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Authors

Kassardjian, Ari
Moatamed, Neda

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Original Article
Expression of immune checkpoint regulators, cytotoxic T lymphocyte antigen 4 (CTLA-4), and CD137 in cervical carcinoma

Ari Kassardjian, Neda A Moatamed

Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, Los Angeles 90095-1732, California, USA

Abstract: Immune checkpoint inhibitors have a significant role in oncology. One of these immune checkpoints 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 the activated immune cells. Clinical trials have also been set for anti-CD137 in several malignancies. We assessed CTLA-4 and CD137 expression on a tissue microarray (TMA) comprising of 99 core tissues which included normal, non-neoplastic, and neoplastic cervical lesions. 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 infiltrates. Overall, CTLA-4 was positive in 30% (30/100) of the cervical malignancies. Sub-categorically, 20% of invasive endocervical adenocarcinomas, 63% of adenosquamous carcinomas, and 31% of squamous cell carcinomas were positive for CTLA-4 with a tendency toward lower grade squamous cell carcinomas (SCCs). CD137 was positive in 100% lymphoplasmacytic infiltrates of endocervical adenocarcinomas, 90.5% of SCCs, and 87.5% of adenosquamous carcinomas. This study has found a significant expression of CTLA-4 in cervical cancer cells and CD137 positivity of lymphoplasmacytic infiltrates with potential for future targeted immunotherapy.

Keywords: Immune checkpoint, cytotoxic T-lymphocyte-associated protein 4, CD137, PD-L1, cervix, carcinoma, cervix

Introduction

Among gynecologic malignancies, cervical carcinoma is 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 most cervical cancers. The most common histologic types of cervical cancer include squamous cell carcinoma which is the major type (70%), and endocervical adenocarcinoma which is less common (about 25%); the other types or variants constitute 5% [2].

Due to improved and increased availability of the cervical cancer screening tests, the precancerous lesions are detected and treated at ear-

lier stages. Early-stage cervical cancer can be cured with surgery, while concurrent chemoradiation is the treatment for locally advanced disease. Patients with recurrent or metastatic cancer, however, have limited treatment options (NCCN). In recent years, there have been significant developments in anti-cancer immunotherapies targeting certain immune checkpoints which have shown to be a promising therapeutic strategy against advanced malignancies. Unlike radiation and standard chemotherapies, which aim to directly interfere with tumor cell growth and survival, immunotherapies target the tumors indirectly by enhancing the anti-tumor immune response that may spontaneously arise in the tumors [3]. The programmed death-1/programmed death-ligand-1 (PD-1/PD-L1) immune regulatory axis has emerged as a major target of immunotherapy in

several malignancies. Our group and others have shown that PD-L1 is positive in a significant number of cervical cancers [4, 5].

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 cancer, including melanoma and prostate carcinoma. It has already resulted in significant favorable outcomes, representing a major milestone in cancer immunotherapy [6-11]. CTLA-4 acts as a negative regulator of the T-cell activation by indirectly diminishing signaling through the co-stimulatory receptor CD28 [12-14]. 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 [15]. CTLA-4 expression has been shown in various neoplasms including nasopharyngeal carcinoma, esophageal carcinoma, non-small cell lung cancer, and breast cancer [16-21].

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 [22]. 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) [23, 24]. 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 activities, with effective antitumor response [25-28].

The aims of this investigation were to observe and compare the expression of CTLA-4 and CD137 in the cervical tissues and evaluate the CTLA-4 immunohistochemistry (IHC) reactions as we had previously done in breast cancer [21].

Materials and methods

Ethics statement

This study was reviewed and approved by the Institutional Review Board at the David Geffen School of Medicine at UCLA (IRB#15-001035).

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 (Supplementary Tables 1, 2, 3, 4, 5 and 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. The sections were 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 along tonsil tissue as control. 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 [21].

Intensity 0: If there was no reaction in the cytoplasm.

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

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

Intensity 3+: If a high number of cytoplasmic granules had a reaction.

