Expression of Immune Checkpoint Regulators, Cytotoxic T Lymphocyte Antigen 4 (CTLA-4) and CD137 in Cervical Carcinomas

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Abstract

Introduction: Immune checkpoint inhibitors in cancer therapy has a significant role in oncology. One of these immune checkpoint mediators is cytotoxic T-lymphocyte associated protein 4 (CTLA-4). Inhibition of the CTLA-4 pathway has already led to the FDA approval of Ipilimumab (anti-CTLA-4), a targeted therapy for melanoma and other malignancies.

CD137 is an inducible, costimulatory receptor of the tissue necrosis factor receptor superfamily expressed on activated immune cells. Clinical trials had also been set for anti-CD137 in several malignancies.

We investigated the expression of CTLA-4 and CD137 antibodies in benign and malignant uterine cervical tissues.

Method: We assessed CTLA-4 and CD137 expression on a tissue microarray (TMA) comprising of 100 normal, non-neoplastic, and neoplastic cervical tissues. When detected as strong granular cytoplasmic reaction in the epithelial cells, CTLA-4 expression was scored as positive. For CD137, the results were recorded based on the presence or absence of staining reaction on the cell membranes of the lymphoplasmacytic infiltrate.

Result: Overall, CTLA-4 was positive in 30% (30/100) of the cervical malignancies. Subcategorically, 20% of invasive endocervical adenocarcinomas, 62.5% of adenosquamous carcinomas, and 31% of squamous cell carcinomas were positive for CTLA-4 with a tendency toward lower grades SCCs. CD137 was positive in lymphoplasmacytic infiltrates of all endocervical adenocarcinomas, 90.5% of squamous cell carcinoma, and 87.5% cores of adenosquamous carcinomas.

Conclusion: This study has found a significant expression of CTLA-4 in cervical cancer cells and CD137 positivity of lymphoplasmacytic infiltrates with potentials for future targeted immunotherapy.

Introduction

Among gynecologic malignancies, cervical carcinomas are the fourth most common cancer cause of death worldwide with an estimated 570,000 cases diagnosed and 311,000 deaths in 2018 [1]. Human papillomavirus (HPV) is central to the development of the cervical neoplasia and can be detected in the vast majority of cervical cancers. The most common histologic types of cervical cancer include squamous cell carcinoma which is the major type (70%) while adenocarcinomas, originating from the glandular epithelium, is less common (about 25%) and the remaining (5%) constituting other types or variants [2].

Improvements and increased availability of the cervical cancer screening tests has allowed earlier detection and treatment of the precancerous lesions. Consequently, a survival improvement has been observed in patients with cervical cancers over recent decades, mostly in developed countries where

preventive strategies have been developed. Early-stage cervical cancer can be cured with surgery, while concurrent chemoradiation is the treatment for the lesions in locally advanced stages. Patients with recurrent or metastatic cancers however, have limited treatment options. Immunotherapy is shown to be a promising therapeutic strategy against advanced malignancies. In recent years, there has been a significant improvement in immune check point proteins leading to development and implementation of targeted anti-cancer immunotherapies. Unlike radiation therapy and standard chemotherapy, which aim to directly interfere with tumor cell growth and survival, immunotherapies target the tumor indirectly by enhancing the anti-tumor immune responses that may spontaneously arise in tumors [3]. The programmed death-1/programmed death-ligand-1 (PD-1/PD-L1) immune regulatory axis has emerged as a new immunotherapy in several malignancies. Our group has shown that PD-L1 is positive in a significant number of cervical cancers [4].

Although cancer cells can be immunogenic, tumor progression is associated with the evasion of the immunosurveillance and the promotion of tumor tolerance. The Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) represents a crucial immune checkpoint, the blockade of which can potentiate anti-tumor immunity. The anti-CTLA-4 antibody, ipilimumab, was the first immune checkpoint inhibitor to be tested and approved by the FDA for the treatment of cancers, including melanoma and prostate carcinoma, and has already resulted in significant favorable outcomes, representing a major milestone in cancer immunotherapy [5–10]. CTLA-4 acts as a negative regulator of the T-cell activation by indirectly diminishing signaling through the co-stimulatory receptor CD28 [11–13]. Studies have demonstrated that CTLA-4 expression, on certain tumor cell lines, causes apoptosis of CTLA-4-expressing tumor cells after interaction with soluble CD80 or CD86 recombinant ligands [14]. CTLA-4 expression has been shown in various neoplasms including nasopharyngeal carcinoma, esophageal carcinoma, non-small cell lung cancers, and breast cancers [15–20].

In addition to checkpoint blockade agents such as ipilimumab for CTLA-4 expressing tumors, agents targeting the tumor necrosis factor (TNF) superfamily of costimulatory receptors have gained attention in recent years [21]. CD137 is an inducible, costimulatory receptor of the TNF receptor superfamily expressed on activated immune cells, including effector and regulatory T cells, natural killer (NK) cells, and dendritic cells (DCs) [22, 23]. Recent studies indicate that the addition of anti-CD137 antibodies can supplement the antitumor efficacy of immune checkpoint inhibitors by enhancing cytotoxic T-cell and NK-cell activity, with effective antitumor responses [24–27].

