Tadalafil Augments Tumor Specific Immunity in Patients with Head and Neck Squamous Cell Carcinoma

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Abstract

Purpose: To determine if phosphodiesterase 5 (PDE5) inhibitors can augment immune function in patients with head and neck cancer through inhibition of myeloid-derived suppressor cells (MDSC).

Experimental Design: We performed a randomized, prospective, double blinded, placebo controlled, phase II clinical trial to determine the in vivo effects of systemic PDE5 inhibition on immune function in patients with head and neck squamous cell carcinoma (HNSCC).

Results: Tadalafil augmented immune response, increasing ex vivo T-cell expansion to a mean 2.4-fold increase compared with 1.1-fold in control patients ($P = 0.01$), reducing peripheral MDSC numbers to mean 0.81-fold change compared with a 1.26-fold change in control patients ($P = 0.001$), and increasing general immunity as measured by delayed type hypersensitivity response ($P = 0.002$). Tumor-specific immunity in response to HNSCC tumor lysate was augmented in tadalafil-treated patients ($P = 0.04$).

Conclusions: These findings demonstrate that tadalafil augments general and tumor-specific immunity in patients with HNSCC and has therapeutic potential in HNSCC. Evasion of immune surveillance and suppression of systemic and tumor-specific immunity is a significant feature of head and neck cancer development. This study demonstrates that a PDE5 inhibitor, tadalafil, can reprogram tumor-specific immune suppression in patients with head and neck cancer, with potential for therapeutic application. Clin Cancer Res; 21(1); 30–38. ©2015 AACR.

Introduction

Head and neck squamous cell carcinoma (HNSCC) affects more than 50,000 individuals annually in the United States (1). Immune response is of critical importance in malignant transformation for a variety of solid tumors (2). Patients with HNSCC demonstrate significant impairment in immune recognition of tumor cells, and evasion from immune response is a significant factor in HNSCC carcinogenesis (3).

In vitro evidence suggests that immune-mediated tumor cell killing occurs through mechanisms distinct from chemotherapy-mediated killing and is a significant component of the therapeutic response (4). In a study of colorectal cancer, characteristics of the immune infiltrate were more predictive of clinical outcomes than the classic TNM staging (5). In ovarian cancer, 5-year overall survival was 38.7% for patients with an intratumoral T-cell infiltrate compared with 4.5% in those without detectable intratumoral T cells (6). In addition, the presence of intratumoral regulatory T cells was associated with reduced survival in ovarian cancer (7). There is a high rate of anergy for patients with HNSCC, with PPD nonresponsiveness in 50% of patients, and anergy has been associated with poor survival (8–17).

The immune system of cancer-bearing hosts has developed a complex network ultimately leading to the development of tumor-induced tolerance that includes regulatory T cells (Tregs), natural killer T cells (NKT), tumor-associated macrophages (TAM), indoleamine 2,3-dioxygenase (IDO), and myeloid-derived suppressor cells (MDSC) (18). However, MDSCs play an increasingly critical link between the innate and adaptive immune system through their ability to influence the fate of Tregs in antigen-specific T-cell tolerance (19–21). MDSCs are induced by tumor-derived GM-CSF, VEGF, and IL6 and ultimately lead...
Translational Relevance

Patients with head and neck squamous cell carcinoma (HNSCC) demonstrate significant impairment in immune recognition of tumor cells, and evasion from immune response is a significant factor in HNSCC carcinogenesis. These data demonstrate that tadalafl is effective in reversing suppression of immune recognition of head and neck cancer in patients taking daily tadalafl. Tadalafl was chosen due to a favorable toxicity profile and the ability of this drug to provide long-acting effect with once daily dosing. Ongoing clinical trials include the use of tadalafl to chronically modulate immune responses in combination with conventional therapy in HNSCC and to determine whether vaccine responses can be augmented with this approach.