For staining evaluation, the same scoring system previously developed by our group, for PD-L1 in cervical [5] and CTLA-4 in breast [21]

tissues, was used. 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+ and/or 3+; Expression: *Low-Positive.*

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

Score "2b"-≥50% of cells with Intensity of 2+ and/or 3+; Expression: *Positive.*

Since 1+ intensity had been observed in normal epithelial tissue (squamous epithelial lining of tonsil), only "1b" and "2b" reactions were interpreted as "positive".

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 using tonsil as control. The results were recorded based on presence or absence of the staining reactions on the cell membranes of the lymphoplasmacytic cells. Any expression intensity on ≥5% of cells within the lymphoplasmacytic infiltrate of the tumors was considered as "Positive".

Statistical analysis

2×2 tables for nonparametric Fisher Exact testing were employed to compare the selected sets of data. Also, "Negative" and "Positive" interpreted results for CTLA-4 were converted to binary "zeros" and "ones" for Student's *t*-test analysis. A result of less than 5% positivity for CD137 among the lymphoplasmacytic cells population was considered as negative and designated as "0" otherwise "1". Microsoft (Redmond, WA) Office-365 Excel sheets and StatPlus, version7 (https://www.analystsoft. com) were used for tabulation of the data and the statistical analyses and the results are shown in **Table 1**.

Study design

Based on the histopathology diagnoses, the cores were categorized into three groups: Group I, squamous cell carcinoma; Group II, adenosquamous carcinoma; Group III, endocervical adenocarcinoma. Group I was further subcategorized as IA, carcinoma grade I; 1B, carcinoma grade II; and 1C, carcinoma grade III. Using the designed scoring method, CTLA-4 expression findings were recor*ded for each group and tabulated in their respective* supplementary tables. The available clinical and demographic information was extracted from the Abcam product datasheet and added alongside the findings. Statistical tests were carried out to compare two sets of data at a time. To reject the null hypothesis, a two-tailed *p*-value ≤ 0.05 was considered asignificant difference between two groups.

Results

All CTLA-4 reactive cores showed cytoplasmic granules as had been observed previously [21], all with 1+ or 2+ intensities. We did not observe any reaction with 3+ intensity. All CD137 reactions were cytoplasmic (**Figure 1**) or on cell membranes of the lymphoplasmacytic cells which had been similarly observed by others [29]. No such reaction was observed on the tumor cells.

Of the 99 TMA cores, four cases were benign cervical tissues (2 normal and 2 with cervicitis) listed in Supplementary Table 1. In one case (case # 2, Supplementary Table 1), 40% of the cells had a 1+ intensity reaction (**Figure 2**), however, all 4 cases were interpreted as "Negative" per established criteria. Except for the same case which had a few positive lymphoid cells, the CD137 reaction was negative in the other 3 cases (Supplementary Table 1). Remaining 95 cores were malignant which will be discussed below.

Group I, Squamous cell carcinoma (SCC)

Of the 99 cores, 74 were SCC. Twenty-nine cores (39.2%) had no CTAL-4 expression (score "0"), 22 cores (29.7%) had 1+ intensity (scores: "1a" and "2a"), and 23 cores (31.1%) had 2+ intensity (scores: "1b" and "2b") reac-

Table 1. Summary of cores with interpretive positivity of the immune checkpoints in the groups and subgroups of uterine cervical carcinoms and related statistical analysis results

Group	Carcinoma	Median Age	Total (n)	CTLA-4 Positive (n)	CTLA-4 Positive (%)	CD137 Positive (%)
Control	Benign	57	4	0	0%	25%
Group I						
Squamous cell carcinomas, all		44	74	23	31%	91%
	IA-SCC, grade I	46	7	3	43%	86%
	IB-SCC, grade II	44	52	16	31%	96%
	IC-SCC, grade III	39	15	4	27%	73%
Group II						
Adenosquamous carcinoma		40	8	5	63%	88%
Group III						
Endocervical adenocarcinoma		40	13	2	15%	100%
Groups I, II, and III		44	95	30	32%	91%

Student's *t*-test: "Benign" versus Group I, II, and III (two-tailed, unequal variance): CTLA-4: *P*-value <0.0001, CD137: *P*-value =0.075.

