Expression of PD-1 and PD-L1 in Anaplastic Thyroid Cancer Patients Treated With Multimodal Therapy: Results From a Retrospective Study

Ashish V. Chintakuntlawar,[1] Kandelaria M. Rumilla,[2] Carin Y. Smith,[3] Sarah M. Jenkins,[3] Robert L. Foote,[4] Jan L. Kasperbauer,[5] John C. Morris,[1,6] Mabel Ryder,[1,6] Samer Alsidawi,[1,7] Crystal Hilger,[1] and Keith C. Bible[1]

[1]Division of Medical Oncology, Mayo Clinic, Rochester, Minnesota 55905; [2]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota 55905; [3]Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota 55905; [4]Department of Radiation Oncology, Mayo Clinic, Rochester, Minnesota 55905; [5]Department of Otorhinolaryngology, Mayo Clinic, Rochester, Minnesota 55905; [6]Division of Endocrinology, Mayo Clinic, Rochester, Minnesota 55905; and [7]Division of Hematology, Mayo Clinic, Rochester, Minnesota 55905

Context: Anaplastic thyroid cancer (ATC) is rare and a highly fatal malignancy. The role of programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) as prognostic and/or predictive markers in ATC is unknown.

Objective: Multimodal therapy offers the best chance at tumor control. The objective of this study was to detect potential associations of PD-1/PD-L1 axis variables with outcome data in ATC.

Design: Retrospective study of a uniformly treated cohort.

Setting: Single institution retrospective cohort study.

Patients or Other Participants: Sixteen patients who received intensity-modulated radiation therapy (15 had preceding surgery) were studied.

Main Outcome Measure: Patients treated with multimodal therapy were followed and assessed for overall survival (OS) and progression-free survival (PFS).

Results: All samples demonstrated PD-1 expression in inflammatory cells whereas tumor cells were primarily negative. PD-L1 was expressed on ATC tumor cells in most samples and showed mainly membranous staining. High PD-1 expression (>40% staining) in inflammatory cells was associated with worse overall survival (OS; hazard ratio, 3.36; 95% confidence interval, 1.00 to 12.96; $P < 0.05$) and trended toward worse PFS, whereas high PD-L1 expression in tumor cells (>33% staining) trended toward worse PFS and OS.

Conclusion: PD-1/PD-L1 pathway proteins are highly expressed in ATC tumor samples and appear to represent predictive markers of PFS and OS in multimodality-treated ATC patients. (*J Clin Endocrinol Metab* 102: 1943–1950, 2017)

Thyroid cancer incidence is rising, in large part due to indolent small papillary thyroid cancers, most of which have excellent long-term prognoses (1). However, a small group of patients with thyroid cancer do have advanced disease, and their mortality rates are much higher. Anaplastic thyroid cancer (ATC), the most lethal of any human cancer, accounts disproportionally for deaths related to thyroid cancer.

Abbreviations: ATC, anaplastic thyroid cancer; CI, confidence interval; DTC, differentiated thyroid cancer; HR, hazard ratio; OS, overall survival; PD-1, programmed death-1; PD-L1, programmed death ligand-1; PFS, progression-free survival.

ATC is a subset of thyroid cancer that is undifferenti-ated, exhibits rapid growth, and is almost universally fatal (2). To be effective, treatment of ATC must be instituted expeditiously and requires multidisciplinary input. Doxo-rubicin is the only Food and Drug Administration–approved systemic therapy option, but it has very limited efficacy (3). To date, the most efficacious approach in-volves chemoradiation therapy with or without surgical resection. Data from our group has demonstrated that in stage IVA and IVB ATC, multimodal therapy with chemoradiation is associated with improved outcomes (4). However, most of these patients, and nearly all stage IVC patients, ultimately die of their disease. Newer ther-apies are therefore desperately needed.

Malignancies often subvert the inherent mechanisms of immune system regulation to "hide" from active immune surveillance. Checkpoint inhibitors, such as cytotoxic T lymphocyte–associated protein 4 inhibitors and pro-grammed death ligand-1 (PD-L1) inhibitors, have shown efficacy in overcoming this immune suppression in a subset of patients with melanoma, lung cancer, and many other malignancies (5–7). The roles of these checkpoint in-hibitors in thyroid cancer remain unknown, but they are being investigated. Recent reports demonstrate expres-sion patterns of programmed death-1 (PD-1) and PD-L1 in differentiated thyroid cancer (DTC) (8–10); however, the role of PD-1 and PD-L1 as predictive and/or prognostic markers is lacking in ATC.

We have previously reported on a small cohort of ATC patients treated with a multimodal therapy protocol who experience apparently improved overall survival (OS) (11). We have since treated additional patients using the same approach, validating initial results (4). The objec-tive of this retrospective study was to examine the roles of PD-1 and PD-L1 in defining the prognosis of this uni-formly treated cohort of ATC patients.

Methods

Patients and clinical data

This is a single-institution, retrospective cohort study ap-proved by the Mayo Clinic Institutional Review Board. Only patients who had provided prior informed consent for research purposes, received all or part of their care at the Mayo Clinic, and had tissue available for analysis were included. Patients were identified from our institutional database, and only pa-tients treated with a multimodal protocol using chemoradiation with or without surgery were included. Patients were treated between 1 January 2003 and 31 December 2015.

Inclusion criteria included patients with newly diagnosed, his-tologically confirmed ATC. Pathological samples were rereviewed by K.M.R. for the study. Patients with poorly differentiated thyroid cancer, or tumors that were diagnosed only at autopsy or by death certificate, were excluded. Patients who were treated under the Radiation Therapy Oncology Group trial 0912 were also excluded.

