The Oncologist®

Immuno-Oncology

Clinical Activity of Nivolumab for Human Papilloma Virus-Related Juvenile-Onset Recurrent Respiratory Papillomatosis

Ben C. Creelan,[a] M. Usman Ahmad,[c] Frank J. Kaszuba,[a] Farah K. Khalil,[b] Allison W. Welsh,[d] Metin Ozdemirli,[e] Nazaneen N. Grant,[f] Deepa S. Subramaniam[g]

Departments of [a]Thoracic Oncology and [b]Anatomic Pathology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, USA; [c]Morsani College of Medicine, University of South Florida, Tampa, Florida, USA; [d]Foundation Medicine Inc., Cambridge, Massachusetts, USA; Departments of [e]Pathology and [f]Otolaryngology, [g]Division of Hematology/Oncology, Georgetown Lombardi Comprehensive Cancer Center, MedStar Georgetown University Medical Center, Washington, D.C., USA

Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Recurrent respiratory papillomatosis • Nivolumab • Debulking surgical procedures • Debridement • Benign neoplasms • Tracheal stenosis • Tumor-infiltrating lymphocytes

Abstract

Background. Juvenile-onset recurrent respiratory papillomatosis (JO-RRP) is a human papilloma virus-mediated progressive benign neoplasm that affects children and young adults. Primary management consists of regular surgical debulking to maintain airway patency and vocal function. Like condyloma acuminata, JO-RRP is associated with immune dysregulation, and T cells isolated from papillomas express an anergic phenotype. Therefore, we hypothesized that programmed death protein 1 axis inhibition could stabilize tumor growth.

Materials and Methods. We treated two patients with refractory JO-RRP using nivolumab, with the primary objective of assessing clinical activity. We explored baseline papilloma features using immunohistochemistry and comprehensive genomic profiling.

Results. Both patients experienced symptomatic improvement, and interval laryngoscopies revealed a reduction in papillomatosis burden. One patient has not required subsequent surgical debridement for almost 2 years. On pathologic examination of pretreatment papillomas from both cases, infiltrating T cells were evident in the papilloma stroma, and papilloma programmed death ligand 1 expression was absent. Papilloma mutational load ranged between three and six mutations per megabase for each case. From on-treatment biopsy tissue, a higher amount of intraepithelial T cells and programmed death ligand 1 expression were detected in the papilloma.

Conclusion. Nivolumab appears to have promising activity in JO-RRP, and further clinical investigation with more patients in clinical trials is warranted. ***The Oncologist*** 2019;24:829–835

Implications for Practice: To the authors' knowledge, this article is the first report describing clinical activity with a programed cell death-1 (PD-1) inhibitor to treat a rare but detrimental type of respiratory tract epithelial neoplasm that afflicts young adults. Two patients were treated, and tumor features, such as mutational load, were examined with the intent to stimulate future hypotheses for translational research. The safety and activity of PD-1 inhibitors in this population still need to be corroborated in clinical trials and should not yet be adopted into clinical practice.

Introduction

Juvenile-onset (JO) recurrent respiratory papillomatosis (RRP) is a benign neoplasm of the larynx that affects children and young adults, with an incidence of 4.3 in 100,000 [1]. It is putatively mediated by vertical transmission of human papilloma virus (HPV) during infancy, predominantly the HPV-6 and -11 genotypes [2]. Although JO-RRP is histologically benign, the papillomas often enlarge and obstruct the airway if untreated [3]. Currently, JO-RRP is primarily managed with repetitive surgical debridement, with an average requirement of four to five resections per year for each patient [4]. Despite advances in surgical techniques, scarring and tracheal stenosis inevitably occur, and no consensus treatment approach exists. Relatively few systemic therapies have consistently shown activity in RRP [1]. Systemic bevacizumab induced responses in a case series of five patients, but progression after treatment discontinuation was common [5]. Therefore, more effective systemic therapies for JO-RRP are needed.

Correspondence: Ben C. Creelan, M.D., M.S., H. Lee Moffitt Cancer Center, 12902 Magnolia Dr. FOB1, Tampa, Florida 33612, USA. Telephone: 813-745-3050; e-mail: ben.creelan@moffitt.org Received August 14, 2018; accepted for publication February 8, 2019; published Online First on March 6, 2019. http://dx.doi.org/10.1634/theoncologist.2018-0505

