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ORIGINAL ARTICLE

Blockade of the programmed death ligand 1 (PD-L1) as potential therapy for anaplastic thyroid cancer

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Abstract

Purpose Anaplastic thyroid carcinoma (ATC) is a rare, highly aggressive form of thyroid cancer (TC) characterized by an aggressive behavior and poor prognosis, resulting in patients' death within a year. Standard treatments, such as chemo and radiotherapy, as well as tyrosine kinase inhibitors, are ineffective for ATC treatment. Cancer immunotherapy is one of the most promising research area in oncology. The PD-1/PD-L1 axis is of particular interest, in light of promising data showing a restoration of host immunity against tumors, with the prospect of long-lasting remissions.

Methods In this study, we evaluated PD-L1 expression in a large series of TCs (20 cases) showing a progressive dedifferentiation of the thyroid tumor from well differentiated TC to ATC, employing two different antibodies [R&D Systems and VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody]. We also tested the anti PD-L1 mAb in an in vivo animal model.

Results We found that approximately 70–90% of ATC cases were positive for PD-L1 whereas normal thyroid and differentiated TC were negative. Moreover, all analyzed cases presented immunopositive staining in the endothelium of vessels within or in close proximity to the tumor, while normal thyroid vessels were negative. PD-L1 mAb was also effective in inhibiting ATC growth in an in vivo model.

Conclusions These data suggest that immunotherapy may be a promising treatment specific for ATC suggesting the need to start with clinical TRIALs.

Keywords PD-L1 · PD-1 · Anaplastic thyroid cancer · Immunotherapy

Introduction

Cancer immunotherapy is one of the most promising research area in oncology [1]. One approach is the blockade of immune check-points (ICPs), a series of inhibitory pathways crucial for maintaining self tolerance which are deregulated in cancer [1]. Because ICPs are initiated by ligand-receptor interactions, they can be blocked by antibodies. Such mechanisms represent an opportunity to enhance antitumor immunity. In particular, the blockade of the programmed cell death protein 1 (PD-1) has been investigated with promising results in several cancers [2–4]. PD-1 (B7-1) is a cell-surface glycoprotein normally expressed by macrophage lineage cells and T cells. The binding of PD-1 to one of its ligands, PD-L1 or PD-L2, can inhibit cytotoxic T-cell immune responses, leading to tolerance of cells expressing PD-L1 or PD-L2 [5]. PD-L1 is constitutively expressed by tumor cells as a result of

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oncogenic signaling or dynamic IFNγ expression in tumor microenvironments [6].

Thyroid cancer (TC) is mostly curable with conventional therapies including surgery, hormonal therapy and radioiodine providing clinical remission in nearly 90% of cases. However, a small group of TCs is more aggressive, developing distant metastases and being refractory to conventional therapies [7]. This group includes about 10% of well differentiated TCs, most of the poorly differentiated TCs and the anaplastic TC (ATC). In particular, ATC represents 1–2% of thyroid malignancies and is believed to arise from terminal dedifferentiation of papillary or follicular TC [8]. ATC is characterized by high-mitotic rate, lymphovascular invasion, and undifferentiated features which confer on ATC an extremely aggressive behavior and a very poor prognosis, resulting in patients' death within a year [9]. Tyrosine kinase inhibitors are ineffective in the treatment of ATC [9–11].

A few studies have addressed the expression of PD-L1 in TC both in papillary TC (PTC) and poorly differentiated/ATC [12–18]. Interestingly, finding a correlation between PD-L1 expression and the aggressive behavior of thyroid disease, they concluded that identification of PD-L1 expression may be exploited to uncover a new therapeutic strategy for patients with refractory TC.

In this study we evaluated PD-L1 expression by immunohistochemistry in a large series of TCs, from the poorly differentiated forms of cancer to the ATC, employing two different antibodies [VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody and the anti-PD-L1 mAb of the R&D Systems]. We also tested the efficacy of anti PD-L1 antibody as tools for TC immunotherapy in an in vivo animal model.

Materials and methods

Patients and tissues

The study group comprises 20 patients with ATC from the Histopathological sections of the University Hospital of Siena and from Arezzo Hospital, where the histopathological diagnoses were performed. Two cases (10%) were biopsies from a neck inoperable mass, while 18 (90%) were surgical resections of thyroid tumors. All samples but three (85%) also included normal thyroid gland, which was useful to check PD-L1 immunoreactivity in normal thyroid tissue, while a progressive dedifferentiation of the thyroid tumor was seen in 13 samples (65% of cases). This striking peculiarity of the present files often allowed us to observe different aspects of the tumors in the same section, i.e., different histological grades as well as different degrees of immunoreactivity. Among these 13 samples, 12 (87.5%) dedifferentiated from the insular variant of papillary TC to ATC, 1 (12.5%) evolved directly from papillary TC to ATC.

