailto:mac@dendreon.com)).

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