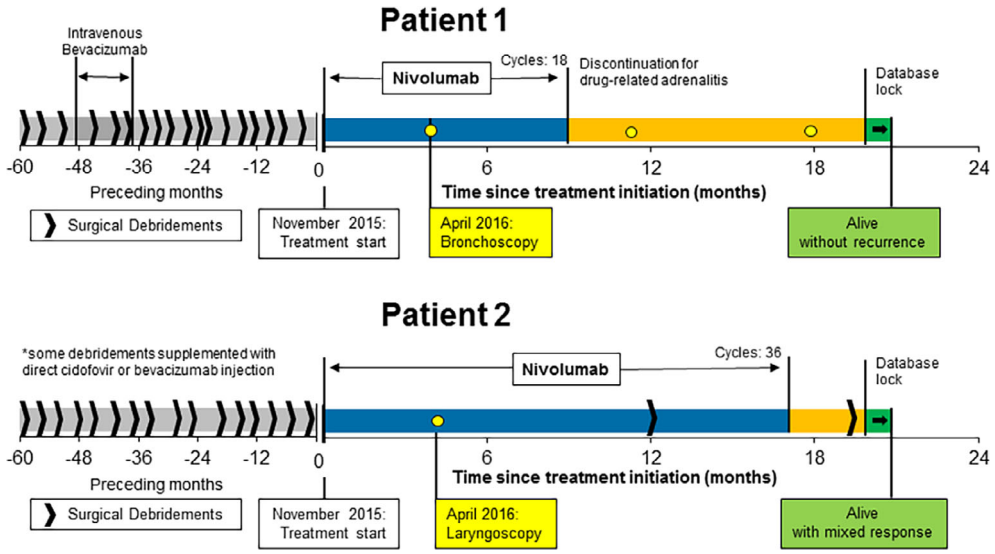

Figure 1. Patient treatment diagram showing clinical course with nivolumab.

Similar to condyloma acuminatum, JO-RRP is more common in immunosuppressed populations and is linked to susceptible class II human leukocyte antigen and killer-cell immunoglobulin-like receptor alleles [6]. Like other HPV-mediated neoplasms, viral integration with insertional mutagenesis may be a central etiologic component of JO-RRP [7]. Within papilloma cells, viral proteins create immunogenic epitopes that recruit infiltrating lymphocytes [6]. However, papilloma cells may secrete cytokines, which impair an effective type 1 T helper cell-like response [8]. T cells isolated from papillomas express an exhausted phenotype, and elevated programed death ligand 1 (*PD-L1*) mRNA expression has been observed in papilloma cells compared with normal laryngeal tissue [9]. Therefore, abrogation of programed cell death-1 (PD-1) signaling may enable T-cell recognition and effector activity against RRP. PD-1 inhibitors have demonstrated sustained responses in HPV-mediated malignancies, including carcinomas of the oropharynx [10], anus [11], and cervix [12]. We hypothesized that a PD-1 inhibitor would lead to papilloma regressions in patients with refractory JO-RRP. We treated two patients with JO-RRP using nivolumab, with the primary objective of assessing the clinical activity.

Subjects, Materials, and Methods

Clinical Treatment

This study was conducted at the H. Lee Moffitt Cancer Center and Research Institute in Tampa, FL, and Georgetown Lombardi Cancer Center in Washington, DC. We obtained informed consent for nivolumab clinical treatment for both patients. The study was performed in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. Nivolumab was obtained from a commercial patient assistance program and administered parenterally every 2 weeks at 3 mg/kg. Adverse events and outcome were assessed retrospectively based upon clinic documentation and graded according to Common Terminology Criteria for Adverse Events, version 4. Follow-up time from start of nivolumab treatment was 1.7 years for each patient.

Computed Tomography

Axial images were obtained on a standard-definition multislice 64-detector row scanner (Siemens Medical Solutions, Erlangen, Germany). Slices of 2.5 mm thickness through the chest and abdomen were collected after the administration of nonionic iohexol intravenous contrast (Omnipaque; GE Healthcare).

Immunohistochemistry

Papilloma tissue was collected and preserved in formalin 10% buffer for 24 hours and embedded in paraffin (FFPE). Suitable areas were selected by a pathologist and sectioned into 5 µm slides for immunohistochemistry. CD3 (Cell Marque, rabbit polyclonal) and PD-L1 (Merck, clone 22C3) analyses were performed with Dako Link 48 autostainer after high pH heat-induced epitope retrieval digestion. For p16 overexpression, a diffuse staining pattern was considered as positive, and focal staining pattern was considered as negative. FFPE tissue sections were run through Leica Jung Autostainer XL using Program 1 (routine hematoxylin and eosin stain) with Eosin Y (Sigma-Aldrich: E4382) and Harris Hematoxylin (Poly Scientific: S212) reagents. For CD3 antibodies, positive controls were performed on benign lymph node tissue obtained using the same fixation and retrievals methods as described above. Cellular localization was determined using high-power settings for each of the tumor markers. A commercial probe cocktail (800-2220, Ventana HPV II Family 6) was used to evaluate HPV genotype. HPV was also assessed with in situ hybridization staining of epithelial cell nuclei with supplied control slides and in accordance with the manufacturer's instructions (782-2839, Ventana).