Information for each patient was collected from the elec-tronic medical records, including demographic characteristics, pathologic characteristics, and details of treatment modalities, response, and follow-up for vital status. Survival endpoints and follow-up were documented through January 2016. Standard American Joint Committee on Cancer staging was used to define stages IVA, IVB, and IVC.

Immunohistochemistry

Pathologist-assessed tissues were blinded to patient out-comes. For conventional microscopic examinations, represen-tative sections of the tumor were fixed in formalin, routinely processed, embedded in paraffin, cut at 4 μm, and stained with hematoxylin and eosin. Additional sections were cut for im-munohistochemistry per the clinical method used in the labo-ratory at the time. Briefly, PD-L1 staining was performed using the E1L3N clone from Cell Signaling Technology (Danvers, MA) at a 1:750 dilution with a 64-minute Cell Conditioning Solution 1 pretreatment for antigen retrieval with OptiView and adenosine 5′-monophosphate detection (diaminobenzidine) on the BenchMark XT platform from Ventana Medical Systems (Tucson, AZ). PD-1 (CD279) staining was performed using the NAT105 clone from Abcam (Cambridge, MA) at a 1:200 di-lution with a 32-minute Cell Conditioning Solution 1 pre-treatment for antigen retrieval with OptiView detection (diaminobenzidine) on the BenchMark XT platform from Ventana Medical Systems (Tucson, AZ). Appropriate positive and negative controls were included in each sample run to assure reliability of IHC assessment. Stains were initially scored for intensity of staining (0 to 3), percentage of cells staining (0% to 100%), cell type, and stain localization from which an Allred-like score was calculated for all samples (12). A score of 0–5 was given as follows: no staining (0), ≤1% (1), 1% to 10% (2), 11% to 33% (3), 34% to 66% (4), and 67% to 100% (5), re-spectively. Intensity of staining was scored as: 0, negative; 1, weak; 2, intermediate; and 3, strong. Scores from percentage staining and intensity were added to get the final score. Samples were considered positive if the Allred-like score was ≥3. PD-L1 high vs low and PD-1 high vs low for tumor and inflammatory cells, respectively, were determined arbitrarily with respect to the median percentage staining for each group. For example, median percentage staining for PD-L1 in tumor cells was 33%, and hence ≤33% was designated as PD-L1 low and >33% was designated as PD-L1 high.

Statistical analysis

To calculate survival endpoints, the date of diagnosis (date of biopsy, imaging, or clinic visit documenting diagnosis), date of locoregional recurrence and/or distant metastasis (date of biopsy or the date of imaging or clinic visit documenting recurrence), and date of death or last follow-up were used. OS was considered based on death from any cause. Progression was defined as the earliest date of development of any locoregional relapse, distant metastasis, or death. Patients were followed from the date of diagnosis to the earliest date for each endpoint, or were censored at last follow-up. OS and progression-free survival (PFS) were summarized using the Kaplan–Meier method, and the median survival time was reported. Hazard ratios, 95% profile likelihood-based con-fidence intervals, and likelihood ratio tests were calculated using Cox proportional hazards models. All analyses were

performed using SAS version 9.4 (SAS Institute, Cary, NC) or R version 3.1.1 (13), and *P* values of <0.05 were considered statistically significant.

Results

We identified 54 patients from our records with the diagnosis of ATC in the prespecified study period. Five patients were excluded, as they were treated as part of the Radiation Therapy and Oncology Group protocol, and one patient did not have research authorization and was excluded. Of the remaining 48 patients, 18 were treated with palliative radiation therapy or best supportive care and did not receive multimodal therapy, and they were also excluded from analysis. Sixteen of the remaining 30 patients had sufficient tissue for testing. Patient and treatment characteristics are noted in Table 1. Eleven patients were male (69%), and median age was 58 (range, 37 to 83) years. Most patients had stage IVB disease (n = 13, 81%). There were two (13%) with stage IVC and one (6%) with stage IVA disease at diagnosis. Of the 16 samples, 10 (63%) showed pure ATC with no

evidence of prior DTC, whereas the remaining six (38%) showed evidence of concomitant DTC in the same pathologic samples.

Treatment consisted of surgical resection, if feasible, followed by chemoradiation. Fifteen patients (94%) underwent surgical resection. Complete surgical resection with negative margins (R0 resection) was achieved in four (27%), resection with microscopic positive margins (R1) was achieved in eight (53%), and three (20%) had R2 resection. One (6%) patient initiated chemoradiation after diagnosis by biopsy and did not undergo surgical resection. All patients (n =16, 100%) received chemoradiation therapy. Median dose of radiation therapy was 66 Gy (range, 46 to 70). Ten (63%) patients received docetaxel and doxorubicin as concurrent chemotherapy. Other regimens used were cisplatin (n = 2, 13%), carboplatin plus paclitaxel (n = 2, 13%), and doxorubicin (n = 2, 13%). All patients completed intended chemoradiation therapy.