2×2 table Fisher Exact statistical tests (CTLA-4)	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	1.0
SCC, grade III	11	4	
SCC, grade I	4	3	0.63
SCC, grade III	11	4	
SCC, all grades	51	23	0.12
Adenosquamous carcinoma	3	5	
SCC, all grades	51	23	0.33
Endocervical adenocarcinoma	11	2	
Adenosquamous carcinoma	3	5	0.056
Endocervical adenocarcinoma	11	2	

SCC, squamous cell carcinoma; Positive includes "1b", and "2b" scores.

tions. The cores with the scores of "1b" and "2b" were interpreted as "positive". An example of the reaction in this group is shown in **Figure 3**. Sixty-seven (90.5%) cores showed positive expression of CD137 in the lymphoplasmacytic infiltrates in the tumor stroma (**Figure 1**).

Subgroup IA, SCC Grade I

Among the 74 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 expressions with 1+ intensity (score "1a" and "2a"). The remaining 3 cores (42.9%) had 2+ intensity reaction for CTLA-4 scored as "1b" and "2b" which were interpreted as "Positive". All but one core had expression of CD137 (85.7%) within the lymphoplasmacytic infiltration (Supplementary Table 2).

Subgroup IB, SCC Grade II

Fifty-two cores had grade-II SCC lesions of which 21 (40.4%) had no reaction for CTLA-4, and thus scored as "0". Fifteen cores had the reactions for CTLA-4 (28.9%) with 1+ intensity (score of "1a" and "2a"). The remaining 16 cores (30.8%) were positive for CTLA-4 with a score of either "1b" or "2b", collectively interpreted as "Positive". All 52 cores had lymphoplasmacytic infiltration which had expressed CD137 except for one (Supplementary Table 3).

Subgroup IC, 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". Five cores (33.3%) had 1+ intensity with score of "1a" and "2a". Only four
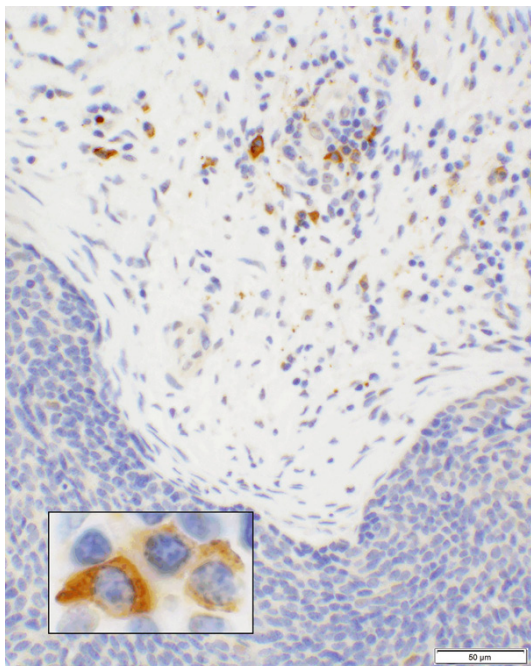
Figure 1. CD137 expression in lymphoplasmacytic cell infiltrates. A photomicrograph of a case showing squamous cell carcinoma (blue cells in lower segment of the picture) that are negative for CD137 while the infiltrating cells in the stroma show positive granular cytoplasmic staining of lymphoplasmacytic cells (20× objective). A high magnification of the lymphoplasmacytic cells is shown in the inset (100× objective).
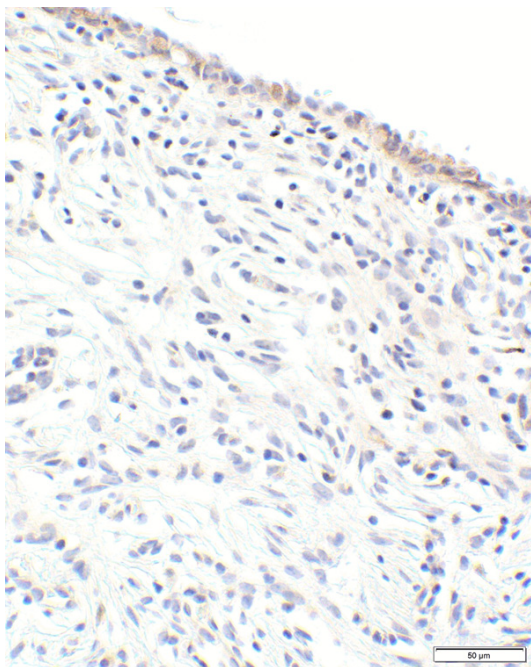