Tumor DNA Sequencing

Suitable FFPE blocks containing papillomas were selected for DNA extraction. Comprehensive genomic profiling (CGP) was

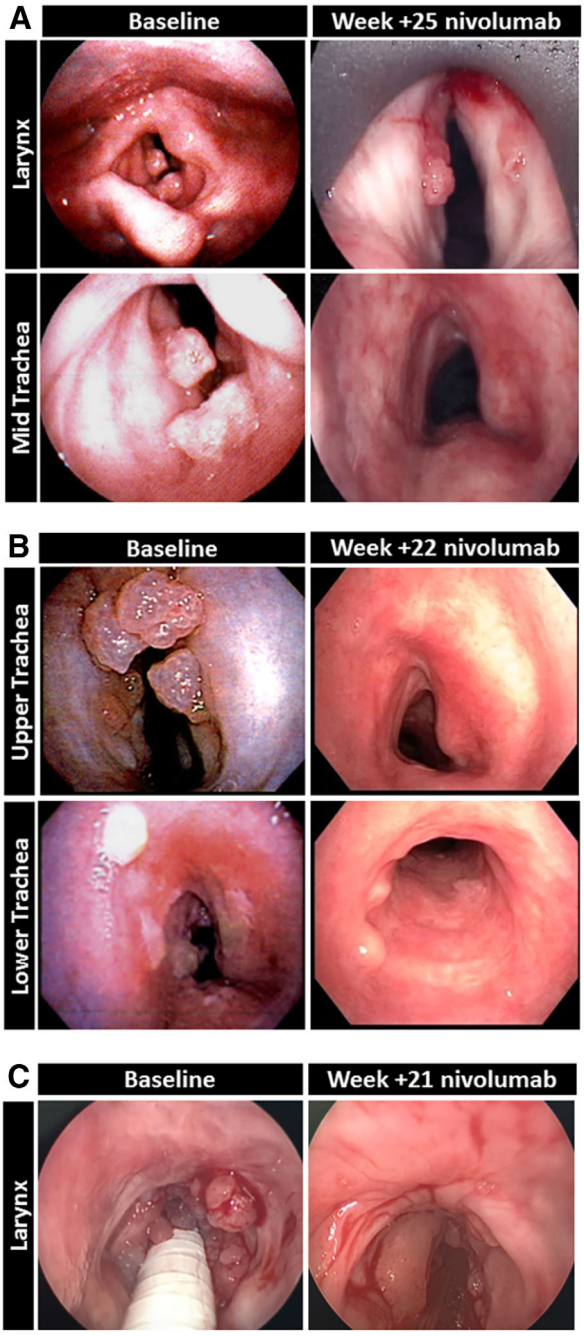

Figure 2. Representative pre- and posttreatment intraoperative photos showing improvement of tracheal papillomatosis after nivolumab. Direct fiberoptic laryngoscopies **(A)** and bronchoscopies (**B**) for Patient 1. Papillomas had improved or resolved at weeks 22 to 25, with residual stenosis. Intraoperative laryngoscopies **(C)** for Patient 2. At baseline, an endotracheal tube transverses the true vocal folds, which are poorly defined because of scarring and papilloma burden. On evaluation after nivolumab, papillomas occupied a smaller mucosal surface.

performed in a Clinical Laboratory Improvement Amendments-certified, College of American Pathologists-accredited laboratory (Foundation Medicine, Cambridge, MA). DNA was extracted and hybrid capture-based next-generation sequencing was performed (FoundationOne assay) on the full coding region of 315 genes (plus introns from 28 genes), which identified genomic alterations (substitutions, indels, copy number amplifications, and rearrangements). Tumor mutation burden (TMB) was calculated as mutations per megabase (Mb) and included the total number of base substitutions, indels, with germline alterations removed. Both synonymous and non-synonymous somatic alterations were counted, because synonymous alterations may have been reflective of the overall level of mutational processes present [13]. The presence of HPV DNA was assessed with polymerase chain reaction (PCR), using an HPV array with a set of type-specific primers, including HPV 6, 11, 16, and 18. This method was previously reported [7].

Statistics

A Poisson experiment was used to compare the number of surgery days before and after nivolumab (http://stattrek.com/online-calculator/poisson.aspx). A Poisson experiment examines the number of times an event occurs during a specified interval [14]. The Poisson random variable (n) was defined as the number of days of surgery over the time period after nivolumab, and the average rate of events (μ) was defined as the mean number of surgery days over an identical duration, averaged for the 5 years before nivolumab treatment. The cumulative probability that the true frequency of successes would be predicted to fall within the range of 0 and n, p ($X \leq n$), was calculated. A cumulative probability of $p < .05$ was considered significant.