Table 1 Synopsis of patient's characteristics

Case #	Sex	Age	Normal thyroid tissue Yes/No	Presence of differentiated TC
1	F	81	Y	No
2	M	85	Y	No
3	M	74	Y	PTC; insular carcinoma
4	F	79	Y	No
5	F	54	Y	PTC; insular carcinoma
6	F	78	Y	PTC; insular carcinoma
7	M	69	Y	PTC
8	F	82	Y	PTC; insular carcinoma
9	M	79	Y	PTC; insular carcinoma
10	F	87	Y	PTC; insular carcinoma
11	F	85	Y	PTC; insular carcinoma
12	M	76	N	No
13	M	78	Y	PTC; insular carcinoma
14	M	77	Y	No
15	M	60	Y	PTC; insular carcinoma
16	F	33	Y	PTC; insular carcinoma
17	M	72	Y	PTC; insular carcinoma
18	M	75	N	No
19	F	75	N	No
20	F	71	Y	PTC; insular carcinoma

According to the World Health Organization (WHO) classification of tumors, carcinomas were classified as well-differentiated (PTC), poorly differentiated (insular variant of PTC), and anaplastic [19]. A synopsis of patient's characteristics is reported in Table 1. Supplementary Figure 1 shows representative images of the files studied.

The histopathological diagnosis was based on the histomorphological aspect of the tumor and on clinical data. Every case showed a high proliferative index (Ki67, 40–70%), positivity for TP53, negativity for TTF-1; other tumors that could display a similar morphology were excluded by performing immunohistochemical markers such as CD34, CD31, desmin, smooth-muscle actin, S-100 protein, HMB45, Melan A, and CD45. Such analyses were performed at the time of the original pathological report and therefore different clones and products were used.

Informed consent to use biological specimens for investigational procedures was obtained from all patients or their relatives and the study was conducted in accordance with our local ethical committee.

Immunohistochemistry

All samples were fixed in buffered formalin for 12–48 h. After fixation, the surgical samples were examined, sampled for pathological diagnosis, and paraffin-embedded. Significant selected block(s) were used for the study. Criteria used for block selection were tumor areas with scarce or absent necrotic phenomena and, when possible, transition areas including within single blocks normal thyroid tissue, well differentiated thyroid tumor, poorly differentiated thyroid tumor, and ATC. In most cases, to document the progression of the neoplastic disease two different slides were required and used. Immunohistochemistry was carried out employing two different antibodies and two different revealing methods.

In one protocol, deparaffinized sections underwent antigen retrieval with citrate buffer (pH 6.0) for 10 min at 98°. After rinsing in phosphate-buffered saline (PBS), unspecific protein–protein interactions were quenched with 5% bovine serum albumin in PBS for 10 min. Then, sections were incubated with anti-PD-L1 mAb (CAT #MAB1561, R&D Systems, Minneapolis, MN) for 60 min, followed by ImmPRESS AP reagent kit anti-mouse IgG for 30 min according to manufacturer instructions (Vector, Burlingame, CA). Upon washings, alkaline phosphatase reaction was developed for 30 min with Vector Red alkaline phosphatase substrate kit (Vector, Burlingame, CA) additioned with 2 mM levamisole. Finally, sections were counterstained with hematoxylin. Negative controls were carried out replacing the primary antibody with PBS.

In the second protocol, immunohistochemistry was carried out with BenchMark IHC/ISH instruments using VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody which is intended for laboratory use in the detection of the PD-L1 protein in formalin-fixed, paraffin-embedded tissue. Tissues were stained with OptiView DAB IHC Detection Kit on a VENTANA BenchMark Series automated staining instrument. To evaluate and better locate the reaction in neoplastic cells consecutive sections were always stained with H&E.

Any staining in necrotic areas was disregarded. Normal human term placenta tissue was used as a positive control for PD-L1 staining. We considered a sample positive for PD-L1 expression only if ≥25% of the cells were positively stained. When only scattered neoplastic cells were focally positively immunostained (about 10%), the case was classified as negatively stained, yet we mentioned such a positivity.

Evaluation of the immunohistochemical reactions was independently performed by two expert pathologists (PT and RO). Results were compared.

Cell culture

Human 8505c ATC cells were obtained from Sigma-Aldrich and maintained in high-glucose DMEM supplemented with 10% FCS. 8505c cells are known to carry the V600E BRAF mutation.

Animal model

Studies were conducted in compliance with institutional guidelines and regulations. In particular, the study received the approval by the local ethical committee for animal health (Organismo Per il Benessere Animale-OPBA, University of Siena) and by the Italian Ministry of Health (authorization #776/2017-PR). All experiments have been performed following the 3Rs principle: replacement, reduction, and refinement as Guidelines for the welfare and use of animals in cancer research [20]. Twelve 5-weeks-old female BALB/c nude mice were purchased from Envigo and maintained in our animal facility in pathogen-free condition. Six animals were inoculated with 6×10^6 ATC cells subcutaneously and treated with PD-L1 mAb (Ventana) i.p. at 0.1 mg/dose/mouse on days 1, 3, 5, and 7 after the appearance of a palpable mass. To assess tumor growth, tumor diameters were measured with a calliper and tumor volume calculated using the formula $\text{width}^2 \times \text{length}/0.52$. The remaining six animals, seemingly inoculated with ATC cells, were left untreated (without administration of anti-PD-L1 antibody) and used as a control group. Since data were not normally distributed, Mann–Whitney test was used for statistical analysis. $p < 0.05$ was considered significant.