Figure 2. CTLA-4 expression in normal endocervical glands. Photomicrograph of benign endocervical tissue (case #2, Supplementary Table 1) with 1+ (light/

weak) cytoplasmic reaction of the epithelial lining which was interpreted as "Negative" (20× objective).

cores (26.7%) were interpreted as "Positive" for CTLA-4 with scores of "1b" and "2b". Although all 15 cores had lymphoid infiltrates, only 11 (73.3%) had expressed CD137 (Supplementary Table 4).

Group II, adenosquamous carcinoma

Eight of the cores in the TMA fell into this group (Supplementary Table 5). One core had no reaction for CTLA-4, and thus scored as "0". Two cores had 1+ intensity reaction (score of "1a" and "2a") and were counted as "Negative" in the Interpretation column. Five cores were positive (62.5%) for CTLA-4 with an intensity of 2+ and were scored as "1b" and "2b" and interpreted as "Positive". All but one core had expression of CD137 (87.5%) within the lymphoplasmacytic infiltrate (Supplementary Table 5).

Group III, endocervical adenocarcinoma

This group comprised of 13 cores. One core was scored as "0" since it had no reaction for CTLA-4. Additional 10 cores had 1+ intensity (score of "1a" and "2a") and subsequently interpreted as "Negative". Only 2 cores were positive (62.5%) with an intensity 2+, scored as "1b" and "2b", and interpreted as "Positive". An example of a positive case is shown in **Figure 4**. All cores had expression of CD137 (100%) within the lymphoplasmacytic infiltrate (Supplementary Table 6).

Statistical results

Student's *t*-tests were performed on 4 benign versus 95 malignant cores using the "Negative" and "Positive" interpretive criteria as described for CTLA-4 and CD137. Since the compared core numbers were 4 versus 95, the analyses used unequal variance. The obtained two-tailed *P*-values were <0.0001 and 0.075 for CTLA-4 and CD137, respectively (**Table 1**). Fisher Exact (2×2 table) analysis was used to identify the differences between the groups and the subgroups. "Positive" CTLA-4 expression was present in 31% of SCCs, where the expressions were 43%, 31%, and 27% in grades I, II, and III, respectively (**Table 1**). Regardless of higher rates of expressions in the lower grades, the grade comparison in SCC resulted in insignifi-
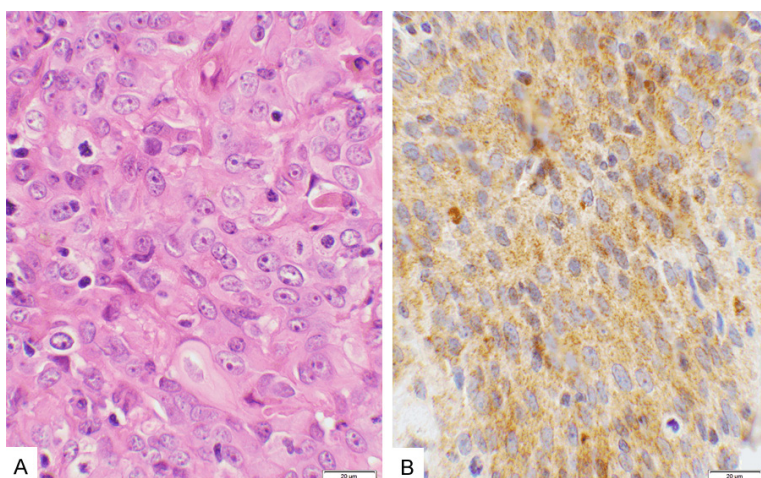
Figure 3. Score 2b in squamous cell carcinoma. A. An example of squamous cell carcinoma stained with hematoxylin & eosin, showing the malignant cell nuclei with prominent nucleoli. B. CTLA-4 stain of the same case showing a moderate cytoplasmic granular reaction (2+ intensity) involving more than 90% of the cells, scored as "2b", and interpreted as "Positive" (40× objective).
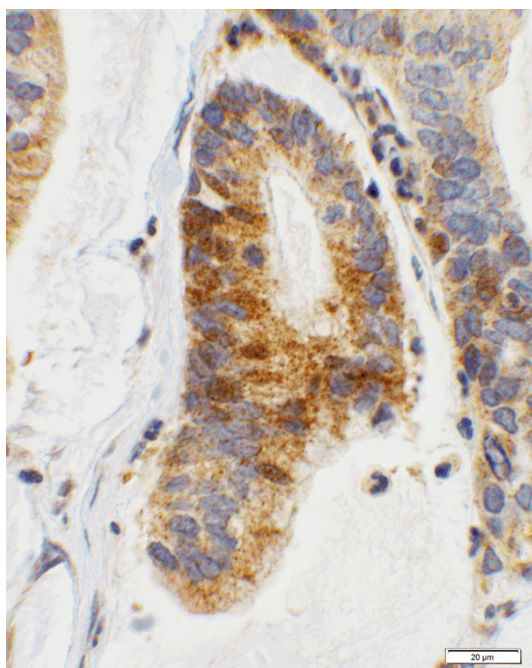