As studies involving the expression of CTLA-4 in benign and malignant cervical tissues are still in the preliminary stages, we studied expression of CTLA-4 in cervical neoplasms juxtaposed with that of CD137 expression within the lymphoplasmacytic infiltrate of the tumor microenvironment. We used tissue microarrays to elucidate the correlation of CTLA-4 expression with tumor reactivity and CD137 expression within the lymphoplasmacytic infiltrate and thus the possibility of their use in combination immunotherapy. The aims of the investigation were to observe and compare the expression of CTLA-4 and CD137 in cervical tissue and to establish a scoring system for evaluation of the CTLA-4 immunohistochemistry (IHC) reactions as we have previously done for CTLA-4 expression in breast

cancer [19]. Potentially, tumor expression of CTLA-4 and CD137 can provide a rationale for screening of the tumor samples to identify candidates for the targeted therapies and further provide grounds for investigation of the anti-immune checkpoint treatments. The introduction of the scoring systems for CTLA-4 and CD137 expression are intended to facilitate a more systematic correlation between the tumor expressivity and potential stratification of the responses to the cancer treatments.

Materials And Methods

Ethics statement

Institutional Review Board at the David Geffen School of Medicine at UCLA had approved this study (IRB#15-001035). No human consents were need owing to the nature of the investigation which was carried out on commercially obtained tissue microarray sections.

Tissue microarray

Tissue microarray (TMA) glass slides of formalin-fixed paraffin embedded human cervical tissues were obtained from Abcam Inc. (Cambridge, MA). The TMA included 99 cores of both normal and neoplastic cervical tissues. The average size of each core, after fixation and paraffin embedding, was about 1 mm. Each core had been derived from one patient with her respective age listed in the table (Tables 1-6). Hematoxylin and eosin (H&E) stain was applied to one slide for histopathologic assessment. Two of the authors (AK and NAM) evaluated the cores for immunohistochemistry (IHC) grading and diagnostic accuracy. Histopathologic diagnoses were made per established criteria and nomenclature published by the World Health Organization [2]. Per Abcam's specifications, all tissues had been fixed in 10% buffered formalin solution for 24 hours and had been further processed using identical standard procedures. Sections were freshly cut upon order and were placed on Superfrost-Plus or Starfrost adhesive glass slides. At the microscopic examinations, the sections appeared to be 4-6 µm in thickness.

CTLA-4 immunohistochemistry

Mouse anti-human CTLA-4 monoclonal antibody (clone F8) was obtained from Santa Cruz Biotechnology (Dallas, TX). IHC was carried out on one of the TMA slides employing the anti-CTLA-4 antibody at 1:100 dilution, adhering to the general guidelines recommended by the Santa Cruz Biotechnology including appropriate controls. The results were recorded based on the intensity of the staining reaction on the cytoplasm, as well as the estimated percentage of positive tumor cells as was previously described and photomicrographed in detail on female breast tissues [19]:

Intensity 0: If there was no reaction in cytoplasm

Intensity 1+: If a low number of cytoplasmic granules had the reaction

Intensity 2+: If a moderate number of cytoplasmic granules had the reaction

CD137 immunohistochemistry

Rabbit anti-human CD137 polyclonal antibody (ab197942) was obtained from Abcam. It was applied to one of the TMA slides for IHC staining at 1:100 dilution and adhering to the general guidelines using appropriate positive and negative controls. The results were recorded based on the presence or absence of staining reaction on the cell membranes of the lymphoplasmacytic infiltrate. Any expression level of CD137 ≥ 5% within the lymphoplasmcytic infiltrate of the tumor microenvironment was considered as "Positive" for CD137 expression.

Statistical analysis

A 2 x 2 table for nonparametric Fisher Exact testing was employed to compare the selected groups and the subgroups. For completeness, no adjustments for multiple comparisons across groups and subgroups were made, owing to the exploratory nature of this study using a novel scoring system for CTLA-4 and PD-L1 expression. Microsoft (Microsoft, Redmond, WA) Office-365 Excel sheets and Statistica (version 13) were used for tabulation of the data and the statistical analyses.

Study design

For CTLA-4 IHC staining evaluation, the same scheme previously developed by our group for CTLA-4 scoring in breast carcinomas was used [19]. Three categories of expression were designated for CTLA-4 staining, "*Negative*", "*Low-Positive* (LoPos)", and "*Positive*" as defined in the scoring system below:

*Score "**0**"* - **100%** of cells with Intensity of **0**; Expression: ***Negative***.

*Score "**1a**"* - **<50%** of cells with Intensity of **1+**; Expression: ***Low-positive***.

*Score "**1b**"* - **<50%** of cells with Intensity of **2+**; Expression: ***Low-Positive***.

*Score "**2a**"* - **≥50%** of cells with Intensity of 1+; Expression: ***Positive***.

*Score "**2b**"* - **≥50%** of cells with Intensity of **2+**; Expression: ***Positive***.

For CD137 IHC staining evaluation, any positive cell membrane staining of ≥ 5% of the lymphoplasmacytic infiltrate was considered as "*Positive*". Based on the histopathology diagnoses, the cores were divided into three groups: **Group I**, squamous cell carcinoma (Grade I, II and III); **Group II**, adenosquamoous carcinoma; **Group III**, endocervical adenocarcinoma. Using the designed scoring method, CTLA-4 expression findings were recorded for each group and tabulated in their respective tables. The clinical and demographic information was extracted from the Abcam product datasheet and added alongside the findings. The statistical test was carried out to compare two sets of data at a time. A two-tailed *p*-value of 0.05 or less was considered a significant statistical difference between the two compared groups.