Results

PD-L1 expression in ATC: comparison between two different antibodies

ATC cells showed an intense and diffuse plasma membrane and/or cytoplasm positivity (Fig. 1a) in 13/20 (65%) cases analyzed with VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody. In all immunoreactive cases, the reaction was strong and consistent, being positively stained at least 80% of undifferentiated cells. Seven out of 20 (35%) ATC cases were negative (Fig. 1b), even though in 1 case an intense positive reaction could be observed in isolated scattered cells (about 10% of neoplastic cells). Well-differentiated TC of the papillary histotype (13 cases) was always negative as shown in Fig. 1c in which we could observe the transition from the negative PTC to the positive ATC. Ten poorly differentiated insular tumors were completely negative, while two showed isolated scattered positive cells (Supplementary Figure 2). Normal thyroid tissue was analyzed in 17 cases. All of them were negative for PD-L1 expression (Fig. 1d). Similarly, Fig. 1e showed a case of ATC with cords of neoplastic cells, positively stained, embedded in a fibrous stroma which was negative

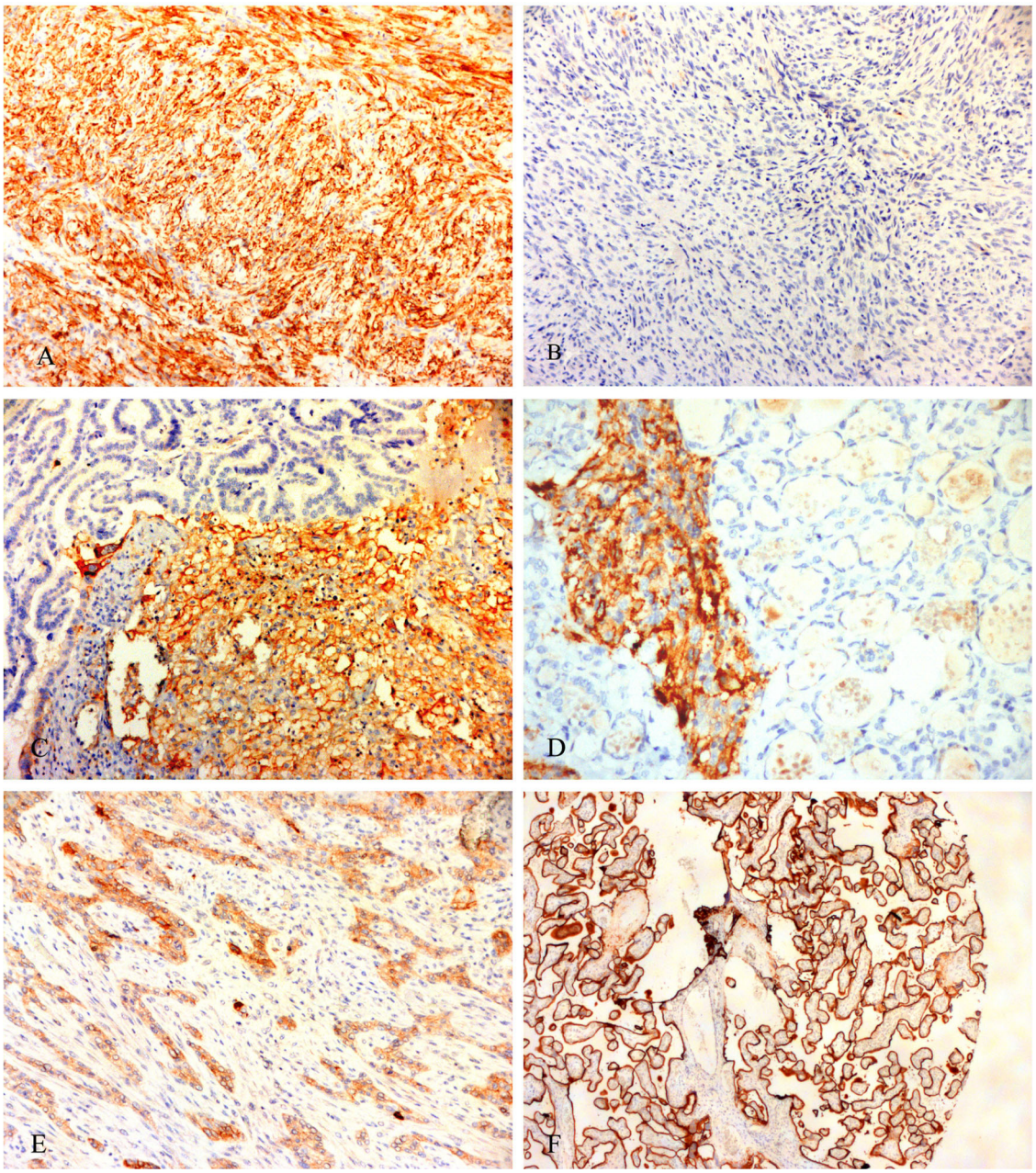

Fig. 1 **a** ATC, VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody. Strong and consistent positive stain of neoplastic cells. Original magnification ×40. **b** ATC, VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody. Negative case. Most of the cells are completely negative, even though a minority of them (2–3%) show a weak positivity. Original magnification ×40. **c** Transition area between PTC and ATC, VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody. In the upper left corner PTC cells are not stained, whereas in the lower right corner ATC cells show a strong staining. Original magnification ×100. **d** ATC, VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody. An island of positively stained ATC cells infiltrates an area of normal thyroid tissue which is unstained. Original magnification ×100. **e** ATC, VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody. Cords of positively stained cancer cells can be seen embedded within the unstained fibrous stroma. Original magnification ×40. **f** Placenta, VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody. The positive control displays a strong staining

for PD-L1 expression (case 10, Table 1). Positive control carried out on placenta sections showed a strong reaction on villous epithelium (Fig. 1f).