to T-cell immune dysfunction through mechanisms including the production of arginase-1 (Arg1) and inducible nitric oxide synthase (NOS2). These two pathways lead to T-cell dysfunction through the downregulation of the ζ-chain of the T-cell receptor and nitrosylation of the tyrosines on the TCR of CD8 cells (22, 23). Studies of MDSC-mediated immune suppression show that MDSCs exploit the metabolism of l-arginine (l-Arg) to render lymphocytes unresponsive to antigen stimulation (24, 25). NO-mediated suppression of T-cell activation is modulated by IL2 receptor signaling pathways and by direct proapoptotic effects (26, 27). The ability of NOS inhibitors to reverse MDSC-induced immunosuppression both in vivo and in vitro confirms the immunoregulatory role of NO.

Functional elimination of MDSCs can overcome the immunosuppressive state (28), and phosphodiesterase 5 (PDE5) inhibitors block both nitric oxide as well as arginase 1 production and restore tumor-specific T-cell function (Fig. 1; ref. 29). In vitro activation of peripheral T cells from patients with HNSCC is increased with short-acting PDE5 inhibitors (29).

We hypothesized that PDE5 blockade may reverse tumor-associated immune suppression in patients with HNSCC and designed a randomized, placebo controlled, phase II biomarker endpoint trial in patients with HNSCC to define the effects of an oral PDE5 blocking agent on peripheral T-cell activation, global immune status, and MDSC function. We used tadalafl due to a favorable toxicity profile and the ability of this drug to provide long-acting PDE5 blockade with once daily dosing.

Materials and Methods

Trial design

We performed a double blinded, randomized two-arm placebo controlled study in which patients with biopsy proven HNSCC were randomized to tadalafl 20 mg once a day (20 mg daily) for at least 10 days before their definitive therapy or to placebo before definitive therapy in a 5:3 ratio (Supplementary Fig S1). To define general systemic immunity, delayed type hypersensitivity (DTH) to Candida was determined before initiation of therapy and 2 days before the end of therapy. Induration and erythema were assessed at 48 hours. This clinical trial was approved by the Johns Hopkins Medical Institutions Review Board (Clinicaltrials.gov identifier, NCT00894413), and all participants gave written informed consent to participate in the trial. Exclusion criteria included presence of existing immune suppression; a full list of inclusion and exclusion criteria are listed in Supplementary Materials. The majority of patients did not undergo surgical resection and were treated with primary radiation therapy (Supplementary Materials).

U266B1 (U266) and NCI-H292 (H292) myeloma cell lines were purchased from ATCC and passaged as directed. Briefly, U266 and H292 were cultured in complete RPMI media (Life Technologies) in T75 flasks at a concentration of 1 × 10^6 cells/mL and 5 × 10^5 cells/mL (respectively) with media renewal 2 to 3 times weekly. Cells are passaged no more than three total passages before aliquots are made for storage in the vapor phase of liquid N_2. Cells are characterized by staining with human CD38 and CD138/C2 (BD Biosciences) and mycoplasma testing is conducted in the laboratory on a regular basis using the PCR-based MycoDect kit from Greiner Bio-One. Head and neck cell lines are mycoplasma free based on the PCR-based MycoDect Kit from GreinerBio-One North America, Inc. Cell lines are authenticated at Johns Hopkins Genetics Research Core Facility using a Short Tandem Repeats Profile Report for Cell Line Authentication [A Promega PowerPlex 16HS kit was used to PCR amplify 15 short tandem repeat (STR) loci plus a gender determining marker, Amelogenin]. The PCR product was electrophoresed on an ABI Prism 3730xl Genetic Analyzer using an Internal Lane Standard 600. Data was analyzed using the GeneMapper v 4.0 software (Applied Biosystems). Cell lines were confirmed within 6 months of lysate preparation. To produce lysate, cells are grown in large cultures so that at least 50 to 100 × 10^6 cells can be obtained. Cell pellets are made by centrifuging the cellular culture. The cell pellets are exposed to a series of a minimum of eight freeze/thaw cycles in dry ice/isopropanol bath and then sonicated to further shear the cellular membrane. Protein concentration analysis is performed using a BSA protein kit (Thermo-Fisher Scientific).