Figure 4. Score 2b in endocervical adenocarcinoma. An example of endocervical adenocarcinoma showing 2+ staining with CTLA-4 where there are a moderate number of granules in the cytoplasm (40× objective).

cant *P*-values of 0.67, 1.0, and 0.63 (**Table 1**). Comparing SCC to adenosquamous-carcinoma and endocervical-adenocarcinoma yielded *P*-values of 0.12 and 0.33. Although a higher percentage of the adenosquamous carcinomas

(63%) had expressed CTLA-4, when tested against endocervical adenocarcinoma, the resulting *P*-value was 0.056 (**Table 1**).

Discussion

At the outset, the results of this study indicate that CTLA-4 is expressed in 32% of cervical carcinomas which is significantly (*P*-value <0.0001) higher than occurring in normal or benign cervical tissues. Expression of CTLA-4 has a propensity for lower grade SCCs; however, the difference between grades did not reached statistical significance (**Table 1**). Endocervical adenocarcinomas exhibit the lowest (15%) and adenosquamous carcinoma the highest (63%) rates of "Positive" CTLA-4 expression with a Fisher Exact *P*-value of 0.056 which indicates a near-significant difference (**Table 1**). The rate of the reactivity of CTLA-4 immune checkpoint is similar, if not identical, to that of PD-L1 under similar experimental conditions [5]. On the other hand, CD137 is expressed in more than 90% of the lymphoplasmacytic infiltrates of the tumor microenvironment, although it is also expressed in benign cervical tissues (Supplementary Table 1), for which, the Student's *t*-test returned a *P*-value of 0.075 when benign cores compared to the malignant ones. This statistically insignificant *P*-value may be due to the small size of the benign cores or may also be due to its common expression in both benign and malignant conditions.

Expression of PD-L1 and CTLA-4 in more than 30% of the cervical cancers helps in expanding the arsenal in the immunotherapy against this neoplasm. Also, with emergence of the monoclonal antibody, CD137 can serve as an adjuvant to the immune checkpoints in immunotherapy of the cancer since it is expressed in more than 90% of the cancer cases (**Table 1**).

The PD-1 pathway involves PD-L1 and PD-L2 by binding to the PD-1 receptors of the T-cells which shield the cells from the attack by the inactivating killer T-cells. Pembrolizumab, a humanized monoclonal antibody, binds to the PD-1 receptors, shielding it from the ligands.

When unable to bind to the receptor, PD-L1 and/or PD-L2 expressing cells will be exposed to the attack by the immune cells. In this process, not only the tumor cells will be the subject of the strikes, but also normal cells may get damaged during the process, leading to the autoimmune side effects [30].

The cytotoxic T-lymphocyte-associated antigen 4 or CTLA-4 molecules negatively regulate T-cell activation by triggering inhibitory pathways which dampen T-cell activity when bound to their ligands (CD80/CD86) [31]. CTLA-4 is an immune checkpoint inhibitor, best known for its activity and survival effects on metastatic melanoma, as well as other solid tumors [32, 33]. Blocking CTLA-4 results in promotion of T-cell activation and proliferation [10, 19]. Ipilimumab, a recombinant human monoclonal antibody, acts as such blocking agent. Therefore, by blocking, the overall immune responses are enhanced leading to subsequent tumor killing by the T-cells [34]. The side effects of this treatment include fatigue, diarrhea, pruritis, rash, and colitis [35].