When the anti-PD-L1 mAb from R&D systems was tested, we observed that 4 out of 17 (23.5%) normal thyroid cases analyzed displayed a positive staining for PD-L1 and 18/20 (90%) ATC samples had a diffuse and strong membrane positivity (Fig. 2a). Again, PTCs were negative whereas the infiltrating ATCs were positive for PD-L1 (Fig. 2b). Insular variant showed focal PD-L1 positive staining in

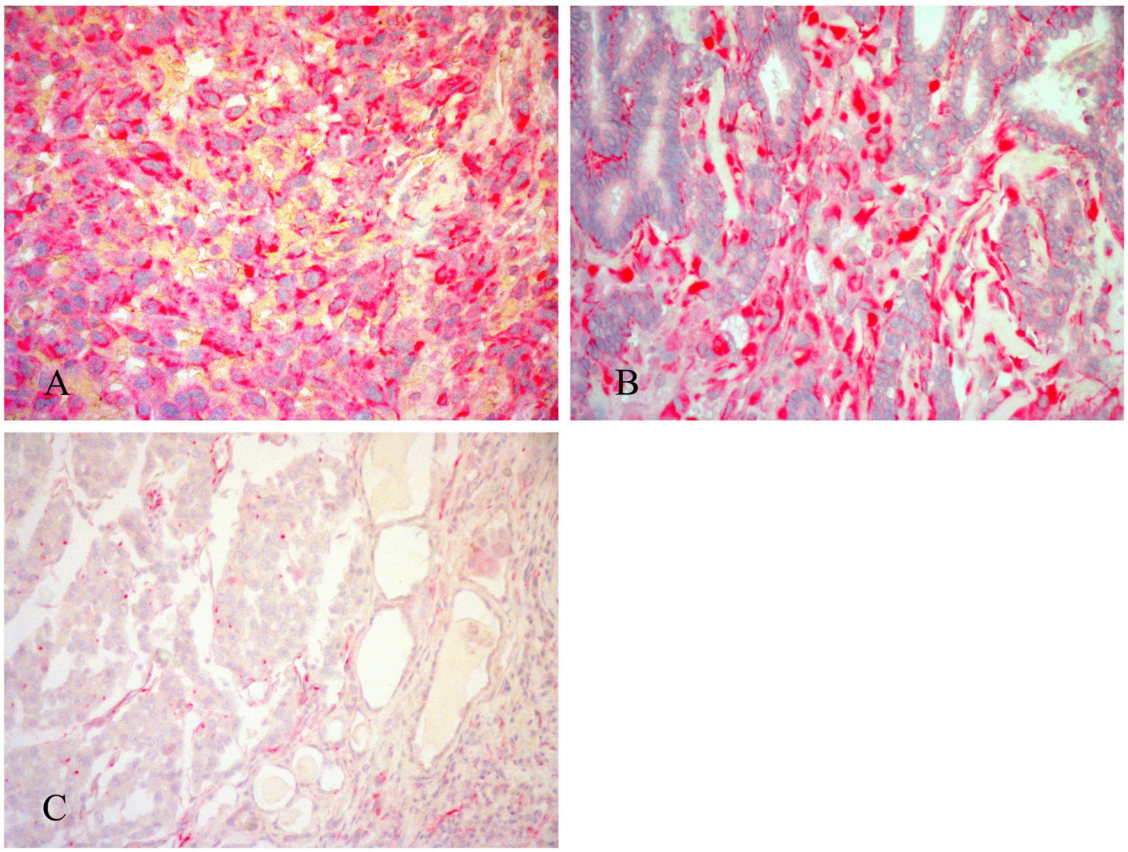

Fig. 2 **a** ATC, anti-PD-L1 mAb R&D Systems (#MAB1561). ATC cells show a strong and diffuse positive staining. Original magnification ×100. **b** Transition area between PTC and ATC, anti-PD-L1 mAb R&D Systems (#MAB1561). An area of PTC (unstained cells) is infiltrated by highly PD-L1 immunoreactive ATC cells in the stroma. Vascular endothelial cells are also positive. Original magnification ×100. **c** Poorly differentiated insular tumor, anti-PD-L1 mAb R&D Systems (#MAB1561). Scattered tumor cells show dots of positive reaction. Endothelial cells are also PD-L1 immunoreactive. Original magnification ×40

plasma membrane, restricted to the basal part of the cell in 36% of the cases. All analyzed cases presented immunopositive staining in the endothelium of vessels within or in close proximity to the tumor, while normal thyroid vessels were negative. Figure 2c shows insular thyroid cells (on the left) and normal thyroid (on the right) both negative for PD-L1 expression while endothelial cells are positive.

For both antibodies used, we considered a sample positive if ≥25% of cells were PD-L1 immunoreactive.