Statistical analyses

The study was powered for 40 patients, 25 to tadalafl and 15 to placebo, calculated to provide 81% power for the statistical detection of a 0.8 SD difference in post-pre change between the two groups at one-sided Type I error rate of 0.05. The study was terminated as per protocol after 40 patients were randomized. Of these 40 patients, 32 patients completed the full course of treatment and had both evaluable baseline and day 10 to 14 immune assessments. For comparison of treated and control group demographic variables values were calculated using the Fisher exact tests for categorical variables and Wilcoxon rank-sum tests for continuous variables.

Pre- and posttreatment change in the immune response outcome was quantified as a fold change (post/pre) and data were log transformed to improve normality (Table 1). A Wilcoxon rank-sum test was performed for the comparison of immune response endpoints between the two arms due to the skewed distributions. Because only increased immunity would warrant further study of the regimen, statistical tests for the immune response outcomes were one-sided unless otherwise indicated and statistical significance was considered at P < 0.05. A multivariable linear regression analysis was further performed adjusting for baseline clinical variables, including pretreatment values, tobacco exposure, site, HPV status, and clinical T stage. The primary analyses (n = 32) included patients who were randomized and completed full course of treatment and had baseline and postbaseline (day 10) assessments. These analyses were repeated in an intent-to-treat population, including all patients who were randomized...
where a conservative approach of baseline-observation-carried-forward, i.e., assuming no change in response to tadalafil administration, was applied to impute missing outcome data.

Forty-five patients were initially screened in the study; 5 were excluded at the time of screening due to the presence of distant metastases (n = 3), high serum calcium (n = 1), or a second cancer (n = 1). Eight patients (20%) withdrew after enrollment (of these, 4 voluntarily discontinued before starting therapy due to trial logistics or enrollment in a competing trial, 2 due to findings of distant metastases/other cancers during routine staging after randomization before starting therapy, and 2 due to adverse events while on study related to study drug side effects (back pain). Patients with oropharynx HNSCC without available HPV status were excluded from analyses of HPV status and outcomes (n = 3); the remaining patients from nonoropharyngeal sites were considered HPV negative. Analyses were carried out using SAS (version 9.3; SAS Institute) and R statistical software (version 2.15.2).
Table 1. Immune response outcomes between treatment arms

<table>
<thead>
<tr>
<th>Immune response</th>
<th>Placebo</th>
<th>Tadalafil</th>
<th>Fold change</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INOS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>14</td>
<td>17</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>1.00 (0.28)</td>
<td>1.10 (0.28)</td>
<td>0.81 (0.56)</td>
<td>0.66 (0.43)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>1.02 (0.99)</td>
<td>1.17 (1.34)</td>
<td>1.06 (1.47)</td>
<td>0.90 (1.10)</td>
</tr>
<tr>
<td><strong>MDSC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>15</td>
<td>17</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>41.7 (16.7)</td>
<td>54.2 (20.0)</td>
<td>44.8 (19.9)</td>
<td>0.81 (0.13)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>51.2 (8.0)</td>
<td>128.0 (2.0)</td>
<td>117.9 (1.9)</td>
<td>0.81 (0.13)</td>
</tr>
<tr>
<td><strong>CD8</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>14</td>
<td>15</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>22.1 (17.4)</td>
<td>19.6 (12.5)</td>
<td>19.5 (13.5)</td>
<td>0.99 (0.32)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>21.1 (4.6)</td>
<td>1.05 (0.35)</td>
<td>1.16 (0.45)</td>
<td>0.99 (0.32)</td>
</tr>
</tbody>
</table>

NOTE: Fold changes between treatment arms were compared and P values calculated using two-sided exact Wilcoxon rank-sum tests.

* P < 0.05.
** P < 0.01.
*** P < 0.001.