Samples were stained for both PD-1 and PD-L1, with tumor cells and inflammatory cells in stroma independently assessed and scored. Representative hematoxylin and eosin staining for an ATC is shown in Fig. 1(a). PD-1 staining

Table 1. Patient and Treatment Characteristics by PD-1 High (>40%) vs Low (≤40%) Expression in Inflammatory Cells, and PD-L1 High (> 33%) vs Low (≤ 33%) Expression in Tumor Cells

	PD-1 Expression in Inflammatory Cells [N (%)]		PD-L1 Expression in Tumor Cells [N (%)]		Total Patients [N (%)]
	High (>40%)	Low (≤40%)	High (>33%)	Low (≤33%)	
Sex					
Male	5 (71)	6 (67)	5 (83)	6 (60)	11 (69)
Female	2 (29)	3 (33)	1 (17)	4 (40)	5 (31)
Race					
White	6 (86)	9 (100)	5 (83)	10 (100)	15 (94)
Black	1 (14)	0	1 (17)	0	1 (6)
Median age, y	55 (range, 45–71)	60 (range, 37–83)	60 (range, 50–71)	57 (range, 37–83)	58 (range, 37–83)
Histology					
Pure ATC	3 (43)	7 (78)	3 (50)	7 (70)	10 (63)
With DTC	4 (57)	2 (22)	3 (50)	3 (30)	6 (38)
AJCC stage					
IVA	0	1 (11)	0	1 (10)	1 (6)
IVB	6 (86)	7 (78)	5 (83)	8 (80)	13 (81)
IVC	1 (14)	1 (11)	1 (17)	1 (10)	2 (13)
Surgery					
Yes	7 (100)	8 (89)	6 (100)	9 (90)	15 (94)
No	0	1 (11)	0	1 (10)	1 (6)
Resection status					
R0	3 (43)	1 (11)	2 (33)	2 (20)	4 (25)
R1	3 (43)	5 (56)	4 (67)	4 (40)	8 (50)
R2	1 (14)	2 (22)	0	3 (30)	3 (19)
Biopsy only	0	1 (11)	0	1 (10)	1 (6)
Chemoradiation	7 (100)	9 (100)	6 (100)	10 (100)	16 (100)
Median radiation dose, Gy	64 (range, 46–66)	66 (range, 58–70)	63 (range, 46–66)	66 (range, 58–70)	66 (range, 46–70)
Chemotherapy					
Docetaxel plus doxorubicin	5 (71)	5 (56)	5 (83)	5 (50)	10 (63)
Carboplatin plus paclitaxel	2 (29)	0	1 (17)	1 (10)	2 (13)
Cisplatin	0	2 (22)	0	2 (20)	2 (13)
Doxorubicin	0	2 (22)	0	2 (20)	2 (13)

Abbreviation: AJCC, American Joint Committee on Cancer.

was highly discordant in tumor cells vs inflammatory cells. Most tumor cells lacked PD-1 staining (PD-1 positive, n = 1; PD-1 negative, n = 15). In contrast, all samples showed positive staining for PD-1 in inflammatory cells (n = 16). Median PD-1 percentage staining in inflammatory cells was 40% (range, 9% to 80%), and the median Allred-like score was 5.5 (range, 3 to 7). Representative images of low ($\leq$40%) and high ($>$40%) PD-1 expression on inflammatory cells is shown in Fig. 1(d) and 1(e), respectively.

PD-L1 staining in tumor cells was positive in most patients (n = 13). The staining was mostly membranous and relatively strong, with median PD-L1 staining of 33% (range, 0% to 100%), and the median Allred-like score was 6 (range, 0 to 8). PD-L1 staining in inflammatory cells was positive in seven of 16 patients, with both median percentage staining and median Allred-like scores of 0. We could not definitively determine whether PD-1 expression in inflammatory cells correlated with PD-L1 staining in tumor cells (data not shown). Representative images of low ($\leq$33%) and high ($>$33%) PD-L1 expression on tumor cells is shown in Fig. 1(b) and 1(c), respectively.

Patient and treatment characteristics based on PD-1 expression in inflammatory cells ($\leq$40% vs $>$40%) and PD-L1 expression in tumor cells ($\leq$33% vs $>$33%) are shown in Table 1. None of the differences in various characteristics in each group was statistically significant. Details of survival characteristics are noted in Table 2. At last follow-up, 14 of 16 patients had died and all died of disease-related complications. Median PFS was 123 days for PD-L1 tumor cell–positive patients vs

1388 days for PD-L1 tumor cell–negative patients [hazard ratio (HR), 1.38; 95% confidence interval (CI), 0.42 to 6.20; P = 0.62; Fig. 2(a)]. Similarly, median OS was 175 days for PD-L1 tumor cell–positive patients vs 1995 days for PD-L1 tumor cell–negative patients [HR, 1.35; 95% CI, 0.41 to 6.10; P = 0.64; Fig. 2(b)]. When the same data were analyzed by percentage staining ($\leq$33% vs $>$33% PD-L1 staining), median PFS [Fig. 2(c)] was 453 days for $\leq$33% PD-L1 positive vs 118 days for $>$33% PD-L1 positive tumor cell staining patients (HR, 3.04; 95% CI, 0.90 to 10.80; P = 0.07). Similarly, median OS [Fig. 2 (d)] was 1474 days for $\leq$33% PD-L1 positive vs 149 days for $>$33% PD-L1 positive tumor cell staining patients (HR, 3.72; 95% CI, 0.91 to 18.25; P = 0.07).

Median PFS per PD-L1 staining in inflammatory cells (positive vs negative) was also statistically nonsignificant [Fig. 3(a); 202 days for PD-L1 positive vs 119 days for PD-L1 negative; HR, 0.78; 95% CI, 0.26 to 2.28; P = 0.66]. Similarly, median OS was 953 days for PD-L1 inflammatory cell positive vs 175 days for PD-L1 inflammatory cell negative [Fig. 3(b); HR, 0.65; 95% CI, 0.19 to 1.95; P = 0.44]. Median PD-L1 staining percentage in inflammatory cells was 0, and analysis of patients with no staining (0%) vs $\geq$1% was also not significant for both PFS and OS (data not shown).