Results

Of the 99 cores of cervical tissue examined on the TMA slide, four cases were benign cervical tissue (including cervicitis) and 95 cores were malignant. The malignant cores included 7 cases of squamous cell carcinoma (grade I), 52 cases of squamous cell carcinoma (grade II), 15 cases of squamous cell carcinoma (grade III), 8 cases of adenosquamous carcinoma and 13 cases of endocervical adenocarcinoma. The patient's median age was 44 years old. Lymphoplasmacytic infiltration was observed in the majority of the cores which were recorded in the respective tables (Supplement Tables 1–6). Staining of ≥ 5 % within the lymphoplasmacytic infiltrate with CD137 was considered as positive expression. All 4 benign cervical samples were histopathologically verified and interpreted as "negative" with a score of "0" or "1a"(Supplement Table 1). The 1 benign case with a score of "1a" had epithelial staining of 1 + intensity for CTLA-4 which was comparable to the reaction on the normal control epithelial cells (Fig. 1). Therefore, 1 + intensities were considered as "Negative" in all groups and were designated as such under a column labeled as "Interpretation" in their respective tables. As a result, positive/over-expression of CTLA-4 reactions were confined to 2 + intensities with scores of "1b or less than 50% expression" and "2b or greater than 50% expression" for this study.

Group I, Squamous cell carcinoma (SCC)

Of the 99 cores, 74 were SCC. Twenty nine cores (39.2%) had no CTAL-4 expression and were given a score of "0". In addition, 22 cores (29.7%) had 1 + intensity reaction with CTLA-4 staining. Due to similarity with the 1 + reaction in the normal cervical tissue core, these cores were also considered as "Negative" in the "Interpretation". Further details of the findings are described under the tumor-grade subgroups below where scores of "1b" and "2b" were counted as positive/over-expressed in 23 cores (**31.1%**). An example of the reactions in this group is shown in (Fig. 2A &2B). Sixty seven (90.5%) SCC cores shoed positive expression of CD137 in lymphoplasmacytic infiltration around the tumor (Fig. 3).

SCC, Grade I

Among the 73 SCC cores, 7 cores were grade I tumors, of which 2 (28.6%) had no CTLA-4 expression and scored as "0". Two additional cores (28.6%) had expression with a 1 + intensity (score 1a and 2a), similar to the benign and normal tissues, and thus were counted as "Negative" in the "Interpretation". The remaining 3 cores (**42.9%**) were positive for over expression of CTLA-4 (2 + intensity) and scored as 1b and 2b. All but one core had expression of CD137 within the lymphoplasmacytic infiltration (85.7%). The presence of lymphoplasmacytic infiltration and their CD137 expression are listed in Supplement Table 2.

SCC Grade II

Fifty-two cores had grade-II lesions of which 20 (40.4%) had no reaction for CTLA-4, and thus scored as "0" (#1–21, Supplemental Table 3). Additional 7 cores had reactions, with 1 + intensity (score of "1a") and six cores with 1 + intensity (score of "2a") similar to the benign and normal tissues, thus were counted as "Negative" in the "Interpretation" (#22–36, Supplemental Table 3). The remaining 16 cores were positive

for over-expression of CTLA-4 (**30.8%**) with a score of either “1b” or “2b” (2 + expression) (#37–52, Supplemental Table 3). Fifty of the cores (96.2%) had expression of CD137 within the lymphoplasmacytic infiltration. The presence of lymphoplasmacytic infiltration and their CD137 expression are listed in Supplement Table 3.

SCC, Grade III

This subgroup was comprised of 15 cores of which 6 (40%) had no reaction for CTLA-4, and thus scored as “0”. Additional 2 cores (#7–8; Table 4) had 1 + intensity (score of “1a”) and 3 cores (#9–11, Supplementa Table 4) had 1 + intensity (score of “2a”) and subsequently counted as “Negative” in the “Interpretation”. Only four cores were positive (**26.7%**) for CTLA-4 over-expression with one being “Low-Positive” (#12; Supplemental Table 4). Eleven of the cores (73.3%), had expression of CD137 within the lymphoplasmacytic infiltration. The presence of lymphoplasmacytic infiltration and their CD137 expression are listed in Supplement Table 4.

Group II, Adenosquamous cell carcinoma

Eight of the cores on the TMA fell in this group (Supplemental Table 5). One core had no reaction for CTLA-4, and thus scored as “0”. Additional 2 cores (#2–3; Supplemental Table 5) had 1 + intensity (score of “1a” and “2a”) and subsequently counted as “Negative” in the “Interpretation”. Five cores were positive (**62.5%**) for CTLA-4 over-expression with two being “Low-Positive” (#4–5; Supplemental Table 5). All but one core had expression of CD137 within the lymphoplasmacytic infiltration (87.5%). The presence of lymphoplasmacytic infiltration and their CD137 expression are listed in Supplement Table 5.