Results

Patient 1

A 41-year-old female nonsmoker was initially diagnosed with JO-RRP at age 2. She had been previously treated with multiple systemic therapies over several decades, including interferon-α, celecoxib, indole-3-carbinol, and bevacizumab. Nonetheless, the patient continued to require repetitive surgical microlaryngoscopy debridements for her RRP every 3 to 5 months; therefore, we treated her with intravenous nivolumab (Fig. 1). She experienced improvement in vocal quality and stridor. Compared with a baseline laryngoscopy performed 2 days before her first treatment, a regression in size and number of papilloma lesions was evident on both bronchoscopy (Fig. 2A) and subsequent laryngoscopy (Fig. 2B). In addition, surveillance computed tomography (CT) of the thorax showed reduction of a faintly visible papilloma in the distal trachea (Fig. 3A). After completing 9 months of nivolumab treatment, the patient remains in remission, receiving regular CT scans and visits every 3 months. She has not required any other JO-RRP treatment or surgery since receiving nivolumab. Over the time studied, her probability of surgery was lower compared with the preceding 5 years ($p = .022$). She did experience an adverse event of grade 2 adrenal insufficiency at month 9, requiring supplemental hydrocortisone. We electively discontinued nivolumab after this adverse event and opted for surveillance.

An archival pretreatment biopsy of a bronchial papilloma showed no mitotic figures or high-grade dysplasia (Fig. 4A). Squamous epithelium overlying fibrovascular cores with elongated nuclei were present and characteristic of papillomatosis. No mitotic figures or high-grade dysplasia was identified.

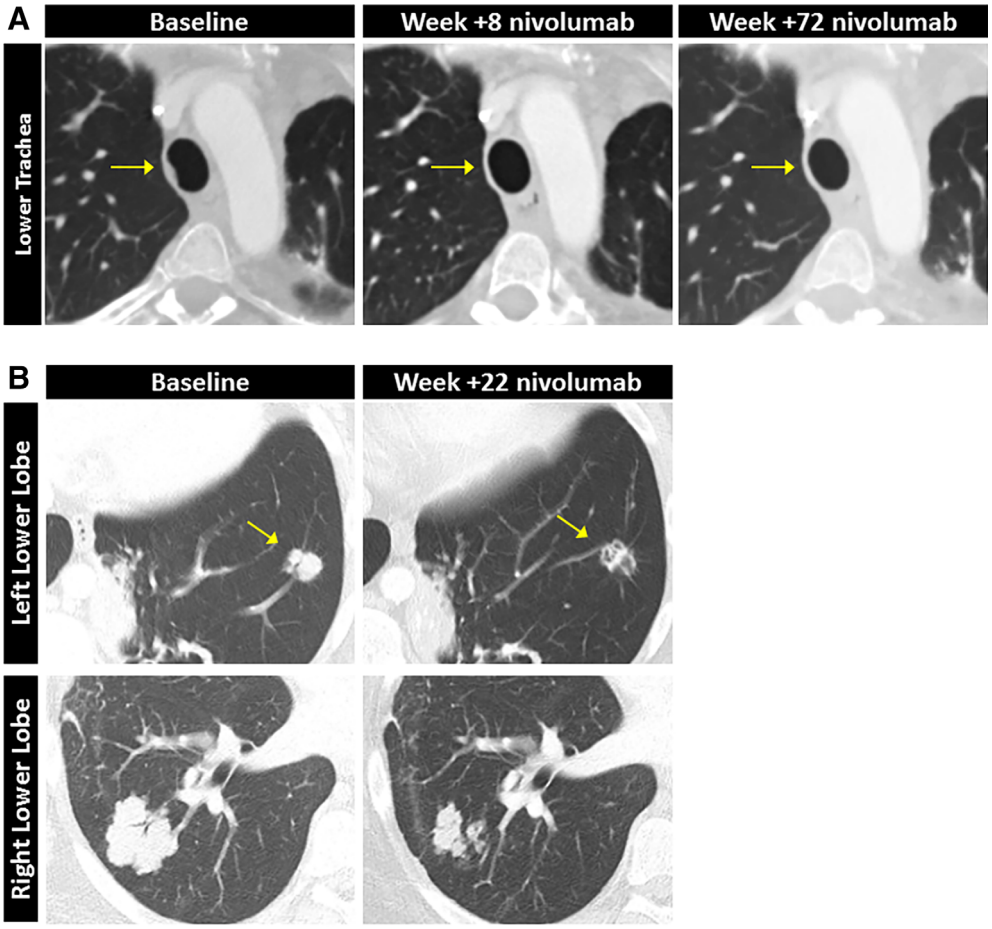

Figure 3. Pre- and posttreatment computed tomography (CT) images of Patients 1 and 2. **(A):** Pre- and posttreatment CT axial images of Patient 1 showing improvement of a faintly visible squamous papilloma in distal trachea. **(B):** Pre- and posttreatment CT axial images of Patient 2 showing improvement of parenchymal lung papillomas consisting of biopsy-confirmed recurrent respiratory papillomatosis.