Comparison for PD-1 positive vs PD-1 negative in tumor cells (n = 1 vs n = 15, respectively) was not performed due to the inadequate number of positive patients. PD-1 staining in inflammatory cells was positive in all samples. However, PFS in patients with $\leq$40% PD-1–positive inflammatory cells was 250 days vs 123 days in patients with $>$40% PD-1–positive inflammatory cells [Fig. 3(c); HR, 1.96; 95% CI, 0.66 to 5.85; P = 0.22]. OS was significantly different in these two groups [Fig. 3(d); 2059 days for $\leq$40% PD-1 positive vs 175 days for $>$40% PD-1 positive; HR, 3.36; 95%, CI 1.00 to 12.96; P < 0.05].

Discussion

ATC is a highly lethal cancer with median survival historically reported to be 4 to 6 months (14–17). Treatment of AJCC stage IVA and IVB patients with chemoradiation therapy with or without surgery can provide long-term control, but is very toxic (11, 18–20). Multimodal therapy has been consistently offered at our institution for these selected patients, providing a uniformly treated cohort for analysis.
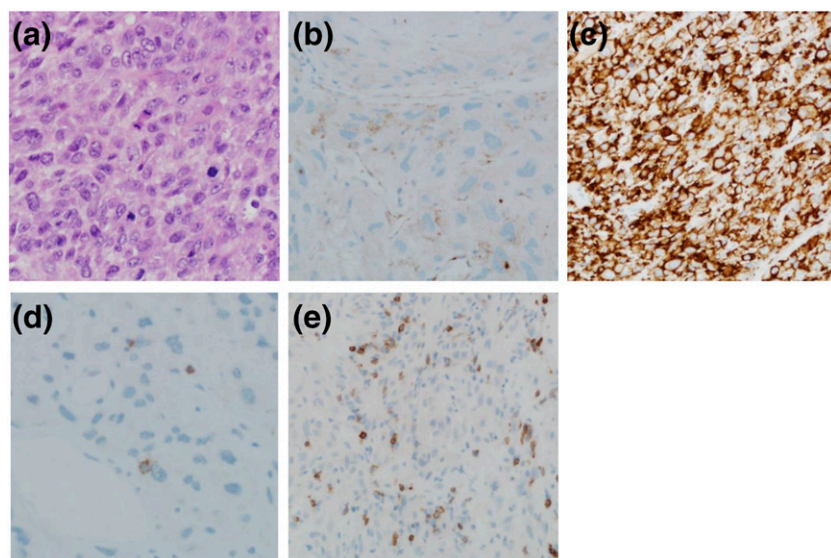

Figure 1. Representative photomicrographs showing (a) hematoxylin and eosin–stained section of ATC, (b) section of ATC tumor mass showing low PD-L1 expression, (c) section of ATC tumor mass showing high PD-L1 expression, (d) section showing low PD-1 expression on inflammatory cells, and (e) section showing high PD-1 expression on inflammatory cells. Original magnification: a, c, and e, 200$\times$; b and d, 400$\times$.

Table 2.   **PFS and OS of the PD-1 and PD-L1 Staining Groups**

Outcome	Groups	N (No. Events)	Median Days	1-Year Survival % (95% CI)	2-Year Survival % (95% CI)	Hazard Ratio (95% CI)	P Value
PFS	PD-L1 tumor						
	Negative	3 (3)	1388	66.7 (13.3, 100.0)	66.7 (13.3, 100.0)	Reference	0.62
	Positive	13 (12)	123	23.1 (0.2, 46.0)	15.4 (0.0, 35.0)	1.38 (0.42, 6.20)	
	PD-L1 tumor percentage staining						
	≤33%	10 (9)	453	50.0 (19.0, 81.0)	40.0 (9.6, 70.4)	Reference	0.07
	>33%	6 (6)	118	0.0 (NA)	0.0 (NA)	3.04 (0.90, 10.80)	
	PD-L1 inflammatory cells						
	Negative	9 (9)	119	33.3 (2.5, 64.1)	22.2 (0.0, 49.4)	Reference	0.66
	Positive	7 (6)	202	28.6 (0.0, 62.0)	28.6 (0.0, 62.0)	0.78 (0.26, 2.28)	
	PD-1 inflammatory cell percentage staining						
	≤40%	9 (8)	250	44.4 (12.0, 76.9)	33.3 (2.5, 64.1)	Reference	0.22
	>40%	7 (7)	123	14.3 (0.0, 40.2)	14.3 (0.0, 40.2)	1.96 (0.66, 5.85)	
OS	PD-L1 tumor						
	Negative	3 (3)	1995	100.0 (NA)	66.7 (13.3, 100.0)	Reference	0.64
	Positive	13 (11)	175	38.5 (12.0, 64.9)	38.5 (12.0, 64.9)	1.35 (0.41, 6.10)	
	PD-L1 tumor percentage staining						
	≤33%	10 (9)	1474	70.0 (41.6, 98.4)	60.0 (29.6, 90.4)	Reference	0.07
	>33%	6 (5)	149	16.7 (0.0, 46.5)	0.0 (NA)	3.72 (0.91, 18.25)	
	PD-L1 inflammatory cells						
	Negative	9 (9)	175	44.4 (12.0, 76.9)	33.3 (2.5, 64.1)	Reference	0.44
	Positive	7 (5)	953	57.1 (20.5, 93.8)	57.1 (20.5, 93.8)	0.65 (0.19, 1.95)	
	PD-1 inflammatory cell percentage staining						
	≤40%	9 (7)	2059	66.7 (35.9, 97.5)	53.3 (19.4, 87.3)	Reference	<0.05
	>40%	7 (7)	175	28.6 (0.0, 62.0)	28.6 (0.0, 62.0)	3.36 (1.00, 12.96)	

