Emmanuel S. Antonarakis1, David I. Quinn2, Adam S. Kibe3, Daniel P. Petrylak4, Nancy N. Chang5, Erica Dearstyn6, Dwayne Campogan7, Heather Haynes5, Tuyen Vu3, James B. Trager4, Nadeem A. Sheikh5, Charles C. Drake1

1Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, USA; 2Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, USA; 3Brigham and Women’s Hospital, Harvard University, Urologic Surgery, Boston, USA; 4Yale Cancer Center, New Haven, USA; 5Dendreon Pharmaceuticals, Inc., Seattle, USA; 6Nkarta, Inc., San Francisco, USA.

**Lytic CD8+ T cell responses to target antigens induced by sipuleucel-T in men with hormone-sensitive and castration-resistant prostate cancer**

**Background:** Sipuleucel-T is an FDA-approved autologous cellular immunotherapy for patients with asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer (mCRPC), manufactured from peripheral blood mononuclear cells (PBMCs) cultured with the immunogen PA2024, a fusion antigen of prostatic acid phosphatase (PAP) conjugated to granulocyte macrophage colony-stimulating factor. Treatment with sipuleucel-T induces cellular and humoral immune responses to both PA2024 and PAP (Antonarakis, ASCO 2015; Petrylak, ECC 2015). To further elucidate the mechanism of sipuleucel-T–induced immune responses, we evaluated the proliferative and lytic characteristics of PA2024- and PAP-specific CD8+ T cells.

**Methods:** Patient samples were assessed for antigen-specific cellular immune responses by IFN-γ ELISPOT (Enzyme-Linked ImmunoSpot) assays. Cell proliferation was measured with a flow cytometry assay; PBMCs labeled with the membrane dye carboxyfluorescein succinimidyl ester (CFSE) were cultured with PA2024 or PAP, and progressive dilution of CSFE indicated antigen-specific cellular proliferation. Phenotypic analyses were then performed to identify whether proliferating T cells were CD4+ (T helper [T_{H}]) or CD8+ (cytotoxic T lymphocytes [CTLs]). To assess whether CD8+ T cells were engaged in CTL activity, loss of intracellular granzyme B (GzB), indicating exocytosis of this apoptosis-mediating enzyme, was also measured. Patient samples were from 2 sipuleucel-T clinical trials (NCT01431391; NCT01981122) in hormone-sensitive prostate cancer and mCRPC patients.

**Results:** Sipuleucel-T generated PA2024 and PAP-specific IFN-γ ELISPOT responses (N=118), and both CTL and T_{H} subpopulations demonstrated proliferation responses to PAP and PA2024 (14 patients assessed) 6 weeks post–sipuleucel-T administration (p<0.10). CTL proliferative responses were greater against PA2024 compared with the magnitude of response against PAP, with most patients with CTL responses to PA2024 also having T_{H} responses. CTLs from patients who exhibited PA2024- and/or PAP-specific proliferative responses were assessed for lytic ability. After in vitro antigen stimulation and in all evaluated samples (PA2024, n=14; PAP, n=13), intracellular GzB was significantly decreased compared with a no-antigen control (p<0.05) at 6 weeks post–sipuleucel-T, demonstrating CTL activity.

**Conclusions:** Sipuleucel-T generated PA2024- and PAP-specific cellular responses, which consisted of both T_{H} and CTL responses. Moreover, the lytic activity observed by antigen-specific CTLs provides direct evidence that sipuleucel-T can induce tumor cell lysis against PAP-expressing tumor cells within 6 weeks of sipuleucel-T administration.

**Conflict of Interest:** ESA: Honoraria and consultancy/advisory role with Sanofi, Dendreon, Janssen, and Medivation, research funding from Sanofi, Dendreon, Johnson & Johnson, and Astellas; DIQ: consultancy/advisory role for Astellas Pharma, Dendreon, Novartis, Pfizer, Janssen Oncology, Bristol-Myers Squibb, Medivation, Genentech/Roche, Merck, EMD Serono, and Oncogenex, honoraria from Bayer, Dendreon, Medivation, Astellas Pharma, Novartis, Pfizer, Janssen Oncology, Bristol-Myers Squibb, Genentech/Roche, research funding from Millennium, Genentech/Roche, Sanofi, GlaxoSmithKline; ASK: consultancy/advisory role for Dendreon, Sanofi Aventis, MTG, Profound, Tokai, Janssen; DPP: stock from Bellicum Pharmaceuticals, and Tyme, Inc., honoraria from Bayer, Bellicum Pharmaceuticals, Sanofi,
Johnson & Johnson, Exelixis, Ferring, Millennium, Medication, Pfizer, Genentech, Astellas Pharma, Progenics, and Merck Serono, consultancy/advisory role with Bayer, Bellicum Pharmaceuticals, Dendreon, Sanofi, Johnson & Johnson, Exelixis, Ferring, Millennium, Medivation, and Pfizer, research funding from Oncogenex, Progenics, Johnson & Johnson, Millennium, Dendreon, Sanofi, Progenics, Endocyte, Genentech, Merck, Astellas Medivation, GTX, and Novartis, expert testimony for Celgene and Sanofi, travel expenses from Bayer, Bellicum Pharmaceuticals, Dendreon, Sanofi, Exelixis, Ferring, Medivation, Pfizer, Oncogenex, Progenics, Johnson & Johnson, Millennium, and Celgene; NNC, ED, DG, HH, TV, NAS: employment with Dendreon, stock from Valeant Pharmaceuticals; JBT: employment with Dendreon; CGD: stock in Compugen, NexImmune, Potenza, and Tizvona, consultancy/advisory role for Agenus, Dendreon, NexImmune, ImmunExcite, Janssen, Lilly, Merck, Pierre Fabre, and Roche/Genentech, research funding from Aduro Biotech, Bristol Myers Squibb, Janssen, patents/royalties from Bristol Myers Squibb, AstraZeneca, MedImmune, and Janssen.

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