Immunohistochemistry detected focal p16 expression. In situ hybridization probe for HPV-6 was positive in an episomal pattern, as well as type-specific primers for HPV-6 DNA. CGP revealed an inactivating mutation in the *ARID1A* locus and estimated a TMB of six mutations per Mb. A distribution of T cells was observed primarily in the stroma, and PD-L1 protein expression was absent, with minimal expression in stromal immune cells (Fig. 4B, C). No posttreatment biopsy was available for comparison.

Patient 2

A 27-year old male nonsmoker was initially diagnosed with JO-RRP at age 3 and had previously received interferon-α, methotrexate, intralesional cidofovir, vorinostat, artesunate, recombinant HPV-9 valent vaccine, and intralesional bevacizumab. His papillomas involved the trachea and bronchus, including the lung parenchyma. He had previously received over 350 surgeries for his RRP. Despite prior therapies, he had progressively enlarging papillomas. Therefore, we treated with intravenous nivolumab. Within several weeks, he experienced symptomatic improvement. On subsequent laryngoscopy, an improvement in the total mucosal area and extent of the papillomas was evident (Fig. 2C). Moreover, several papillomas within the lung parenchyma did have mild shrinkage, although some other lung tumors enlarged, consistent with a mixed response (Fig. 3B). At the time of data cutoff, he had required two subsequent microlaryngoscopies. Although his mean frequency of debridement had decreased from 3.6 yearly at baseline to 1.3 yearly after nivolumab, it was not statistically significant (p = .29). He did have intermittent grade 1 skin rash, treated with a topical corticosteroid and moisturizer.

The laryngeal tumor of Patient 2 previously had HPV-11 genotype detected by type-specific primers and confirmed with sequencing [7]. CGP revealed a *PAX5* gene mutation and wild-type *ARID1A* locus. TMB was estimated at three mutations per Mb. Immunohistochemistry showed scattered expression of p16 in 10% of cells. Similar to the RRP of Patient 1, abundant T cells were present in the stroma, but few intraepithelial T cells were detected. PD-L1 expression was absent in papilloma epithelial cells and focally positive on the immune cells in the stroma (Fig. 4D–F). An on-treatment biopsy at week 21 of nivolumab was examined. Compared with pretreatment, the on-treatment biopsy showed increased amounts of both stromal and intraepithelial T cells. In addition, there was increased PD-L1 expression within the epithelial and stromal cells (Fig. 4G–I).