Treatment with PD-L1 mAb reduces tumor growth in in vivo model

To evaluate the potential effect of PD-1–PD-L1 blockade in vivo, athymic nu/nu mice were injected with the anaplastic 8505c cells. After 20 days, mice were sacrificed and the tumor explanted to verify PD-L1 expression by immunohistochemistry. Figure 3a, b shows a strong positive reaction in tumors from two different mice employing the VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody.

As 8505c tumors strongly expressed PD-L1, we repeated the experiment and, after the appearance of a palpable mass (day 7 after inoculation), we started with i.p. injection of PD-L1 mAb (0.1 mg/dose/mouse). As shown in Fig. 3c, mAb was administered on days 1, 3, 5, and 7 after the appearance of tumor masses corresponding to days 7, 10, 12, and 14 after inoculation. Tumor volume was calculated using the formula $\text{width}^2 \times \text{length}/0.52$ and expresses as mm^3. Treatment with anti-PD-L1 mAb significantly ($p < 0.05$) reduced tumor growth starting from day 17 and this difference lasted until day 23 when mice were sacrificed (Fig. 3).

Discussion

ATC represents 1–2% of the thyroid malignancies and is believed to arise from terminal dedifferentiation of follicular or PTC. ATC shows an aggressive behavior and usually death occurs within 1 year from diagnosis. Actually, a treatment for ATC is lacking and neither tyrosine kinase

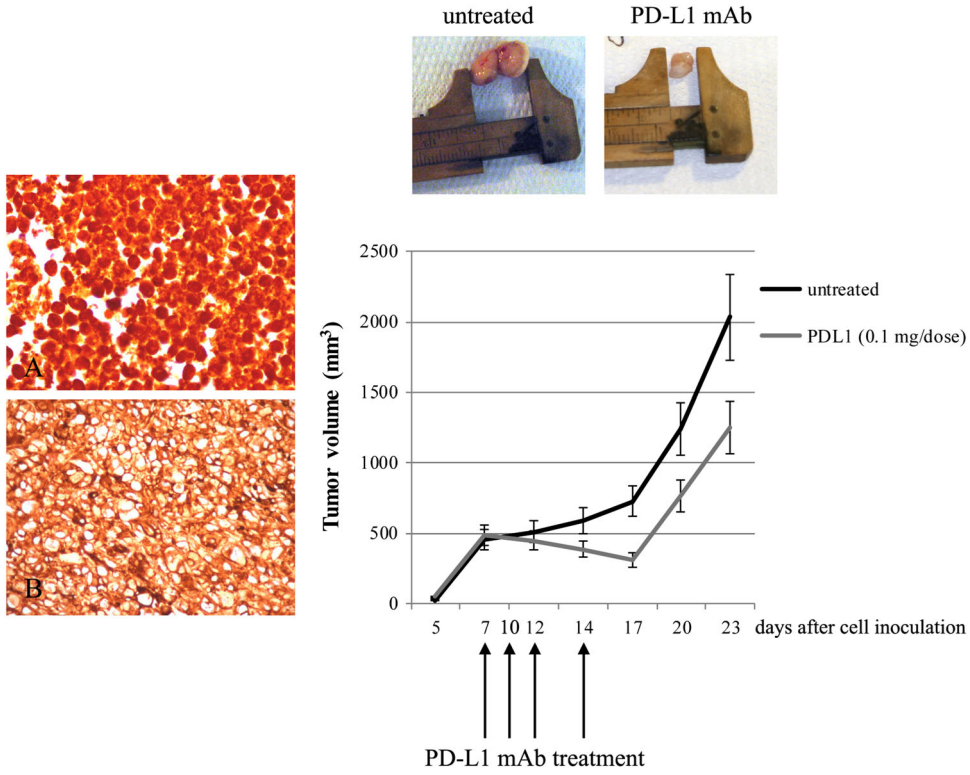

Fig. 3 **a**, **b** Strong positive PD-L1 immunoreaction in tumors from two different mice injected with human ATC cells employing the VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody. **c** Tumor volume calculated using the formula width^2 × length/0.52 and expresses as mm^3. Days of immunotherapeutic treatment are indicate with arrows. Pictures of tumors explanted at day 23 are shown. Starting from day 17, tumors volume of treated and untreated animals show significant differences (*$p < 0.05$)

inhibitors nor standard chemo/radiotherapy are really effective.

The blockade of PD-1 has been investigated with promising results in several cancers [21] including few studies on ATC [12–18].

This report provides further evidence for the usefulness of anti-PD-L1 mAb treatment against ATC and non differentiated TC. A peculiarity of our study is that we compared the efficiency of two different antibodies, the VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody and the anti-PD-L1 mAb of the R&D Systems. We observed that approximately 70% of the ATC cases were positive with the Ventana system and we reached 90% of positive staining with R&D Systems antibody. The latter antibody was apparently more sensitive even in detecting the target in normal thyroid tissue (23.5% of positive sample compared to 0% revealed with Ventana), a finding that has been recently reported also by other investigators [22]. The issue of antibody sensitivity in detecting PD-L1 is important and to a certain extent controversial. Indeed the border between higher sensitivity and lower specificity can be subtle and difficult to set. In our case, the higher sensitivity of the R&D Systems antibody could be also due to a certain degree of nonspecific staining. Additional comparative tests are needed to confirm the reliability of the R&D Systems antibody. For the same reason, though in a different context, comparative studies of immunohistochemistry assays have been devised with four among the most frequently employed antibodies to detect PD-L1 [23, 24]. The monoclonal antibodies tested for this purpose were clones 22C3, 28-8, SP263, SP142, and 73-10. Whereas the former three antibodies showed a comparable sensitivity, clone SP142 was less efficient to stain PD-L1-positive cells and clone 73-10 resulted more sensitive. One of the antibodies tested in the comparative study, clone SP263, was also employed in our research.