Group III, Endocervical adenocarcinoma

This group comprised of 13 cores (Supplemental Table 6). One core had no reaction for CTLA-4, and thus scored as “0”. Additional 10 cores had 1 + intensity (score of “1a” and “2a”) and subsequently counted as “Negative” in the “Interpretation”. Only 2 cores were positive (**62.5%**) for CTLA-4 over-expression with one being “Low-Positive” (#5; Supplemental Table 6). An example of a positive case is shown in (Fig. 4). All cores had expression of CD137 within the lymphoplasmacytic infiltration (100%). The presence of lymphoplasmacytic infiltration and their CD137 expression are listed in Supplement Table 6.

Table 1
Summary of the cores with interpretive CTLA-4 positivity in the groups and subgroups of the uterine cervical carcinoma.

Group	Carcinomas	Median Age	Total (n)	Positive (n)	Positive (%)
Group I					
Squamous cell carcinomas, All		44	74	23	31.1%
	IA - SCC, grade-I	46	7	3	42.9%
	IB - SCC, grade-II	44	52	16	30.8%
	IC - SCC, grade-III	39	15	4	26.7%
Group II					
Adenosquamous carcinoma		40	8	5	62.5%
Group III					
Endocervical adenocarcinoma		40	13	2	15.4%
All Three Groups		44	95	30	31.6%
2x2 table Fisher Exact statistical test			**Negative (n)**	**Positive (n)**	**2-Tailed P-Value**
SCC, grade-I			4	3	0.67
SCC, grade-II			36	16	
SCC, grade-II			36	16	1.00
SCC, grade-III			11	4	
SCC, grade I			4	3	0.63
SCC, grade III			11	4	
SCC, all grades			51	23	0.11
Adenosquamous carcinoma			3	5	
SCC, all grades			51	23	0.33
Endocervical adenocarcinoma			11	2	
Adenosquamous carcinoma			3	5	0.06
Endocervical adenocarcinoma			11	2	

Positive includes 1b, and 2b scores (2 + expression)

Discussion

In this investigation, we compared CTLA-4 overexpression in cervical carcinomas, a commonly used immune checkpoint marker, with the expression of CD137 in the lymphoplasmacytic infiltrate within the tumor microenvironment. Based on the review of the randomly assembled TMA samples, our findings indicate CTLA-4 overexpression in 31.6% of cervical carcinomas and CD137 expression in over 90% of the lymphoplasmacytic infiltrate within the tumor microenvironment. The expression of CTLA-4 had a propensity for lower grade SCCs, however, the percentages of the “Positive” CTLA-4 expression in SCC, grade I (42.9%), grade II (30.8%) and grade III (26.7%%) did not reach statistical significance (Table 1). Endocervical adenocarcinomas exhibited the lowest “Positive” expression level (15.4%) and adenosquamous carcinoma exhibited the highest “Positive” expression level (62.5%), however, these differences were not statistically significant, likely due to the low number of cases. Naturally, in a randomly constructed tissue microarray, the frequency of low prevalent lesions was also low in the TMA samples. Interestingly, the vast majority of cervical cancers exhibited CD137 expression within the lymphoplasmacytic infiltration, even in cases with no CTLA-4 expression. The expression of CD137 was lowest in grade III SCCs (73.3%) versus grade I (85.7%) and grade II (96.2%). Adenosquamous cell carcinoma had expression of CD137 in 87.5% of cases and all endocervical adenocarcinomas in the TMA had expression of CD137 (100%). In all, more than 30% of the patients with the cervical cancers had overexpression of both CTLA-4 and CD137 and thus may become clinically eligible for the CTLA-4 immunotherapy or possible combination immunotherapy with CD137 expression. We have also used the same TMA in our earlier study to evaluate another check point inhibitor and showed that PD-L1 was positive in 37.8% of the cervical carcinomas [4].

We have previously devised a uniform systematic assessment for the CTLA-4 reactions by IHC. The scoring system which is introduced in cervical carcinomas in this study is similar to the CTLA-4 scoring system introduced previously in breast carcinomas [19]. Our scoring system is as follows: Cases with no expression had complete negative staining “Negative” with a score of “0”. If the expression was less than 50%, the expression was interpreted as “Low-Positive” with a score of “1”. If 50% or more of the cells had the protein expression, the specimen was interpreted as “Positive” with a score of “2”. Intensity of 1 + adds the suffix of “a” and the intensities of 2 + and/or 3 + gives a suffix of “b” to the score. Although, there are a wide range of antibodies available against the CTLA-4 protein, the ability of these antibodies to detect overexpression might be variable. In several published studies, the reactions have not been classified based on the intensity of the CTLA-4 IHC. We have shown that the 1 + intensity is seen in normal control benign cervical tissue. Therefore, the 1 + intensity reaction has been interpreted as negative in this study as was also suggested in our previous study with 1 + intensity seen in normal breast tissue [19].

The introduced systematic method, that assigns IHC scores as a percentage of positive tumor cells in relation to the staining intensity, may provide a more objective assessment of the protein expression and a clearer understanding of the roles played by the potential tumor markers in predicting outcome. Most importantly, by evaluating the protein expression quantitatively at the outset, more relevant cutoffs for

tumor positivity can be established for the therapeutic agents in different malignancies. In other words, as new agents are introduced, and/or future clinical studies result in changes of the response rates, dynamic cutoff points can be established for each therapeutic agent in each specific malignancy. Therefore, an objective pathology scoring system is needed for a comprehensive and consistent evaluation of the CTLA-4 reactions.