Recently, immunotherapy has emerged as a new therapeutic approach for patients with cancer, demonstrating efficacy in highly mutated cancers such as melanoma and non–small-cell lung cancer (5, 21). The roles of these immune pathways are now being investigated in thyroid cancer (8, 22); however, their roles in determining prognosis or as a predictive marker remain lacking. We thus explored the role of PD-1 and PD-L1 expression in our cohort of uniformly treated ATC patients. Limitations of our study include small sample size and its single-institution nature; strengths include assessment of uniformly and consecutively treated patients.

In our cohort, most tumor cells were PD-L1 positive. Whether this relates to small cohort size or might reflect a more generalized phenomenon in ATC is unknown. However, recently published data from Bastman et al. (8) similarly showed that six of eight samples from ATC patients highly expressed PD-L1. These limited data suggest that ATCs may be associated with an antitumor immune response that may, however, be blocked or "paralyzed" by high expression of PD-1 and PD-L1 checkpoint molecules that inhibit immune response. PD-L1 may be expressed by the cancer cells themselves, as well as by tumor-associated macrophages (8, 22). The latter have been shown to heavily infiltrate most ATCs and likely contribute to an immunosuppressive tumor microenvironment (23). With no additional stains for subsets of immune cells, we could not comment on the composition of these infiltrates in our ATC patients.

We have shown in ATC that positive staining for PD-L1 in tumor cells or PD-1 staining in inflammatory cells appears predictive of poor prognosis in the ATC patients in terms of both PFS and OS. Despite a relatively small sample size, this is one of the largest uniformly treated patient cohorts, making these findings thought-provoking and potentially important for planning future trials in this rare and highly lethal cancer.

In our cohort, PD-1 staining was mostly seen on immune cells and not on tumor cells. High expression of the PD-1 in these stromal inflammatory cells could be a marker of immune exhaustion as demonstrated by Severson et al. (24) in PD-1– and Tim-3–positive CD8 T cells from patients with DTC. Further comprehensive analyses with specific staining for various subsets of immune cells to characterize the specific population and coexpression of other markers such as Tim-3 in our cohort would be more insightful. In contrast to our study, Batsman et al. (8) demonstrated little expression of PD-1 in DTC and none in ATC samples. Perhaps this is a result of variability and small sample size in both studies. Therefore, additional methods such as analysis from peripheral leukocytes of ATC patients to define "immune signatures" or gene expression markers could be studied to serve as prognostic or predictive markers. However, it is critical to implement these studies prospectively in upcoming clinical studies.

We tried to categorize samples using Allred-like scores to semiobjectively quantify the staining. However, analysis using this score to denote samples as
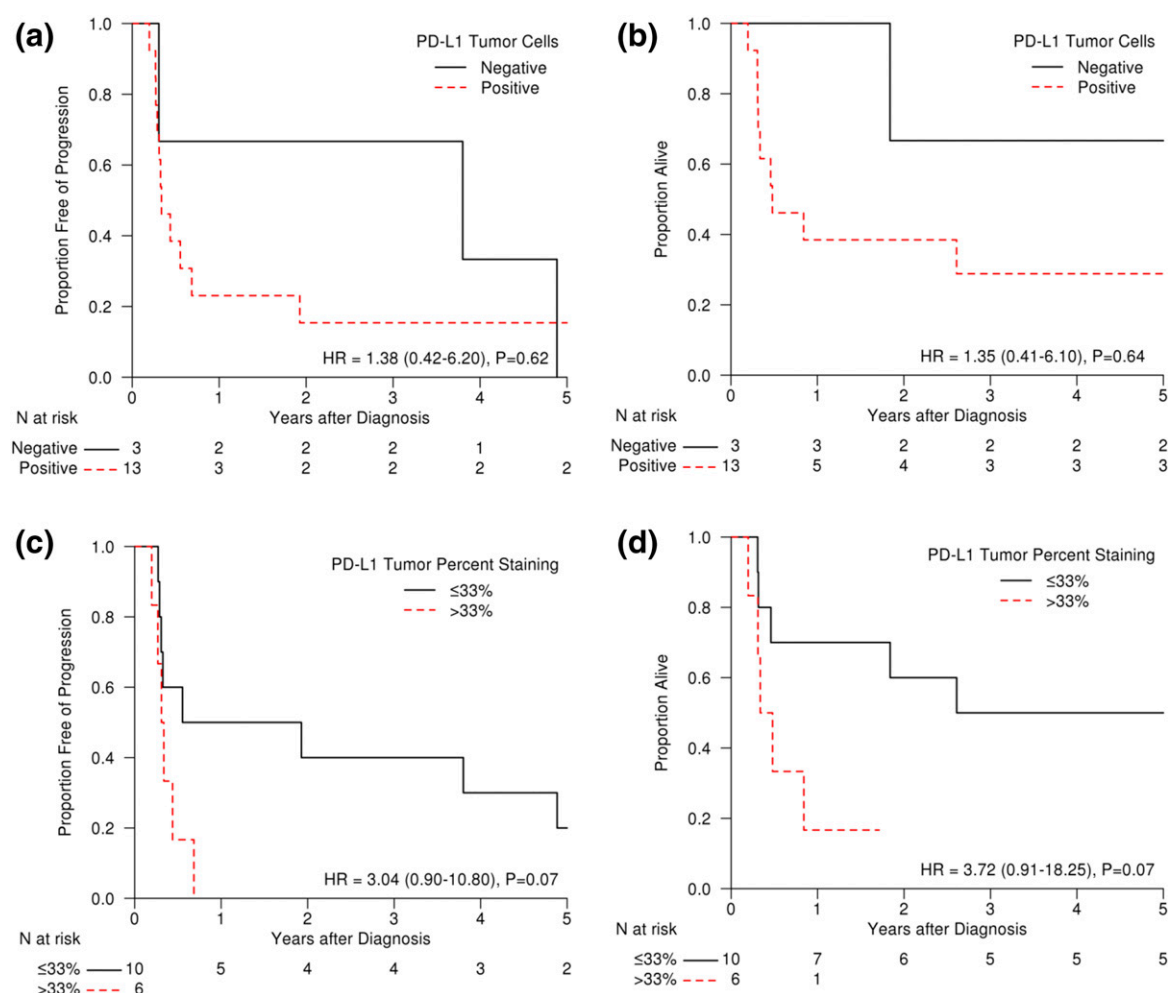
Figure 2. (a) PFS and (b) OS of ATC patients demonstrating positive or negative staining (determined by Allred-like score) for PD-L1 in their tumor cells. (c) PFS and (d) OS of ATC patients demonstrating ≤33% or >33% staining for PD-L1 in their tumor cells.