Results

PDE-5 inhibition inhibits immunosuppressive function

Of the 40 enrolled and randomized patients, 34 started treatment with tadalafl or placebo, 2 of these withdrew before completion of treatment due to back pain, and 32 completed therapy. We analyzed the 32 randomized patients who completed therapy to determine the effects of tadalafl on immune function. The tadalafl-treated group had a higher proportion of smokers and higher T-stage primary tumors (Table 2).

We have previously shown that PDE inhibition suppresses iNOS and arginase-1 (Arg-1) production, thereby inhibiting the immunosuppressive function of MDSCs (29). Inducible NOS (iNOS) and Arg-1 production was determined by quantitative RT-PCR in isolated CD15+ cells. This approach was chosen in light of the fact that CD15 isolation would capture all the CD14 (putative monocytic MDSCs) as well as the CD15+ cells (putative granulocytic MDSCs; Supplementary Fig. S4). Both Arg-1 and iNOS activity was significantly decreased in tadalafl-treated patients with Arg1 showing a mean 0.83-fold change (P = 0.004) in tadalafl-treated patients versus a 1.0-fold change in control patients (Fig. 2). iNOS was significantly reduced in tadalafl-treated patients with a mean 0.66-fold change (P = 0.003) yet slightly increased in control patients 1.02-fold (Fig. 2).

The MDSC phenotype in this study was defined as CD14+ or CD15+/CD33+/HLA-DRlow/IL-4R+ as well as coexpression of CD14+/CD15+/CD33+/HLA-DRlow/IL-4R+ (See Supplementary Data for gating strategy). Inclusion of IL4R+ was validated in earlier studies showing the immunosuppressive function MDSCs expressing this phenotype (21, 30). Although no definitive phenotype has been identified for MDSCs, this phenotype was validated in the accompanying article (Weed and colleagues). We noted both a profound reduction in MDSC numbers with a mean 0.81 change in the treated cohort as well as a 1.28-fold increase in control patients (P < 0.0001, Fig. 2). These findings could suggest that PDE5 inhibition eliminates a positive autocrine feedback loop leading to either a shorter half-life of MDSCs or impaired de novo generation or that in its absence MDSC numbers can continue to increase. We previously showed that MDSC inhibition could reduce Tregs (20). Our analysis demonstrated a statistically significant reduction in Tregs in the tadalafl-treated patients with a relative increase in the placebo group (Treg, mean placebo 1.79, tadalafl 0.84; P = 0.0006).

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Clin Cancer Res; 21(1) January 1, 2015 33

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PDE5 inhibition enhances systemic immunity

Because MDSCs primarily suppress T-cell function, we examined the effect of PDE5 inhibition. Peripheral blood mononuclear cells from both cohorts were stimulated with anti-CD3/CD28 paramagnetic beads, and T-cell expansion was determined by flow cytometry. Tadalafil-treated patients showed a significant increase in T-cell proliferation, with a mean 2.4-fold increase compared with a 1.1-fold increase in control patients \( (P = 0.003; \text{Fig. 3}) \). Tadalafil increased T-cell activation on both CD4+ T cells, mean 1.6-fold in tadalafil-treated versus 1.3-fold in control treated patients \( (P = 0.042) \), and CD8 T cells increased 1.4-fold versus no change \( (P = 0.005) \) as measured by upregulation of CD69 expression on the T-cell subpopulations (Table 3). Other immune response measures, including CD8 effector, CD8 effector memory, CD8 naive, and TCR Zeta populations did not show significant change (Supplementary Data).

To determine the impact of systemic immunity, delayed type hypersensitivity (DTH) reactions were measured to Candida. Tadalafil-treated patients showed a median 4.7-fold increase in DTH reactivity compared with a median 1.4-fold in the placebo-treated patients \( (P = 0.002; \text{Fig. 3}; \text{Table 3}) \).