Antibody-based strategies for cancer treatment have dramatically advanced in the past decade. This highlights the importance of understanding the expression levels of these immune checkpoint regulators in different cancers for effective translational and clinical research. CTLA4 is an immune checkpoint inhibitor, best known for its activity and survival effects in metastatic melanoma, as well as other solid tumors [28, 29]. Checkpoint inhibitors such as CTLA-4 trigger inhibitory pathways which dampen T-cell activity when bound to their ligands (CD80/CD86) [30]. Therefore, blocking of this interaction enhances overall immune responses [31].

Immune checkpoint and costimulatory pathways have different mechanisms with potential for therapeutic synergy approach [32]. The significant point to note is the possible enhanced response rate of immune-checkpoint blockade with combination immunotherapies that promote or block various steps of the immune checkpoint cycle in cancers. This combination strategy improves anti-cancer immunity through the antigen-specific enhancement of CD8 + T-lymphocyte activity and immune memory, but suppresses autoimmunity by increasing the function of regulatory T lymphocytes [33].

CD137 activation results in recruitment of TNFR factors (TRAF1 and TRAF2) which consequently leads to the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), Jun amino-terminal kinases/Stress-activated protein kinases (JNK/SAPK) and p38 mitogen-activated protein kinases (p38 MAPK) pathways. This generates co-stimulatory signals to induce the activities of both $CD4^+$ and $CD8^+$ T lymphocytes [34]. Two clinical trials had been set for anti-CD137 antibodies namely urelumab (BMS-663513) and utomilumab (PF-05082566) [35]. Urelumab showed a promising cancer treatment potential in preclinical studies, however, there was significant liver toxicity of the antibody which caused the clinical trial to be stopped [36]. Recent studies suggested that this antibody is safe at lower doses for a shorter periods of time [37], thus combination therapies of low dose anti-CD137 with other FDA-approved immunomodulators and antibody therapeutics can have great potential of anti-tumor activities and minimize the possibility of systemic toxicities. It has already been shown that agonistic CD137 antibody with combination of anti-CTLA-4 therapy increases the survival of mice in the setting of colon cancer, glioblastomas and prostate cancer [33, 38–40]. This immunotherapeutic combination is promising for future clinical development.

We speculate that these immunological features in cervical cancers might be associated with clinical efficacy of the treatment and may help guide immunotherapeutic strategies in the future. The presence of CTLA-4 and CD137 expression as detected by IHC have the potential to be used not only as a prognostic marker in the cervical cancers, but as a potential predictive marker for the immunotherapeutic responses. Percentage scoring should allow a more thorough assessment of the predictive or prognostic significance

of these immune check point proteins. However, as with all IHC markers, factors such as tissue fixation (both type and duration), the choice of antibody clone, and the IHC staining methodology can dramatically affect test accuracy and reproducibility so these features must be taken into the account [41]. Finally, more studies are warranted to provide further insights into the association of HPV status and the expression level of CTLA-4 and CD137 in cervical cancers. Combining the level of HPV DNA with the expression of CTLA-4 may also provide a novel predictive biomarker of the efficacy of CTLA-4 inhibitors and the prognosis of patients with cervical cancer.

The results of this study have found a significant expression of CTLA-4 (~ 30%) in cervical cancers. Overall, the vast majority of cervical tumors (> 90%) had expression of CD137 within the lymphoplasmacytic infiltrate. Our findings in this study further support future investigations of anti-CTLA-4 and anti-CD137 immunotherapies in the CTLA-4-positive cervical tumors. This study shows that the cervical cancers may express CTLA-4 in the tumor cells and CD137in tumor infiltrating immune cells. These findings support a possible therapeutic role for CTLA-4 and CD137 inhibitors in a subset of cervical carcinomas.

Declarations

Data Availability Statement: All relevant data are within the paper and its Supporting Information files. The original Excel sheet, however, is available upon request.

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Competing interests: The authors declare that they have no competing interests.

Ethics approval and consent to participate: Institutional Review Board at the David Geffen School of Medicine at UCLA has approved this study (IRB#15-001035). The study was performed on glass slides made from tissue microarrays obtained from ABCAM; therefore, no consent was needed for this study.