positive vs negative seemed to not separate the groups prognostically as well as percentage cells staining. The optimal cutoff for choosing PD-L1– or PD-1–positive samples is not known. In the past, major clinical trials have used the PD-L1 cutoff anywhere from 1% (25), 5% (26), 25%, (27) to 50% (28, 29). The reagents and method of scoring used to determine these cutoffs have also varied. Importantly, note membranous vs cytoplasmic staining. In some assays staining of tumor cells only is counted for PD-L1 positivity (26) vs both tumor and immune cells in some assays (25, 28). In our analysis, we used the median percentage staining as cutoff for our survival analysis for both PD-1 and PD-L1 staining to designate them as high vs low. A more rigorous analysis to determine the optimal cutoff will require a larger dataset but will be difficult to obtain in rare disease such as ATC.

In conclusion, high expression of PD-L1 and PD-1 in this cancer suggests that therapies that block these pathways could potentially be effective therapeutic approaches. With limited systemic therapeutic options available, this hypothesis needs to be tested. ATC is a prime target for such therapies, as these cancers have high rates of mutations, including commonly mutated genes such as P53, TERT promoter, and BRAF and also of microsatellite instability genes (30–32). High numbers of mutations (21), mismatch repair deficiency (33), and BRAF mutation (22) all have been shown to be associated with response to anti–PD-1 agents in other contexts. Accordingly, clinical trials of anti–PD-1 agents with chemoradiation therapy are being planned at our institution as well as by the NRG Oncology group.