To further confirm the effect of tadalafil therapy, we performed a multivariate analysis to determine the potential confounding effect of clinical factors on immune measures. We examined potentially confounding factors between cohorts, including T stage, site, tobacco exposure, and HPV status, as this represents a major discriminating factor in HNSCC tumor biology. No immune differences were observed between patients with HPV positive or negative (Supplementary Table S1 and Supplementary Fig. S2).

To confirm the robustness of this analysis, we examined an intent-to-treat analysis in all 40 patients enrolled in the trial regardless of completion of treatment. Univariate analysis showed that in the tadalafil intent-to-treat group there was a significant increase in T-cell expansion \( (P = 0.037) \), systemic response by DTH to Candida \( (P = 0.032) \), CD4+/CD69+ cells \( (P = 0.041) \), and CD8+ cells \( (P = 0.014) \).

Using a multivariate model to control for tobacco exposure, site, HPV and T stage, the increase in T-cell expansion in the tadalafil group remained significant \( (P = 0.022) \). Similarly, the increase in CD8+ T cells in the tadalafil-treated group remained significant \( (P = 0.040; \text{Supplementary Data}) \). We also found a tight correlation between changes in iNOS and Arg-1 expression and TCR zeta expression, MDSC decrease, and enhanced immune response (Supplementary Table S5 and Supplementary Fig. S3). The increase in systemic immunity and increase in CD4+ T cells did not retain significance on multivariate analysis.

We also examined the effect of dosage by examining body mass index (BMI) and body surface area (BSA) in relation to T-cell activation and other immune response parameters in the tadalafil-treated group. We did note a suggestion of decrease in

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**Table 2. Baseline patient characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n = 32)</th>
<th>Placebo (n = 15)</th>
<th>Tadalafil (n = 17)</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>Mean (SD)</td>
<td>58 (12)</td>
<td>55 (6)</td>
<td>60 (15)</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td>Male</td>
<td>29 (91)</td>
<td>13 (87)</td>
<td>16 (94)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td>White</td>
<td>27 (84)</td>
<td>13 (87)</td>
<td>14 (82)</td>
</tr>
<tr>
<td>Tobacco exposure, n (%)</td>
<td>Black/other</td>
<td>5 (16)</td>
<td>2 (13)</td>
<td>3 (18)</td>
</tr>
<tr>
<td>HPV status, n (%)</td>
<td>Never</td>
<td>10 (31)</td>
<td>8 (53)</td>
<td>2 (12)</td>
</tr>
<tr>
<td>Site, n (%)</td>
<td>Oral cavity</td>
<td>7 (22)</td>
<td>1 (7)</td>
<td>6 (35)</td>
</tr>
<tr>
<td></td>
<td>Hypopharynx/yarynx</td>
<td>7 (22)</td>
<td>1 (7)</td>
<td>6 (35)</td>
</tr>
<tr>
<td></td>
<td>Oropharynx</td>
<td>15 (47)</td>
<td>9 (60)</td>
<td>6 (35)</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>3 (9)</td>
<td>0 (0)</td>
<td>3 (18)</td>
</tr>
<tr>
<td>Clinical T stage, n (%)</td>
<td>T0/T1/T2</td>
<td>18 (56)</td>
<td>12 (80)</td>
<td>6 (35)</td>
</tr>
<tr>
<td></td>
<td>T3/T4</td>
<td>13 (41)</td>
<td>3 (20)</td>
<td>10 (59)</td>
</tr>
<tr>
<td>Clinical N stage, n (%)</td>
<td>N0/N1</td>
<td>14 (44)</td>
<td>7 (47)</td>
<td>7 (41)</td>
</tr>
<tr>
<td></td>
<td>N2/N3</td>
<td>17 (53)</td>
<td>8 (53)</td>
<td>9 (53)</td>
</tr>
<tr>
<td></td>
<td>Missing</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>HPV status, n (%)</td>
<td>Negative</td>
<td>16 (50)</td>
<td>6 (40)</td>
<td>10 (59)</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>13 (41)</td>
<td>8 (53)</td>
<td>5 (29)</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>3 (9)</td>
<td>1 (7)</td>
<td>2 (12)</td>
</tr>
</tbody>
</table>

\( \* \) \( P \) values were calculated using two-sided exact \( \chi^2 \) and Fisher exact tests for categorical variables and exact Wilcoxon rank-sum tests for continuous variables, with missing/unknown excluded.
immune effects as BMI and BSA increased potentially due to decreased tissue concentration of tadalafil, but this was not statistically significant. Increase in systemic immunity via DTH showed no variation in relation to BMI or BSA (Supplementary Data).