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Figures

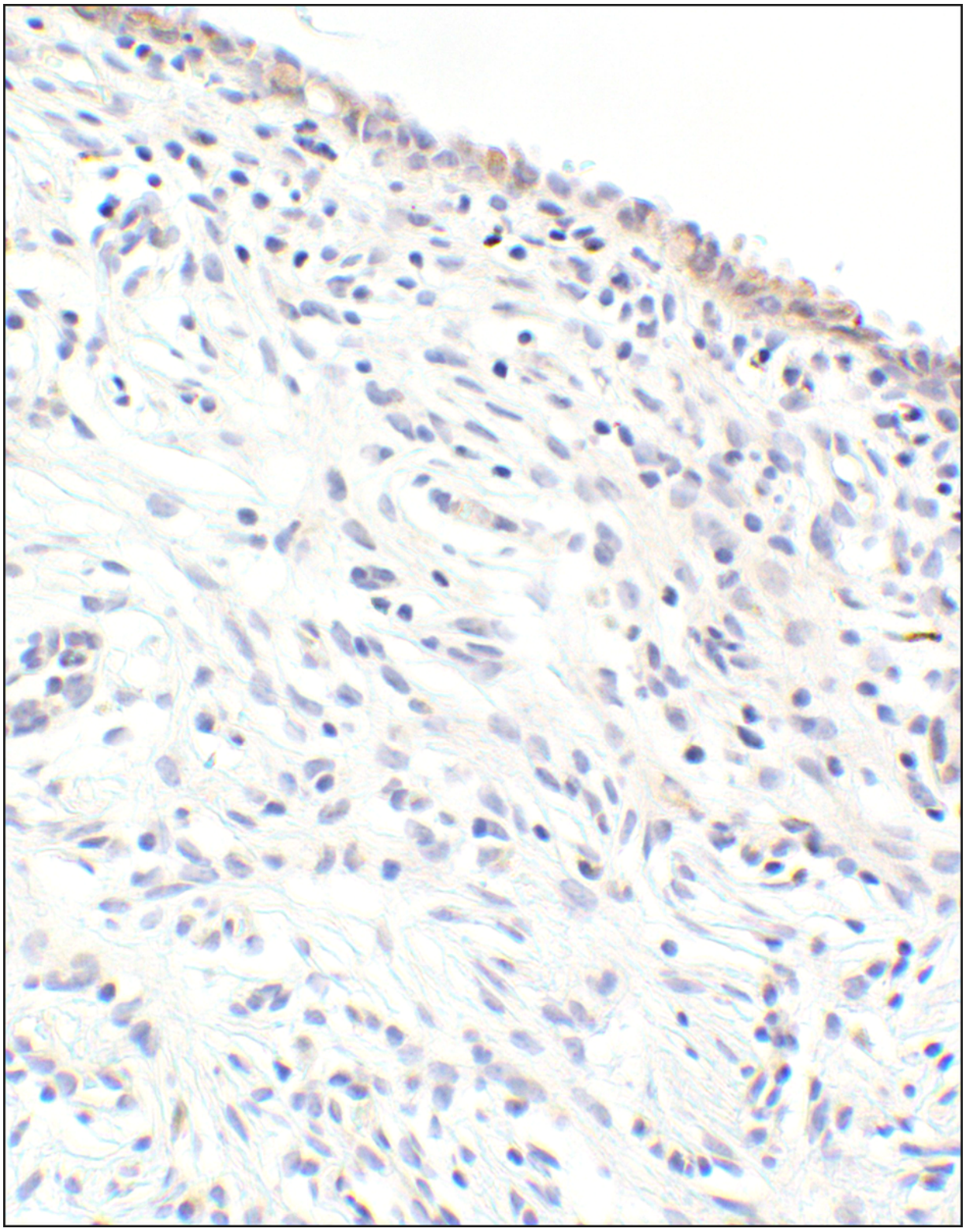

Figure 1

CTLA-4 expression in normal endocervical glands. Photomicrographs of benign endocervical tissue with 1+ (light/weak cytoplasmic staining of CTLA-4) which has been considered a negative expression (objective 20x).