Acknowledgments

Address all correspondence and requests for reprints to: Keith C. Bible, MD, PhD, Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905. E-mail: bible.keith@mayo.edu.
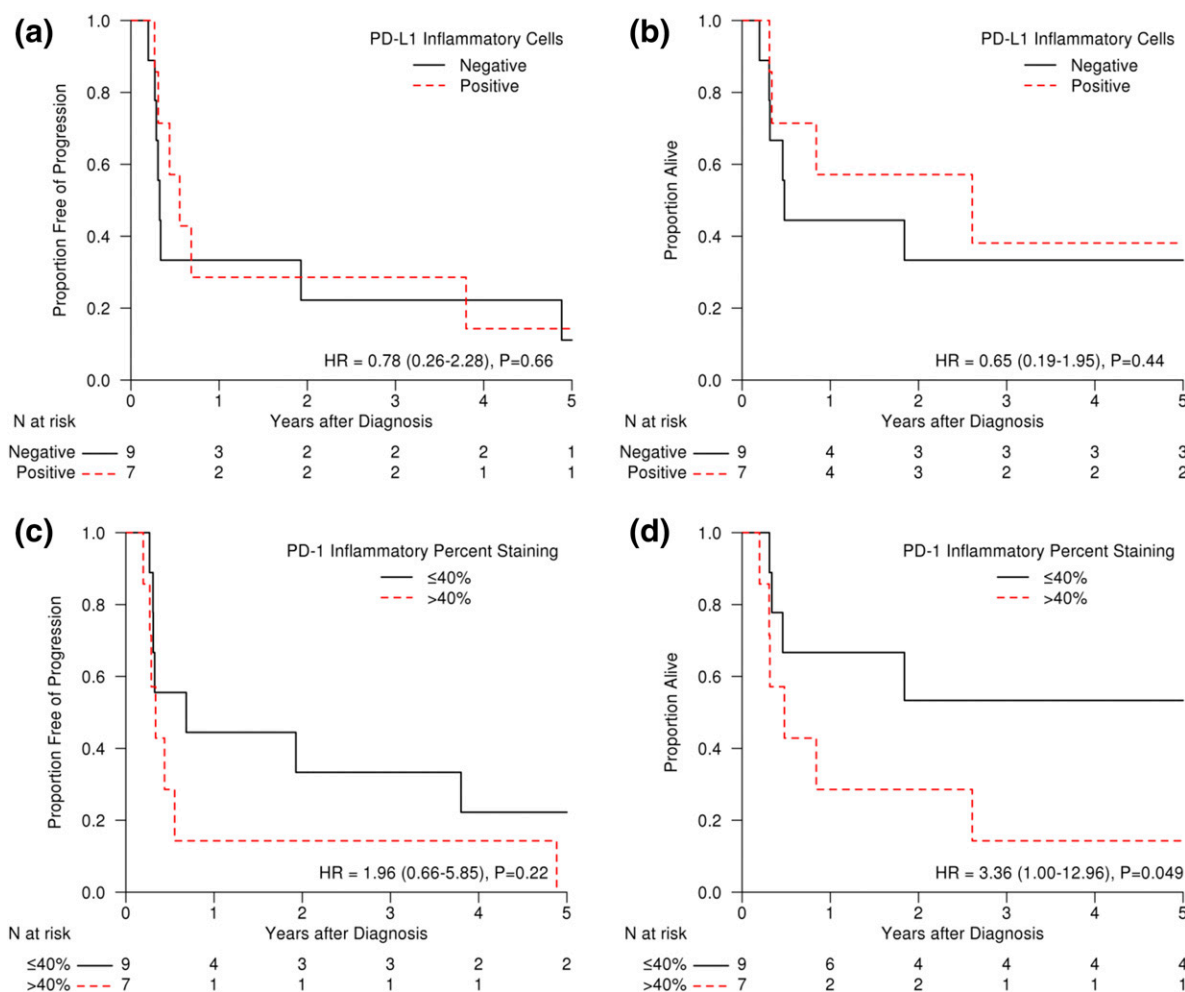
Figure 3. (a) PFS and (b) OS of ATC patients demonstrating positive or negative staining (determined by Allred-like score) for PD-L1 in their inflammatory cells. (c) PFS and (d) OS of ATC patients demonstrating ≤40% or >40% staining for PD-1 in their inflammatory cells.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin.* 2016;**66**(1):7–30.
2. Bible KC, Foote RL, Smallridge RC. Toward improved outcomes in patients with anaplastic thyroid cancer. *Oncology (Williston Park).* 2012;**26**(4):398, 401, 406.
3. Shimaoka K, Schoenfeld DA, DeWys WD, Creech RH, DeConti R. A randomized trial of doxorubicin versus doxorubicin plus cisplatin in patients with advanced thyroid carcinoma. *Cancer.* 1985; **56**(9):2155–2160.
4. Kumar A. Outcomes in response to aggressive multimodal therapy in anaplastic thyroid cancer: the Mayo Clinic experience. In: 15th International Thyroid Congress and 85th Annual Meeting of the American Thyroid Association; 18–23 October 2015; Lake Buena Vista, FL. Oral abstract 72.
5. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell.* 2015;**27**(4):450–461.
6. Allison JP. Immune checkpoint blockade in cancer therapy: the 2015 Lasker-DeBakey Clinical Medical Research Award. *JAMA.* 2015;**314**(11):1113–1114.
7. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. *J Clin Oncol.* 2015;**33**(17):1974–1982.
8. Bastman JJ, Serracino HS, Zhu Y, Koenig MR, Mateescu V, Sams SB, Davies KD, Raeburn CD, McIntyre RC, Jr, Haugen BR, Jr, French JD. Tumor-infiltrating T cells and the PD-1 checkpoint pathway in advanced differentiated and anaplastic thyroid cancer. *J Clin Endocrinol Metab.* 2016;**101**(7):2863–2873.
9. Angell TE, Lechner MG, Jang JK, Correa AJ, LoPresti JS, Epstein AL. BRAF V600E in papillary thyroid carcinoma is associated with increased programmed death ligand 1 expression and suppressive immune cell infiltration. *Thyroid.* 2014;**24**(9):1385–1393.
10. Chowdhury S, Veyhl J, Jessa F, Polyakova O, Alenzi A, MacMillan C, Ralhan R, Walfish PG. Programmed death-ligand 1 over-expression is a prognostic marker for aggressive papillary thyroid cancer and its variants. *Oncotarget.* 2016;**7**(22):32318–32328.
11. Foote RL, Molina JR, Kasperbauer JL, Lloyd RV, McIver B, Morris JC, Grant CS, Thompson GB, Richards ML, Hay ID, Smallridge RC, Bible KC. Enhanced survival in locoregionally confined anaplastic thyroid carcinoma: a single-institution experience using aggressive multimodal therapy. *Thyroid.* 2011;**21**(1):25–30.
12. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod Pathol.* 1998;**11**(2):155–168.
13. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2014.
14. Goutsouliak V, Hay JH. Anaplastic thyroid cancer in British Columbia 1985–1999: a population-based study. *Clin Oncol (R Coll Radiol).* 2005;**17**(2):75–78.

15. McIver B, Hay ID, Giuffrida DF, Dvorak CE, Grant CS, Thompson GB, van Heerden JA, Goellner JR. Anaplastic thyroid carcinoma: a 50-year experience at a single institution. *Surgery*. 2001;130(6): 1028–1034.

16. Sherman EJ, Lim SH, Ho AL, Ghossein RA, Fury MG, Shaha AR, Rivera M, Lin O, Wolden S, Lee NY, Pfister DG. Concurrent doxorubicin and radiotherapy for anaplastic thyroid cancer: a critical re-evaluation including uniform pathologic review. *Radiother Oncol*. 2011;101(3):425–430.