Another characteristic of our series is the selection of cases in which a progressive dedifferentiation of the tumor from PTC (or the insular variant of PTC) to ATC was present. We noted that PTC or poorly differentiate TC are often negative and become positive when tumor dedifferentiates to ATC. By the use of the anti-PD-L1 mAb from R&D system, endothelial cells of vessels in close proximity to or within the tumor appear positively stained. PD-L1 is expressed in basal condition by human and murine blood endothelial cells [25, 26]. Such expression is enhanced by IFNγ and further increases with TNFα [25]. Expression of PD-L1 on tumor-associated CD31-positive cells and on tumor-associated lymph vessels has been previously reported [27, 28]. This finding is intriguing as it unveils an additional possible target for anti-PD-L1 immunotherapy. Actually, the combination of anti-PD-L1 and anti-VEGFR2 was a successful therapy in mice bearing some types of cancer. Apparently, in addition to contrasting the inhibitory

loop PD-L1/PD1, the double treatment promoted the formation of high-endothelial venules and enhanced tumor lymphocyte infiltration [29].

To investigate the role of PD-L1 mAb in vivo, we inoculated BALB/c nude mice with human ATC cells. The genetic background of these mice allows the development of graft tumors. Due to the nu allele on chromosome 11 they have a dysfunctional rudimentary thymus. They display a much reduced lymphocyte population composed almost entirely of B cells and a relatively normal IgM response to thymus-dependent antigens. They also have T-cell precursors in their bone marrow. After injection of human ATC cells, tumors were allowed to grow for 20 days and then analyzed for PD-L1 expression. We demonstrated that human ATC cells implanted in mice are positive for PD-L1. Because of the lack of T cells, this expression is probably due to the presence of PD-L1 on B cells, endothelial tumor vessels and dendritic cells. Then, blocking for 3 weeks PD-1/PD-L1 interaction in vivo using a PD-L1 mAb, we could document a significant reduction in tumor volume in anti-PD-L1 treated animals compared to controls, suggesting that immunotherapy may be a promising treatment specific for ATC. Alternatively, this response could be also linked to an antibody-dependent cell cytotoxic mechanism taking advantage of NK or macrophages released by the hematopoietic organs of nude mice. In order to clarify this issue, the data gathered in vivo need to be corroborated by other independent experiments. At any rate, our data support the idea that PD-L1 can be a good target for immunotherapy.

Actually, there are 4 ongoing clinical trials (ClinicalTrials.gov) addressing the use of immunotherapy agents in TC. Two of them (#NCT03072160, #NCT02628067), employ the antibody Pembrolizumab. In particular, study NCT02628067 is recruiting patients with multiple types of advanced (unresectable and/or metastatic) solid tumors that have progressed on standard of care therapy including TC (histology not specified). Study NCT03072160, on the other hand, is intended for patients who have recurrent or metastatic medullary TC and surgery is no longer a curative option. Study NCT03215095, is recruiting patients affected by histologically or cytologically confirmed thyroid carcinoma of follicular origin (including papillary, follicular, Hürthle cell, or poorly differentiated subtypes and their respective variants) with diagnosis of recurrent and/or metastatic disease; the trial is designed to assess the safety of administering the anti-PD-L1 antibody in combination with radioiodine (131I) upon stimulation/preparation with recombinant human thyroid stimulating hormone. The only clinical trial which is specifically recruiting patients affected with anaplastic TC is study NCT02936102. In this study, patients with triple negative breast cancer, non–small-cell lung carcinoma, endometrial cancer and ATC will be treated with FAZ053 as a single agent or in combination with PDR001, both drugs being anti-PD-L1 antibodies. Taken together these evidences demonstrate that immunotherapy can represent a valid option for advanced and undifferentiated TC, but further dedicated trials are needed.

In summary, we conclude that VENTANA and R&D Systems antibodies are equally useful in revealing PD-L1 in ATC cases and that normal thyroid as well as PTC or its insular variant do not show a detectable levels of expression. The identification of PD-L1 in ATC may have direct therapeutic relevance to patients with refractory TC.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtain from all individual participants included in the study.