PDE5 inhibition enhances tumor specific immunity
To determine the effect of PDE5 blockade on HNSCC-specific immunity, we performed a subanalysis on selected patients with available clinical material to examine tumor-specific immunity to the mixture of HPV\(^+\) and HPV\(^-\) HNSCC tumor lysate: 3 placebo-treated and 3 tadalafil-treated patients. The tadalafil-treated patients demonstrated a significant increase in tumor-specific immunity to HNSCC tumor lysate challenge after tadalafil therapy (\(P = 0.04\)). Interestingly, this response was primarily CD4 restricted despite having observed a greater CD69 upregulation with tadalafil in the CD8 subset and greater proliferation of CD8 cells with PDE5 inhibition (29).

Figure 2.
Tadalafil augments immune function: fold increase in T-cell expansion was determined following anti-CD3/CD28 stimulation. Expansion was significantly greater in the tadalafil-treated arm (2.4-fold) versus placebo (1.3-fold) (\(P = 0.003\)). Systemic immune responsiveness as measured by fold change in the area of induration from a DTH response to Candida with an increase in response in tadalafil-treated patients (\(P = 0.002\)). Change in activated T cells as assessed by CD69 expression: peripheral CD4\(^+\) cells and peripheral CD8\(^+\) cells. Two outliers with a large fold change in the DTH area are suppressed; these are included in Supplementary Fig. S5. Figures reflect data points as in Fig. 2.

Figure 3.
Tadalafil increases HNSCC specific immunity. CFSE-labeled T cells obtained pre- and posttreatment were added to dendritic cells pulsed with the indicated tumor lysate of either a mixture of HPV\(^+\) and HPV\(^-\) HNSCC cell lines or myeloma cell lines (U266 and H929). Tumor specificity was determined after a 7-day coculture by flow cytometry as CFSE\(^-\)/IFN\(\gamma\)/CD3 cells. Shown is the change in tumor specificity in the CD4\(^+\) T cells in the placebo- or tadalafil-treated groups (\(P = 0.04\)). No significant changes in tumor specificity were observed in the CD8\(^+\) T cells.
Despite recent advances in tumor-targeted approaches, it is increasingly clear that the immune system not only is capable of directly imparting antitumor immunity but also plays a critical role in mediating tumor response to chemotherapy as well as potentiating the clinical efficacy of monoclonal antibody therapies such as cetuximab in HNSCC (31, 32). These data reflect an increasing recognition of the complex role the immune system plays in mediating direct antitumor immunity and in potentiating the clinical efficacy of traditional cytotoxic therapies. MDSCs are increasingly recognized as playing a major immunosuppressive role in both the innate as well as adaptive immune responses and are major mediators of immune suppression in many solid tumors, including HNSCC (33).

Because of the critical role of MDSCs in mediating cancer-induced immune suppression, strategies aimed at abrogating their function have significant appeal. Recent approaches include promotion of myeloid differentiation, inhibition of their expansion as well as their elimination through the use of cytotoxic chemotherapy (34). Our rationale for targeting PDE5 inhibition in this setting is to suppress MDSC function through inhibition of the two critical immunosuppressive pathways: iNOS and Arg-1.