CD137 (4-1BB, TNFRSF9) molecule which is a member of tumor necrosis factor family is expressed on a variety of inflammatory cells including lymphocytes of B & T lineage, NK cells, dendritic cells, and neutrophils [24]. Its expression is triggered by activated CD4+ and CD8+ T-cells [36]. CD137 signals are mediated by activation of NF-κB (nuclear factor-kappa B) and by TRAF2 (TNF receptor-associated factor 2) [37]. By binding, urelumab, a humanized IgG4 monoclonal agonist antibody, crosslinks CD137 molecules which results in further activation and proliferation of the T-cells leading to inteleukin-2 secretion, apoptosis, and cytolytic activities [38-41]. These processes will enhance immune activity to eliminate tumor cells [42]. A significant side-effect of the treatment is liver toxicity of the antibody which caused a clinical trial to be stopped [43].

Thus, combination therapies of low dose anti-CD137 with other FDA-approved immunomodulators and antibody therapeutics including anti-PD-1 and anti-CTLA-4 can have great anti-tumor activity and minimize the possibility of systemic toxicity. 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 glioblastoma, colonic, and prostate cancer [42, 44-47]. Immune checkpoint and costimulatory pathways have different mechanisms with potential for therapeutic synergy approach [48]. This study highlights the importance of understanding the expression levels of these immune checkpoint regulators in cervical cancers for effective translational and clinical research.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Neda A Moatamed, Department of Pathology & Laboratory Medicine, David Geffen School of Medicine at UCLA, 10833 Le Conte Avenue, BOX 951732, 13-145 CHS, Los Angeles 90095-1732, CA, USA. Tel: 310-825-0581; Fax: 310-267-2058; E-mail: nmoatamed@mednet.ucla.edu

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Supplementary Table 1. Summary of *benign* cervical tissue cores with CTLA-4 and CD137 expression, scores, and the interpretation

No.	Age	Pathology	CTLA4+ (%)	Intensity	Score	Expression	Interpretation	LPI	LPI: CD137+ (%)
1	64	Normal	0	0	0	Negative	Negative	Present	0
2	64	Normal	40	1+	1a	LoPos	Negative	Present	100
3	50	Cervicitis	0	0	0	Negative	Negative	Present	0
4	48	Cervicitis	0	0	0	Negative	Negative	Present	0

LPI, Lymphoplasmacytic infiltrate; LoPos, low positive interpreted as "Negative".

Supplementary Table 2. Subgroup IA, summary of the cores with squamous cell carcinoma (Grade I) with CTLA-4 and CD137 expression

No.	Age	Stage	CTLA4+ (%)	Intensity	Score	Expression	Interpretation	LPI	LPI: CD137+ (%)
1	44	T1N0M0	0	0	0	Negative	Negative	Present	50
2	37	T1N1M0	0	0	0	Negative	Negative	Present	0
3	45	T3N0M0	5	1+	1a	LoPos	Negative	Present	50
4	68	T1N1M0	80	1+	2a	Positive	Negative	Present	80
5	51	T1N0M0	10	2+	1b	LoPos	Positive	Present	80
6	63	T1N0M0	50	2+	2b	Positive	Positive	Present	80
7	46	T1N0M0	80	2+	2b	Positive	Positive	Present	80

Positive; interpretation includes "1b", and "2b" scores; LPI, Lymphoplasmacytic infiltrate; LoPos, low positive.