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References

1. D.M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy. Nat. Rev. Cancer **12**, 252–64 (2012)
2. H. Borghaei, L. Paz-Ares, L. Horn, D.R. Spigel, M. Steins, N.E. Ready, L.Q. Chow, E.E. Vokes, E. Felip, E. Holgado, F. Barlesi, M. Kohlhäufl, O. Arrieta, M.A. Burgio, J. Fayette, H. Lena, E. Poddubskaya, D.E. Gerber, S.N. Gettinger, C.M. Rudin, N. Rizvi, L. Crinò, G.R. Blumenschein Jr, S.J. Antonia, C. Dorange, C.T. Harbison, F. Graf Finckenstein, J.R. Brahmer, Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N. Engl. J. Med. **373**, 1627–39 (2015)
3. T.K. Choueiri, M.N. Fishman, B. Escudier, D.F. McDermott, C.G. Drake, H. Kluger, W.M. Stadler, J.L. Perez-Gracia, D.G. McNeel, B. Curti, M.R. Harrison, E.R. Plimack, L. Appleman, L. Fong, L. Albiges, L. Cohen, T.C. Young, S.D. Chasalow, P. Ross-Macdonald, S. Srivastava, M. Jure-Kunkel, J.F. Kurland, J.S. Simon, M. Sznol, Immunomodulatory activity of nivolumab in metastatic renal cell carcinoma. Clin. Cancer Res. **22**, 5461–5471 (2016)
4. I. Márquez-Rodas, P. Cerezuela, A. Soria, A. Berrocal, A. Riso, M. González-Cao, S. Martín-Algarra, Immune checkpoint inhibitors: therapeutic advances in melanoma. Ann. Transl. Med. **3**, 267 (2015)
5. H. Dong, S.E. Strome, D.R. Salomao, H. Tamura, F. Hirano, D.B. Flies, P.C. Roche, J. Lu, G. Zhu, K. Tamada, V.A. Lennon, E. Celis, L. Chen, Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat. Med. **8**, 793–800 (2002). Erratum Nat. Med. **8**, 1039 (2002)
6. J.M. Taube, R.A. Anders, G.D. Young, H. Xu, R. Sharma, T.L. McMiller, S. Chen, A.P. Klein, D.M. Pardoll, S.L. Topalian, L. Chen, Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci. Transl. Med. **4**, 127ra37 (2012)