We have previously hypothesized that the effect of PDE5 inhibition was on a destabilization of NOS2 mRNA by reducing the ubiquitous mRNA binding protein, human antigen R (HuR), ultimately leading to a reduction in NO production (29). Ochoa and colleagues recently demonstrated how arginase activity was dependent upon cyclin D3 activity and how stability of cyclin D3 mRNA was dependent upon HuR (35). These latter findings establish a common link between PDE5 inhibition and abrogation of both the NO as well as Arg1 pathways.

These inhibitors are capable of inhibiting MDSC function, augmenting T-cell function, and exerting a measurable antitumor effect in murine models without exerting direct cytotoxic activity on either the MDSCs or tumor (29). Using an agent with no appreciable direct antitumor activity allows us to examine the direct role of MDSC inhibition on global and tumor-specific immunity. In this randomized study, we show that PDE5 inhibition suppresses iNOS and Arg-1 production, enhances global immune responsiveness, augments T-cell function, and generates tumor-specific immunity in HNSCC. These results highlight the critical role of MDSCs as mediators of immune suppressors in HNSCC and suggest the critical importance of inhibiting MDSC function in an effort to augment tumor-specific immunity. This and the article by Weed and colleagues is the first clinical study that demonstrates the ability of PDE5 inhibition to augment immune function in patients with cancer, confirming an earlier observation (36).

One unexpected finding was the observed decrease in MDSC numbers. PDE5 inhibitors are not known cytotoxic agents and the ex vivo addition of PDE5 inhibitors to MDSCs in culture failed to affect their survival (data not shown). This may be due to the short half-life of MDSCs and their endogenous requirement for cytokines produced by the MDSCs to stimulate cell growth in either an autocrine or paracrine manner. It is possible that by reducing iNOS and Arg-1 production, MDSCs alter the tumor microenvironment to either shorten their half-life or prevent the formation of other critical factors required for sustaining or generating additional MDSCs. The ability to adversely affect MDSC function, number, and possibly overall survival with a noncytotoxic agent such as tadalafl is intriguing and could ultimately provide insight into the mechanisms of MDSC generation and persistence in cancer-bearing hosts. These data suggest that MDSCs may serve a critical role in immune response to HNSCC, that PDE5 inhibition is rapid and effective in abrogating MDSC function, and that eliminating MDSC function with PDE5 inhibition can restore both systemic and tumor-specific immunity.

Although we have shown that PDE inhibitors reduce NO2 and ARG1, the full mechanisms underlying these effects remain to be elucidated. One putative mechanism involves the impact of these inhibitors on mRNA stability. cGMP destabilizes NOS2 mRNA by reducing the ubiquitous mRNA-binding protein, human antigen R (HuR). As such, destabilization of NOS2 mRNA via PDE5 inhibition would abrogate NO-mediated immunosuppression more effectively than would competitive inhibition of NO itself. However, because ARG1 mRNA does not possess adenylyl/uridylyl-
and to determine whether vaccine responses can be augmented with this approach.

Disclosure of Potential Conflicts of Interest
P. Serafini and I. Borrello are inventors on a patent, which is owned by Johns Hopkins University, regarding the use of PDE5 inhibitors as immune-modulators. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: J.A. Califano, K.A. Noonan, I. Borrello
Development of methodology: J.A. Califano, K.A. Noonan, I. Borrello
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.A. Califano, Z. Khan, K.A. Noonan, L. Rudra raju, C.G. Gourin, P.K. Ha, M. Levine, M. Tang, R. Blanco, N. Agrawal, W.M. Koch, S. Maru, I. Borrello
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.A. Califano, K.A. Noonan, L. Rudra raju, M. Levine
Study supervision: J.A. Califano

Acknowledgments
The analysis of this article is based on a web database application provided by Research Information Technology Systems (RITS). The authors thank Ervin Griffin for his assistance in processing and storing the samples.

Grant Support
This study was supported by National Cancer Institute Grant 1R21CA156535 and National Cancer Institute/National Institute of Dental and Craniofacial Research Grant P50 CA19032. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 9, 2014; revised October 14, 2014; accepted October 21, 2014; published online January 6, 2015.

References


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