17. Sugitani I, Miyauchi A, Sugino K, Okamoto T, Yoshida A, Suzuki S. Prognostic factors and treatment outcomes for anaplastic thyroid carcinoma: ATC Research Consortium of Japan cohort study of 677 patients. *World J Surg*. 2012;36(6):1247–1254.

18. De Crevoisier R, Baudin E, Bachelot A, Leboulleux S, Travagli JP, Caillou B, Schlumberger M. Combined treatment of anaplastic thyroid carcinoma with surgery, chemotherapy, and hyper-fractionated accelerated external radiotherapy. *Int J Radiat Oncol Biol Phys*. 2004;60(4):1137–1143.

19. Ito K, Hanamura T, Murayama K, Okada T, Watanabe T, Harada M, Ito T, Koyama H, Kanai T, Maeno K, Mochizuki Y, Amano J. Multimodality therapeutic outcomes in anaplastic thyroid carcinoma: improved survival in subgroups of patients with localized primary tumors. *Head Neck*. 2012;34(2):230–237.

20. Mohebati A, Dilorenzo M, Palmer F, Patel SG, Pfister D, Lee N, Tuttle RM, Shaha AR, Shah JP, Ganly I. Anaplastic thyroid carcinoma: a 25-year single-institution experience. *Ann Surg Oncol*. 2014;21(5):1665–1670.

21. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS, Miller ML, Rekhtman N, Moreira AL, Ibrahim F, Bruggeman C, Gasmi B, Zappasodi R, Maeda Y, Sander C, Garon EB, Merghoub T, Wolchok JD, Schumacher TN, Chan TA. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*. 2015;348(6230):124–128.

22. Brauner E, Gunda V, Vanden Borre P, Zurakowski D, Kim YS, Dennett KV, Amin S, Freeman GJ, Parangi S. Combining BRAF inhibitor and anti PD-L1 antibody dramatically improves tumor regression and anti tumor immunity in an immunocompetent murine model of anaplastic thyroid cancer. *Oncotarget*. 2016;7(13):17194–17211.

23. Ryder M, Ghossein RA, Ricarte-Filho JC, Knauf JA, Fagin JA. Increased density of tumor-associated macrophages is associated with decreased survival in advanced thyroid cancer. *Endocr Relat Cancer*. 2008;15(4):1069–1074.

24. Severson JJ, Serracino HS, Mateescu V, Raeburn CD, McIntyre RC, Jr, Sams SB, Haugen BR, French JD. PD-1⁺Tim-3⁺ CD8⁺ T lymphocytes display varied degrees of functional exhaustion in patients with regionally metastatic differentiated thyroid cancer. *Cancer Immunol Res*. 2015;3(6):620–630.

25. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, Patnaik A, Aggarwal C, Gubens M, Horn L, Carcereny E, Ahn MJ, Felip E, Lee JS, Hellmann MD, Hamid O, Goldman JW, Soria JC, Dolled-Filhart M, Rutledge RZ, Zhang J, Lunceford JK, Rangwala R, Lubiniecki GM, Roach C, Emancipator K, Gandhi L; KEYNOTE-001 Investigators. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med*. 2015;372(21): 2018–2028.

26. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012;366(26):2443–2454.

27. Antonia S, Goldberg SB, Balmanoukian A, Chaft JE, Sanborn RE, Gupta A, Narwal R, Steele K, Gu Y, Karakunnel JJ, Rizvi NA. Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study. *Lancet Oncol*. 2016;17(3):299–308.

28. Fehrenbacher L, Spira A, Ballinger M, Kowanetz M, Vansteenkiste J, Mazieres J, Park K, Smith D, Artal-Cortes A, Lewanski C, Braiteh F, Waterkamp D, He P, Zou W, Chen DS, Yi J, Sandler A, Rittmeyer A; POPLAR Study Group. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. *Lancet*. 2016;387(10030):1837–1846.

29. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csoszi T, Fulop A, Gottfried M, Peled N, Tafreshi A, Cuffe S, O'Brien M, Rao S, Hotta K, Leiby MA, Lubiniecki GM, Shentu Y, Rangwala R, Brahmer JR; KEYNOTE-024 Investigators. Pembrolizumab versus chemotherapy for PD-L1–positive non–small-cell lung cancer. *N Engl J Med*. 2016;375(19):1823–1833.

30. Jeon MJ, Chun SM, Kim D, Kwon H, Jang EK, Kim TY, Kim WB, Shong YK, Jang SJ, Song DE, Kim WG. Genomic alterations of anaplastic thyroid carcinoma detected by targeted massive parallel sequencing in a BRAF(V600E) mutation-prevalent area. *Thyroid*. 2016;26(5):683–690.

31. Kunstman JW, Juhlin CC, Goh G, Brown TC, Stenman A, Healy JM, Rubinstein JC, Choi M, Kiss N, Nelson-Williams C, Mane S, Rimm DL, Prasad ML, Höög A, Zedenius J, Larsson C, Korah R, Lifton RP, Carling T. Characterization of the mutational landscape of anaplastic thyroid cancer via whole-exome sequencing. *Hum Mol Genet*. 2015;24(8):2318–2329.

32. Landa I, Ibrahimpasic T, Boucai L, Sinha R, Knauf JA, Shah RH, Dogan S, Ricarte-Filho JC, Krishnamoorthy GP, Xu B, Schultz N, Berger MF, Sander C, Taylor BS, Ghossein R, Ganly I, Fagin JA. Genomic and transcriptomic hallmarks of poorly differentiated and anaplastic thyroid cancers. *J Clin Invest*. 2016;126(3): 1052–1066.

33. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA, Jr. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372(26):2509–2520.