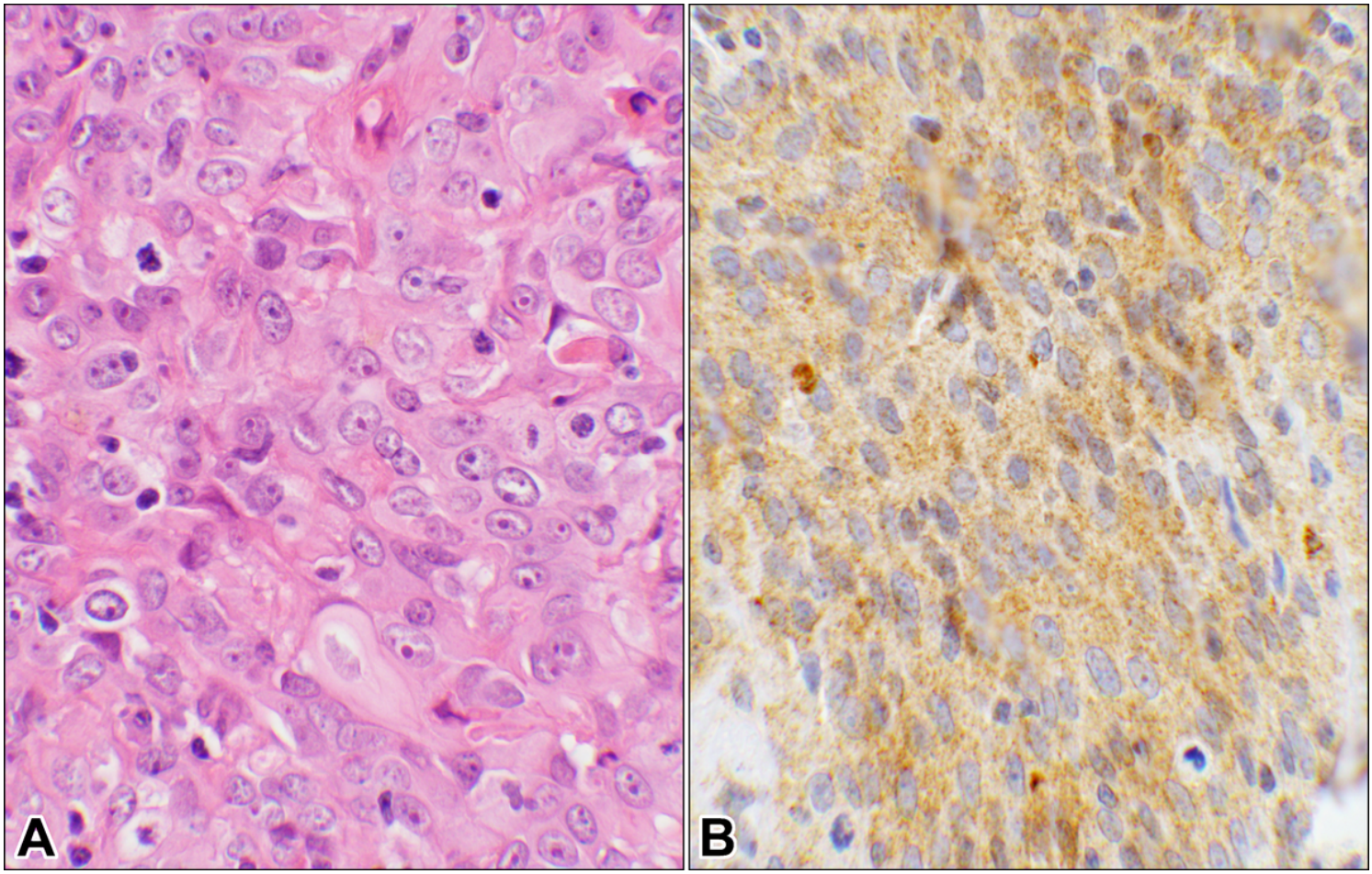

Figure 2

Score 2b in squamous cell carcinoma. An example of squamous cell carcinoma is shown by hematoxylin & eosin stain in Panel A. Panel B shows CTLA-4 with 2+ intensity stain in 100% of the neoplastic cells (40x objective).