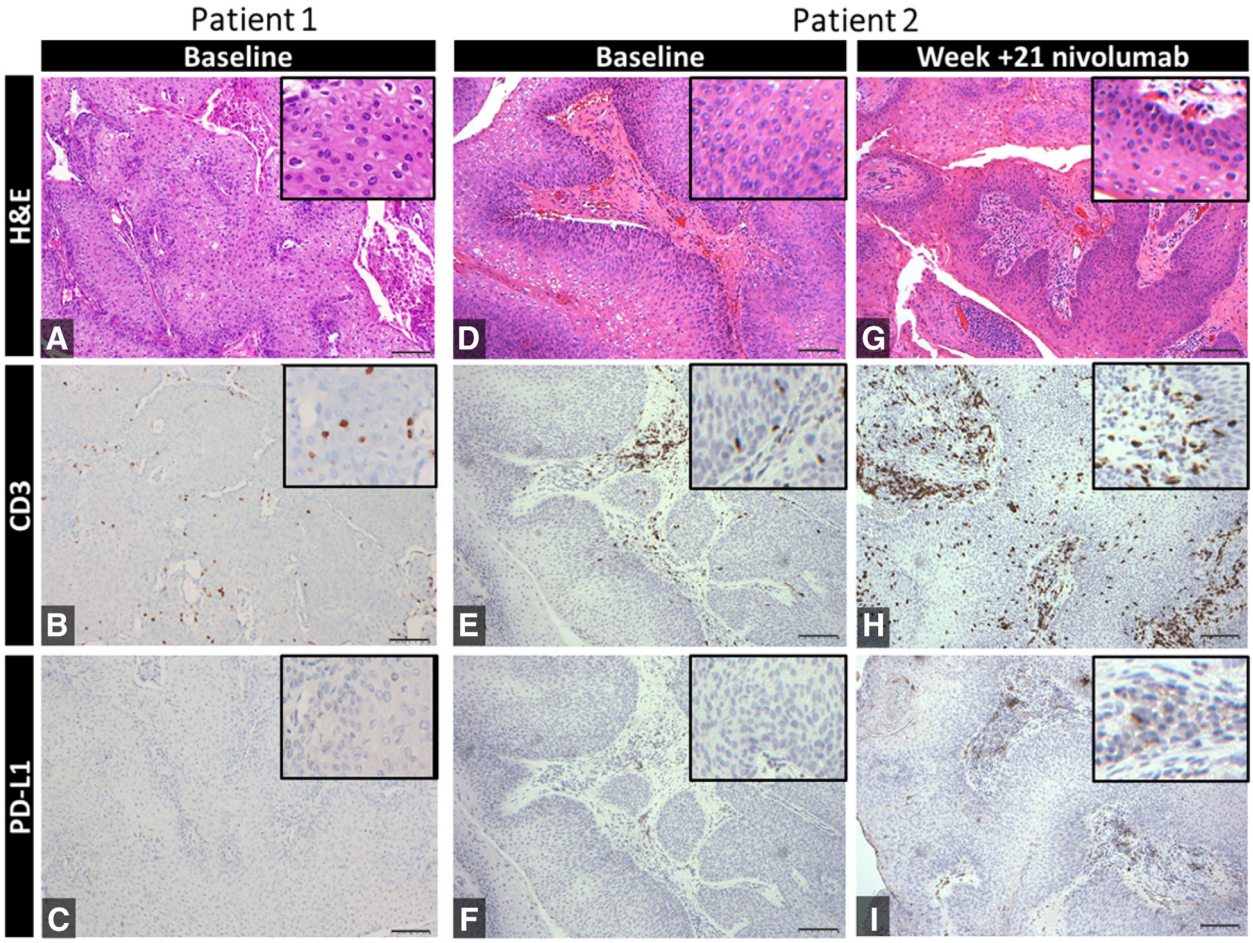

Figure 4. Representative immunohistochemistry of respiratory squamous papilloma biopsies. Pretreatment bronchial papilloma from Patient 1 showing stromal $CD3^+$ T cells **(A)**, few intraepithelial T cells **(B)**, and weak or absent PD-L1 expression **(C)**. Pretreatment tracheal papilloma from Patient 2 showing similar stromal T cells **(D)**, rare intraepithelial T cells **(E)**, and minimal PD-L1 expression **(F)**. On-treatment biopsy of residual tracheal papilloma from Patient 2, showing more abundant T-cell infiltration **(G, H)** and moderate PD-L1 expression **(I)**. Images are ×10 or ×40 magnification with smaller inserts at ×40 or ×100. Bar denotes 100 μm and approximately 50 μm for inserts.
Abbreviations: H&E, hematoxylin and eosin; PD-L1, programmed death ligand 1.

Discussion

There has been intense interest in expanding PD-1 inhibitors into the treatment of histologically benign conditions. In this study, clinical activity was identified using nivolumab to treat two patients with refractory JO-RRP, particularly for Patient 1. This clinical benefit was primarily reduction of papilloma size, subjective symptom improvement, and decreased need for microlaryngoscopy. The presence of HPV-11 in Patient 2 may have caused his less favorable outcome, because the HPV-11 subtype is associated with a more aggressive RRP phenotype [15]. Historically, few treatment options have been effective in controlling refractory JO-RRP. Although intralesional cidofovir has been shown to increase relapse-free interval, the optimal dose and interval remains unknown [3]. Interferon-α may reduce the frequency of surgical excision for RRP but has limited tolerability because of cytokine-related toxicity [16]. Unfortunately, the quadrivalent HPV peptide vaccine has yielded inconsistent results for JO-RRP, with no trials thus far showing a reduction in validated severity scores [17].

In the current report, both patients appeared to have an excluded infiltrate of stromal T cells at baseline and weak PD-L1 expression within the immune cells only. A spatially excluded infiltrate, or type IV microenvironment, may indicate that other suppressive factors are promoting immune tolerance [18]. Within RRP epithelial cells, an abundance of PD-1 and CD69 is detectable on infiltrating T cells, as well as high levels of *PD-L1* mRNA expression [9]. Similar to RRP, the frequency of $PD\text{-}1^+CD4^+$ T cells is significantly increased in recurrent HPV-related genital warts, as compared with primary lesions [19]. Nonetheless, substantial heterogeneity exists in PD-L1 expression between papillomas [20]. Along these lines, the PD-1 axis appears to be one of several pathways in the microenvironment of RRP acting to cause immune suppression, including an imbalance of immature Langerhans cells, defective natural killer cells, and enrichment of $CD4^+$ Helios-negative regulatory T cells [21]. Among patients with RRP, regulatory T cells may be recruited to the papilloma stroma by CC chemokine receptor 4 engagement through aberrant expression of chemokine ligand 17 [9].