Supplementary Table 3. Subgroup IB, summary of the cores with squamous cell carcinoma (Grade II) with CTLA-4 and CD137 expression

No.	Age	Stage	CTLA4+ (%)	Intensity	Score	Expression	Interpretation	LPI	LPI: CD137+ (%)
1	51	T1N0M0	0	0	0	Negative	Negative	Present	80
2	57	T1N0M0	0	0	0	Negative	Negative	Present	80
3	44	T1N0M0	0	0	0	Negative	Negative	Present	50
4	44	T1N0M0	0	0	0	Negative	Negative	Present	50
5	46	T1N0M0	0	0	0	Negative	Negative	Present	50
6	63	T1N0M0	0	0	0	Negative	Negative	Present	80
7	44	T1N0M0	0	0	0	Negative	Negative	Present	50
8	43	T1N0M0	0	0	0	Negative	Negative	Present	80
9	50	T1N0M0	0	0	0	Negative	Negative	Present	50
10	58	T1N0M0	0	0	0	Negative	Negative	Present	50
11	30	T1N0M0	0	0	0	Negative	Negative	Present	50
12	40	T1N0M0	0	0	0	Negative	Negative	Present	50
13	36	T1N1M0	0	0	0	Negative	Negative	Present	50
14	43	T1N1M0	0	0	0	Negative	Negative	Present	30
15	67	T1N1M0	0	0	0	Negative	Negative	Present	50
16	39	T1N1M0	0	0	0	Negative	Negative	Present	40
17	46	T1N1M0	0	0	0	Negative	Negative	Present	50
18	46	T1N1M0	0	0	0	Negative	Negative	Present	50
19	56	T2N0M0	0	0	0	Negative	Negative	Present	80
20	55	T2N0M0	0	0	0	Negative	Negative	Present	80
21	47	T4N1M0	0	0	0	Negative	Negative	Present	80
22	48	T1N0M0	5	1+	1a	LoPos	Negative	Present	50
23	44	T1N0M0	10	1+	1a	LoPos	Negative	Present	50
24	45	T1N0M0	10	1+	1a	LoPos	Negative	Present	50

No.	Age	Stage	CTLA4+ (%)	Intensity	Score	Expression	Interpretation	LPI	LPI: CD137+ (%)
25	40	T1N0M0	10	1+	1a	LoPos	Negative	Present	50
26	37	T1N1M0	10	1+	1a	LoPos	Negative	Present	50
27	45	T1N0M0	20	1+	1a	LoPos	Negative	Present	80
28	41	T1N1M0	30	1+	1a	LoPos	Negative	Present	80
29	30	T1N0M0	80	1+	2a	Positive	Negative	Present	80
30	54	T1N0M0	80	1+	2a	Positive	Negative	Present	50
31	57	T1N0M0	80	1+	2a	Positive	Negative	Present	0
32	37	T1N1M0	80	1+	2a	Positive	Negative	Present	90
33	41	T1N1M0	80	1+	2a	Positive	Negative	Present	90
34	47	T2N0M0	80	1+	2a	Positive	Negative	Present	80
35	50	T2N0M0	80	1+	2a	Positive	Negative	Present	80
36	49	T2N1M0	80	1+	2a	Positive	Negative	Present	50
37	41	T1N0M0	5	2+	1b	LoPos	Positive	Present	80
38	47	T1N0M0	5	2+	1b	LoPos	Positive	Present	50
39	42	T1N1M0	10	2+	1b	LoPos	Positive	Present	50
40	47	T1N1M0	10	2+	1b	LoPos	Positive	Present	80
41	37	T1N0M0	20	2+	1b	LoPos	Positive	Present	50
42	40	T1N0M0	20	2+	1b	LoPos	Positive	Present	50
43	54	T1N0M0	20	2+	1b	LoPos	Positive	Present	50
44	43	T1N0M0	20	2+	1b	LoPos	Positive	Present	50
45	43	T1N0M0	20	2+	1b	LoPos	Positive	Present	50
46	60	T1N1M0	20	2+	1b	LoPos	Positive	Present	50
47	41	T1N0M0	30	2+	1b	LoPos	Positive	Present	50
48	57	T1N1M0	40	2+	1b	LoPos	Positive	Present	0
49	35	T1N0M0	60	2+	2b	Positive	Positive	Present	80
50	35	T1N0M0	60	2+	2b	Positive	Positive	Present	50
51	30	T1N0M0	80	2+	2b	Positive	Positive	Present	50
52	37	T1N0M0	80	2+	2b	Positive	Positive	Present	10

Positive, interpretation includes "1b", and "2b" scores; LPI, Lymphoplasmacytic infiltrate; LoPos, low positive.