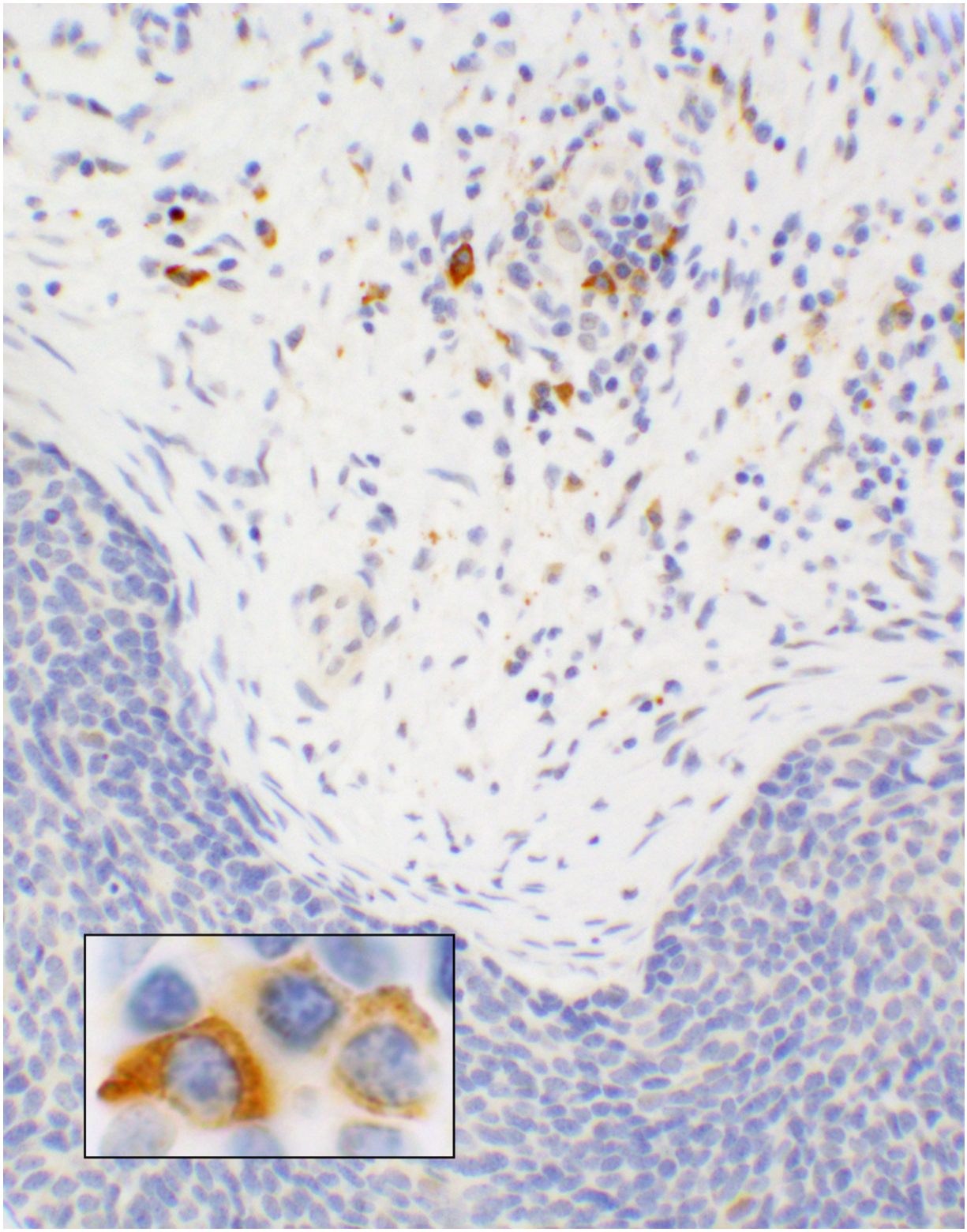

Figure 3

CD137 expression in lymphoplasmacytic infiltrates around the tumor. Photomicrographs of a case showing squamous cell carcinoma being negative for CD137 with positive granular cytoplasmic staining of lymphoplasmacytic cellsCD137 (20x objective). A high magnification of the lymphoplasmacytic cells is shown in the inset (100x objective).

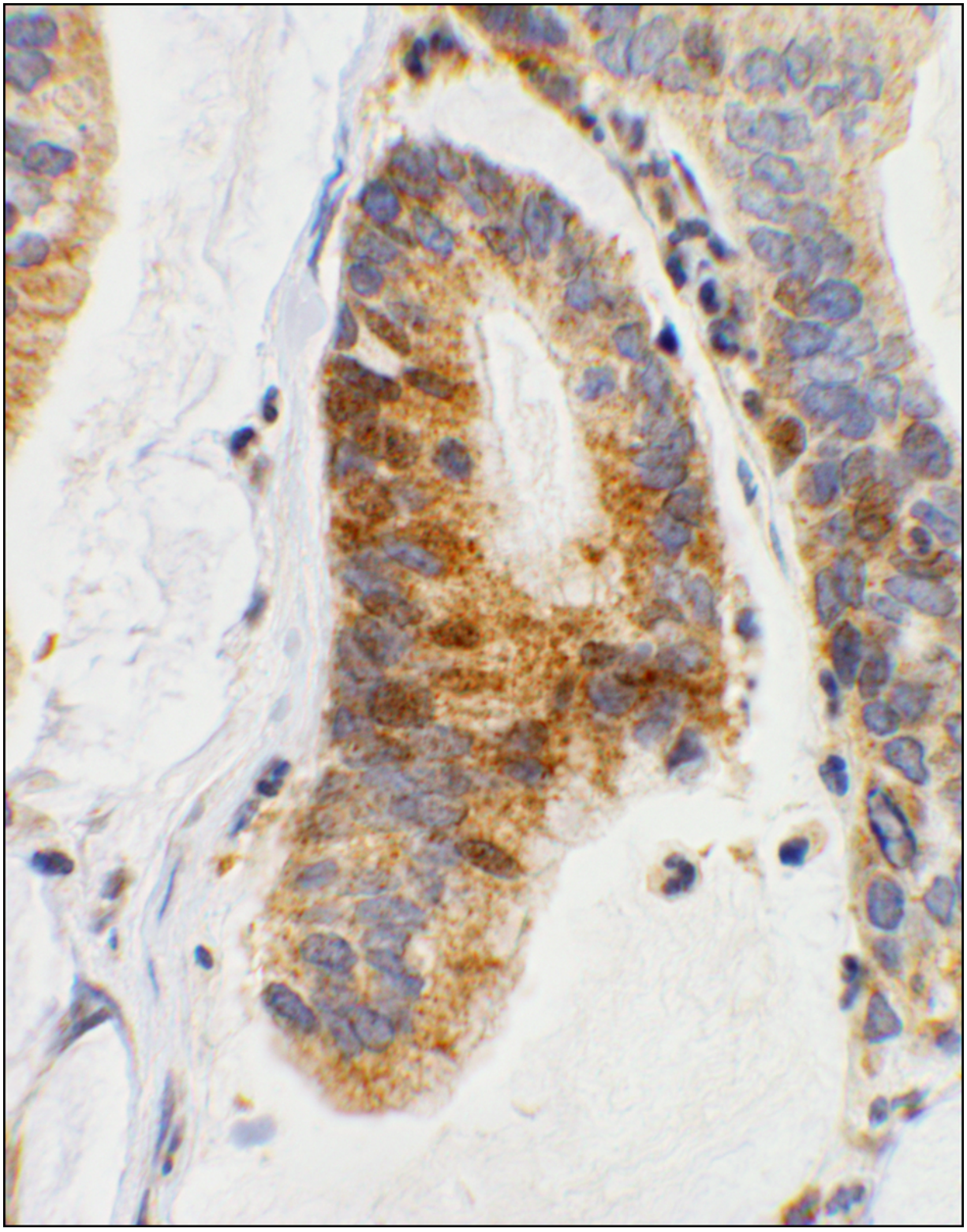

Figure 4

Score 2b in endocervical adenocarcinoma. An example of endocervical adenocarcinoma showing 2+ staining with CTLA-4 (40x objective).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalTable1.docx
- SupplementalTable2.docx
- SupplementalTable3.docx
- SupplementalTable4.docx
- SupplementalTable5.docx
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