Large-scale whole-exome sequencing (WES) of JO-RRP has not been reported to date, and the distribution of mutational load in JO-RRP remains unknown. The mutational load within the papillomas described herein was fairly low, consistent with other histologically benign processes. Along these lines, HPV-positive head and neck cancers (HNSCC) may have lower TMB and higher mutation clonality than HPV-negative HNSCC, regardless of organ site [22]. Likewise, HPV-associated anal squamous cancers are also characterized by low TMB, with a mean of 1.6 to 3.5 mutations per Mb [11]. TMB has correlated with response to PD-1 inhibitors across multiple types of tobacco-related epithelial cancers [23], including HPV-negative HNSCC [24]. However, no predictive value of TMB was detected for PD-1 antibodies in HPV-positive HNSCCs [24]. Therefore, it is possible that effective T-cell responses to HPV-positive squamous neoplasms are due to the dominance of specific virally derived or somatic neoepitopes. Although HPV E6 or E7 reactive tumor-infiltrating lymphocyte was associated with response to adoptive cell transfer in HPV 16- or 17-related cervical cancers, effector T-cell responses were not actually directed against virally associated antigens [25]. Instead, the dominant T-cell clones primarily included T-cell receptors with high affinity for cancer-specific neoantigens and were mostly PD-1$^+$ [25].

This current report was characterized by important limitations, based in part on its retrospective composition. Unfortunately, the immune cells which may have been responsible for the treatment effect could not be further characterized because of paucity of archival tissue. Likewise, cells were not preserved for flow cytometry or functional assays. In addition, TMB was estimated using a fixed number of 315 analyzed genes rather than WES. Nonetheless, this 315-gene panel has been shown to correlate well to mutational load based on WES, even using FFPE samples [13]. Along these lines, fresh tumor biopsies were not available for mRNA extraction and quantitative PCR. Finally, because the clinical visits and surveillance endoscopies were not conducted with an RRP staging tool, we were not able to retrospectively assess disease severity using a validated instrument, such as the Derkay score [1]. A quantitative staging tool would have been useful to measure patient progress and permit comparison between cases. Above all, this report is limited to describing the treatment of only two patients; therefore, definitive conclusions about the efficacy and safety of PD-1 inhibitors for RRP must be reserved for dedicated clinical trials.

Conclusion

Nivolumab showed promising early clinical activity for the two patients with JO-RRP described herein. Further clinical investigation into the safety and response rates for PD-1 axis inhibitors for JO-RRP is still required. To this point, caution is warranted before PD-1 inhibitors can be adopted into practice for JO-RRP. This is because the adverse effect profile of immune checkpoint inhibitors may be more pronounced when applied to the treatment of benign diseases, as compared with advanced cancer. Clinical trials assessing pembrolizumab (NCT02632344) and avelumab (NCT02859454) are currently underway for adults with refractory RRP.

Acknowledgments

We thank Drs. Christine H. Chung, Scott J. Antonia, and Anna R. Giuliano for their thoughtful review. We also thank Sonya J. Smyk, Moffitt Cancer Center, for editorial support. She did not receive any compensation beyond her regular salary. A.W.W. is currently affiliated with Celsius Therapeutics.

Author Contributions

Conception/design: Ben C. Creelan, M. Usman Ahmad, Frank J. Kaszuba, Farah K. Khalil, Allison W. Welsh, Metin Ozdemirli, Nazaneen N. Grant, Deepa S. Subramaniam

Provision of study material or patients: Ben C. Creelan, M. Usman Ahmad, Frank J. Kaszuba, Farah K. Khalil, Allison W. Welsh, Metin Ozdemirli, Nazaneen N. Grant, Deepa S. Subramaniam

Collection and/or assembly of data: Ben C. Creelan, M. Usman Ahmad, Frank J. Kaszuba, Farah K. Khalil, Allison W. Welsh, Metin Ozdemirli, Nazaneen N. Grant, Deepa S. Subramaniam

Data analysis and interpretation: Ben C. Creelan, M. Usman Ahmad, Frank J. Kaszuba, Farah K. Khalil, Allison W. Welsh, Metin Ozdemirli, Nazaneen N. Grant, Deepa S. Subramaniam

Manuscript writing: Ben C. Creelan, M. Usman Ahmad, Frank J. Kaszuba, Farah K. Khalil, Allison W. Welsh, Metin Ozdemirli, Nazaneen N. Grant, Deepa S. Subramaniam

Final approval of manuscript: Ben C. Creelan, M. Usman Ahmad, Frank J. Kaszuba, Farah K. Khalil, Allison W. Welsh, Metin Ozdemirli, Nazaneen N. Grant, Deepa S. Subramaniam

Disclosures

Ben C. Creelan: Roche/Genentech, Takeda, AbbVie, Bristol-Myers Squibb, AstraZeneca, Celgene (C/A), Boehringer Ingelheim (RF); **Farah Khalil:** Merck & Co (C/A); **Allison W. Welsh:** Celsius Therapeutics, Foundation Medicine (E, OI); **Deepa S. Subramaniam:** Takeda, AstraZeneca LLC (C/A), Bristol-Myers Squibb (RF, SAB).