Supplementary Table 4. Subgroup IC, summary of the cores with squamous cell carcinoma (Grade III) with CTLA-4 and CD137 expression

No.	Age	Stage	CTLA4+ (%)	Intensity	Score	Expression	Interpretation	LPI	LPI: CD137+ (%)
1	34	T1N0M0	0	0	0	Negative	Negative	Present	0
2	58	T1N0M0	0	0	0	Negative	Negative	Present	50
3	43	T2N0M0	0	0	0	Negative	Negative	Present	0
4	52	T2N0M0	0	0	0	Negative	Negative	Present	50
5	50	T2N0M0	0	0	0	Negative	Negative	Present	50
6	35	T2N1M0	0	0	0	Negative	Negative	Present	50
7	59	T1N1M0	20	1+	1a	LoPos	Negative	Present	50
8	39	T1N1M0	40	1+	1a	LoPos	Negative	Present	0
9	30	T1N0M0	80	1+	2a	Positive	Negative	Present	80
10	36	T1N0M0	80	1+	2a	Positive	Negative	Present	50
11	45	T2N1M0	80	1+	2a	Positive	Negative	Present	50
12	31	T1N0M0	10	2+	1b	LoPos	Positive	Present	50
13	36	T1N0M0	60	2+	2b	Positive	Positive	Present	0
14	33	T2N0M0	60	2+	2b	Positive	Positive	Present	20
15	58	T1N0M0	70	2+	2b	Positive	Positive	Present	50

Positive, interpretation includes "1b", and "2b" scores; LPI, Lymphoplasmacytic infiltrate; LoPos, low positive.

Supplementary Table 5. Group II, summary of the cores with adenosquamous carcinoma with CTLA-4 and CD137 expression

No.	Age	Stage	CTLA4+ (%)	Intensity	Score	Expression	Interpretation	LPI	LPI: CD137+ (%)
1	27	T2N0M0	0	0	0	Negative	Negative	Present	50
2	48	T1N0M0	20	1+	1a	LoPos	Negative	Present	50
3	36	NA	90	1+	2a	Positive	Negative	Present	90
4	36	T1N0M0	20	2+	1b	LoPos	Positive	Present	50
5	46	T1N0M0	20	2+	1b	LoPos	Positive	Present	90
6	31	T2N0M0	80	2+	2b	Positive	Positive	Present	50
7	44	T2N1M0	80	2+	2b	Positive	Positive	Present	50
8	56	T1N0M0	80	2+	2b	Positive	Positive	Present	0

Positive, interpretation includes "1b", and "2b" scores; LPI, Lymphoplasmacytic infiltrate; LoPos, low positive; NA, not available.

Supplementary Table 6. Group III, summary of the cores with endocervical adenocarcinoma with CTLA-4 and CD137 expression

No.	Age	Stage	CTLA4+ (%)	Intensity	Score	Expression	Interpretation	LPI	LPI: CD137+ (%)
1	42	NA	10	1+	1a	LoPos	Negative	Present	90
2	40	NA	20	1+	1a	LoPos	Negative	Present	5
3	29	NA	30	1+	1a	LoPos	Negative	Present	90
4	48	T1N0M0	20	1+	1a	LoPos	Negative	Present	90
5	33	NA	30	2+	1b	LoPos	Positive	Present	90
6	40	T1N0M0	0	0	0	Negative	Negative	Present	80
7	48	T1N0M0	20	1+	1a	LoPos	Negative	Present	90
8	47	T1N0M0	30	1+	1a	LoPos	Negative	Present	80
9	24	T1N0M0	30	1+	1a	LoPos	Negative	Present	50
10	38	T1N0M0	30	1+	1a	LoPos	Negative	Present	90
11	50	T1N0M0	90	1+	2a	Positive	Negative	Present	90
12	39	T1N1M0	90	1+	2a	Positive	Negative	Present	90
13	39	T2N0M0	80	2+	2b	Positive	Positive	Present	10

Positive, interpretation includes "1b", and "2b" scores; LPI, Lymphoplasmacytic infiltrate; LoPos, low positive; NA, not available.