7. B.R. Haugen, American Thyroid Association Management Guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: what is new and what has changed? Cancer 2017 **123**, 372–381 (2015)
8. Z.W. Baloch, V.A. LiVolsi, Special types of thyroid carcinoma. Histopathology **72**, 40–52 (2018)
9. M.E. Cabanillas, M. Zafereo, G.B. Gunn, R. Ferrarotto, Anaplastic thyroid carcinoma: treatment in the age of molecular targeted therapy. J. Oncol. Pract. **12**, 511–8 (2016)
10. V. Tiedje, M. Stuschke, F. Weber, H. Dralle, L. Moss, D. Führer, Anaplastic thyroid carcinoma: review of treatment protocols. Endocr. Relat. Cancer **25**, R153–R161 (2018)
11. D. Viola, L. Valerio, E. Molinaro, L. Agate, V. Bottici, A. Biagini, L. Lorusso, V. Cappagli, L. Pieruzzi, C. Giani, E. Sabini, P. Passannati, L. Puleo, A. Matrone, B. Pontillo-Contillo, V. Battaglia, S. Mazzeo, P. Vitti, R. Elisei, Treatment of advanced thyroid cancer with targeted therapies: ten years of experience. Endocr. Relat. Cancer **23**, R185–205 (2016)
12. L.L. Cunha, M.A. Marcello, E.C. Morari, S. Nonogaki, F.F. Conte, R. Gerhard, F.A. Soares, J. Vassallo, L.S. Ward, Differentiated thyroid carcinomas may elude the immune system by B7H1 upregulation. Endocr. Relat. Cancer **20**, 103–10 (2013)
13. T.E. Angell, M.G. Lechner, J.K. Jang, A.J. Correa, J.S. LoPresti, A.L. Epstein, BRAF V600E in papillary thyroid carcinoma is associated with increased programmed death ligand 1 expression and suppressive immune cell infiltration. Thyroid **24**, 1385–93 (2014)
14. H. Wu, Y. Sun, H. Ye, S. Yang, S.L. Lee, A. de las Morenas, Anaplastic thyroid cancer: outcome and the mutation/expression profiles of potential targets. Pathol. Oncol. Res. **21**, 695–701 (2015)
15. J.J. Bastman, H.S. Serracino, Y. Zhu, M.R. Koenig, V. Mateescu, S.B. Sams, K.D. Davies, C.D. Raeburn, R.C. McIntyre Jr, B.R. Haugen, J.D. French, Tumor-infiltrating T cells and the PD-1 checkpoint pathway in advanced differentiated and anaplastic thyroid cancer. J. Clin. Endocrinol. Metab. **101**, 2863–73 (2016)
16. S. Chowdhury, J. Veyhl, F. Jessa, O. Polyakova, A. Alenzi, C. MacMillan, R. Ralhan, P.G. Walfish, Programmed death-ligand 1 overexpression is a prognostic marker for aggressive papillary thyroid cancer and its variants. Oncotarget **7**, 32318–28 (2016)
17. S. Ahn, T.H. Kim, S.W. Kim, C.S. Ki, H.W. Jang, J.S. Kim, J.H. Kim, J.H. Choe, J.H. Shin, S.Y. Hahn, Y.L. Oh, J.H. Chung, Comprehensive screening for PD-L1 expression in thyroid cancer. Endocr. Relat. Cancer **24**, 97–106 (2017)
18. M.W. Rosenbaum, B.J. Gigliotti, S.I. Pai, S. Parangi, H. Wachtel, M. Mino-Kenudson, V. Gunda, W.C. Faquin, PD-L1 and IDO1 are expressed in poorly differentiated thyroid carcinoma. Endocr. Pathol. **29**, 59–67 (2018)
19. R. A. DeLellis, R. V. Lloyd, P. U. Heitz, C. Eng, eds. Pathology and Genetics of Tumors of Endocrine Organs. WHO Classification of Tumors, 3rd edn. vol 8, 2004
20. P. Workman, E.O. Aboagye, F. Balkwill, A. Balmain, G. Bruder, D.J. Chaplin, J.A. Double, J. Everitt, D.A.H. Farningham, M.J. Glennie, L.R. Kelland, V. Robinson, I.J. Stratford, G.M. Tozer, S. Watson, S.R. Wedge, S.A. Eccles, An ad hoc committee of the National Cancer Research Institute. Guidelines for the welfare and use of animals in cancer research. Br. J. Cancer **102**, 1555–1577 (2010)
21. J.R. Brahmer, S.S. Tykodi, L.Q. Chow, W.J. Hwu, S.L. Topalian, P. Hwu, C.G. Drake, L.H. Camacho, J. Kauh, K. Odunsi, H.C. Pitot, O. Hamid, S. Bhatia, R. Martins, K. Eaton, S. Chen, T.M. Salay, S. Alaparthy, J.F. Grosso, A.J. Korman, S.M. Parker, S. Agrawal, S.M. Goldberg, D.M. Pardoll, A. Gupta, J.M. Wigginton, Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N. Engl. J. Med. **366**, 2455–2465 (2012)
22. I. Yamauchi, Y. Sakane, Y. Fukuda, T. Fujii, D. Taura, M. Hirata, K. Hirota, Y. Ueda, Y. Kanai, Y. Yamashita, E. Kondo, M. Sone, A. Yasoda, N. Inagaki, Clinical features of nivolumab-induced thyroiditis: a case series study. Thyroid **27**, 894–901 (2017)
23. F.R. Hirsch, A. McElhinny, D. Stanforth, J. Ranger-Moore, M. Jansson, K. Kulangara, W. Richardson, P. Towne, D. Hanks, B. Vennapusa, A. Mistry, R. Kalamegham, S. Averbuch, J. Novotny, E. Rubin, K. Emancipator, I. McCaffery, J.A. Williams, J. Walker, J. Longshore, M.S. Tsao, K.M. Kerr, PD-L1 immunohistochemistry assays for lung cancer: results from phase 1 of the Blueprint PD-L1 IHC assay comparison project. J. Thorac. Oncol. **12**, 208–222 (2017)
24. M.S. Tsao, K.M. Kerr, M. Kockx, M.B. Beasley, A.C. Borczuk, J. Botling, L. Bubendorf, L. Chirieac, G. Chen, T.Y. Chou, J.H. Chung, S. Dacic, S. Lantuejoul, M. Mino-Kenudson, A.L. Moreira, A.G. Nicholson, M. Noguchi, G. Pelosi, C. Poleri, P.A. Russell, J. Sauter, E. Thunnissen, I. Wistuba, H. Yu, M.W. Wynes, M. Pintilie, Y. Yatabe, F.R. Hirsch, PD-L1Immunohistochemistry comparability study in real-life clinical samples: results of Blueprint phase 2 project. J. Thorac. Oncol. **3**, 1302–1311 (2018)
25. M.J. Eppihimer, J. Gunn, G.J. Freeman, E.A. Greenfield, T. Chernova, J. Erickson, J.P. Leonard, Expression and regulation of the PD-L1 immunoinhibitory molecule on microvascular endothelial cells. Microcirculation **9**, 133–145 (2002)
26. N. Rodig, T. Ryan, J.A. Allen, H. Pang, N. Grabie, C. Tatyana, E. A. Greenfield, S.C. Liang, A.H. Sharpe, A.H. Lichtman, G.J. Freeman, Endothelial expression of PD-L1 and PD-L2 downregulates CD8+ T cell activation and cytolysis. Eur. J. Immunol. **33**, 3117–3126 (2003)
27. S. Korehisa, T. Ikeda, S. Okano, H. Saeki, E. Oki, Y. Oda, M. Hashizume, Y. Maehara, A novel histological examination with dynamic three-dimensional reconstruction from multiple immunohistochemically stained sections of a PD-L1-positive colon cancer. Histopathology **72**, 697–703 (2018)
28. L.C. Dieterich, K. Ikenberg, T. Cetintas, K. Kapaklikaya, C. Hutmahcer, M. Detmar, Tumor-associated lymphatic vessels upregulate PDL1 to inhibit T-cell activation. Front. Immunol. **8**, 66 (2017)
29. E. Allen, A. Jabouille, L.B. Rivers, I. Lodewijckx, R. Missiaen, V. Steri, K. Feyen, J. Tawney, D. Hanahan, I.P. Michael, G. Bergers, Combined antiangiogenic and anti-PD-L1 therapy stimulates tumor immunity through HEV formation. Sci. Transl. Med **9** (385), pii: eaak9679 (2017)