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

References

1. Derkay CS, Wiatrak B. Recurrent respiratory papillomatosis: A review. Laryngoscope 2008; 118:1236–1247.

2. Kashima HK, Shah F, Lyles A et al. A comparison of risk factors in juvenile-onset and adult-onset recurrent respiratory papillomatosis. Laryngoscope 1992;102:9–13.

3. Kim HT, Baizhumanova AS. Is recurrent respiratory papillomatosis a manageable or curable disease? Laryngoscope 2016;126:1359–1364.

4. Wilcox LJ, Hull BP, Baldassari CM et al. Diagnosis and management of recurrent respiratory papillomatosis. Pediatr Infect Dis J 2014;33:1283–1284.

5. Wadood Mohammed A. Role of bevacizumab in juvenile onset recurrent respiratory papillomatosis. Otorhinolaryngol Head Neck Surg 2016;1: 42–43.

6. Bonagura VR, Hatam LJ, Rosenthal DW et al. Recurrent respiratory papillomatosis: A complex defect in immune responsiveness to human papillomavirus-6 and-11. APMIS 2010;118:455–470.

7. Yuan H, Myers S, Wang J et al. Use of reprogrammed cells to identify therapy for respiratory papillomatosis. N Engl J Med 2012;367:1220–1227.

8. DeVoti JA, Rosenthal DW, Wu R et al. Immune dysregulation and tumor-associated gene changes in recurrent respiratory papillomatosis: A paired microarray analysis. Mol Med 2008;14:608.

9. Hatam LJ, Devoti JA, Rosenthal DW et al. Immune suppression in premalignant respiratory papillomas: Enriched functional CD4+Foxp3+ regulatory T cells and PD-1/PD-L1/L2 expression. Clin Cancer Res 2012;18:1925–1935.

10. Ferris RL, Blumenschein Jr G, Fayette J et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. N Engl J Med 2016;2016:1856–1867.

11. Morris VK, Salem ME, Nimeiri H et al. Nivolumab for previously treated unresectable metastatic

anal cancer (NCI9673): A multicentre, single-arm, phase 2 study. Lancet Oncol 2017;18:446–453.

12. Frenel JS, Le Tourneau C, O'Neil BH et al. Pembrolizumab in patients with advanced cervical squamous cell cancer: Preliminary results from the phase Ib KEYNOTE-028 study. J Clin Oncol 2016;34(suppl 15):5515A.

13. Chalmers ZR, Connelly CF, Fabrizio D et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 2017;9:34.

14. Le Cam L. An approximation theorem for the Poisson binomial distribution. Pacific J Math 1960;10:1181–1197.

15. Wiatrak BJ, Wiatrak DW, Broker TR et al. Recurrent respiratory papillomatosis: A longitudinal study comparing severity associated with human papilloma viral types 6 and 11 and other risk factors in a large pediatric population. Laryngoscope 2004;114(S104):1–23.

16. Gerein V, Rastorguev E, Gerein J et al. Use of interferon-alpha in recurrent respiratory papillomatosis: 20-year follow-up. Ann Otol Rhinol Laryngol 2005;114:463–471.

17. Hermann JS, Weckx LY, Nürmberger JM et al. Effectiveness of the human papillomavirus (types 6, 11, 16, and 18) vaccine in the treatment of children with recurrent respiratory papillomatosis. Int J Pediatr Otorhinolaryngol 2016;83:94–98.

18. Teng MW, Ngiow SF, Ribas A et al. Classifying cancers based on T-cell infiltration and PD-L1. Cancer Res 2015;75:2139–2145.

19. Chang DY, Song SH, You S et al. Programmed death-1 (PD-1)-dependent functional impairment of CD4+ T cells in recurrent genital papilloma. Clin Exp Med 2014;14:305–313.

20. Liu T, Greenberg M, Wentland C et al. PD-L1 expression and CD8+ infiltration shows heterogeneity in juvenile recurrent respiratory papillomatosis. Int J Pediatr Otorhinolaryngol 2017;95:133–138.

21. Lucs AV, DeVoti JA, Hatam L et al. Immune dysregulation in patients persistently infected with human papillomaviruses 6 and 11. J Clin Med 2015;4:375–388.

22. Chung CH, Frampton GM, Chalmers ZR et al. Genomic profiling of squamous malignancies across anatomic sites. J Clin Oncol 2017;35 (suppl 15):11512A.

23. Rizvi NA, Hellmann MD, Snyder A et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015;348:124–128.

24. Haddad RI, Seiwert TY, Chow LQM et al. Genomic determinants of response to pembrolizumab in head and neck squamous cell carcinoma (HNSCC). J Clin Oncol 2017;35(suppl 15): 6009A.

25. Stevanović S, Pasetto A, Helman SR et al. Landscape of immunogenic tumor antigens in successful immunotherapy of virally induced epithelial cancer. Science 2017;356:200–205.