Response to PD-1 Blockers Alone or Combined with Anlotinib in Treatment of Anaplastic and Poorly Differentiated Thyroid Carcinoma : A Real-world Study to Identify Best-benefited Patients

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Abstract

Background

Anaplastic and poorly differentiated thyroid carcinoma (ATC/PDTC) have poor survival outcomes. Mono-therapy with either immuno-therapy or tyrosine kinase blockers (TKI) is sparsely reported and seems not to significantly affect prognosis or survival. We sought to testify whether combined therapies have a better outcome in real-world settings. Pathological plus PET/CT volumetric biomarkers were analyzed to find crosstalk associated with response.

Methods

Patients receiving PD-1 blockers alone or combined with anlotinib were recruited to undergo sequential PET/CT scanning. Propensity score-matched analysis was carried out for comparison of treatment outcomes. Structure equation modeling was carried out to investigate the crosstalk among sensitive biomarkers. A nomogram was developed and validated using the patients in the same centers.

Results

A total of 69 patients received either combined or mono-therapy with a clinical benefit rate of 88.41% (61 / 69) and tolerable adverse events, with a mean survival time of 14.64 months. The mean survival time of the combined therapy group was 15.67 months. The mean improvement of total lesion glycolysis (TLG) for the combined group was 120.07 (29.42% improved from baseline). Angiogenic marker VEGFR, FGFR, and VEGF mediate the PD-1 blockade impact on TLG improvement.

Conclusion

Anti-angiogenic agents anlotinib could potentiate PD-1 blockade by diminishing angiogenesis or its downstream effects. The combined treatment increased survival and responses could be better evaluated by volumetric PET/CT parameters. Pathological biomarker expression levels were associated with TLG improvement in combined treatment.

Background

Anaplastic thyroid carcinoma (ATC) is a deadly type of cancer with less than 10% of 5-year survival[1 2]. Patients usually present late in staging and distant metastasis occurs early, which is always resistant to multi-modal approaches[3]. Unlike differentiated thyroid cancers responding to effective chemotherapy, there have been little data to suggest effective modalities for ATC, and the median survival time was reported to be less than 6 months[4]. The individual doxorubicin or carboplatin-containing regimens of chemotherapy achieved only less than 25% of the objective response rate (ORR) lasting for 3 to 6 months[5]. Poorly-differentiated carcinoma (PDTC) has a more favorable outcome than ATC but still lacks effective measures with less than 10% of 10-year survival[6 7].

Recent pre-clinical studies suggested heavy angiogenesis of thyroid cancers, which may be potential responders to anti-angiogenic agents[8 9]. Trials of many agents, including lenvatinib and sorafenib, showed moderate effects, but the effects are fluctuating, probably because of small sample sizes. Specifically, a preliminary trial of levantinib showed a favorable response rate (4 of 17 patients), but the trial was aborted due to intolerable adverse events and a median survival of only 10.6 months[10]. Patients with BRAF mutation (accounting for less than 50% of all patients) may benefit from dabrafenib and trametinib, but efficacy is to be determined in larger trials[11 12].

The revolutionizing effects of immune checkpoint inhibitors have changed the baseline regimens of roughly all solid tumors in the past decade because they enable durable anti-tumor effects on cancers and significantly prolong survival[13]. However, trials on ATC management have shown fluctuating response in some studies, and promising results in others, probably because of the unknown tumor microenvironment (TME) in anaplastic histology[14–18]. What is agreed is that a vicious cycle is created by the photomural immune cells and pro-angiogenic agents in the TME, and blocking both simultaneously may reasonably achieve better response[14]. Trials of combined therapy have demonstrated synergistic effects on many solid malignancies but the report of response or survival benefit on ATC/PDTC has been limited to case series or reports[19 20]. Also, several patients fail to benefit from such regimens[20]. Predicting response before treatment, aimed to find resistant and sensitive patients, can be difficult, because the inner workings of angiogenesis and immune TME are too complex to characterize clinically[21]. Attempts of the new method are thus needed to clarify the complex inner workings in combined treatment to illustrate the weights of each sensitive/resistant marker.

Anlotinib is a multi-target tyrosine kinase inhibitor with anti-angiogenic and anti-tumoral effects in preclinical models in ATC, but it has little been reported to have effects on humans so far[9]. Combined with PD-1 blockers, we thus hypothesize an augmented response for ATC treatment and seek to find reasons of sensitivity or resistance by interrogating the crosstalk of biomarkers associated with anti-tumor immunity or angiogenesis. Also, as PET/CT scanning may better reflect tumor bio-aggressiveness than traditional radiological measures, volumetric parameters associated with both uptake ratio and mass size will be applied to measure treatment response.

Methods

Patient Characteristics

The observational study recruited patients diagnosed with poorly undifferentiated thyroid carcinoma (PUDC) and treated with PD-1 inhibitors alone or combined with tyrosine kinase inhibitors in a multi-center database of thyroid cancers, chronologically from May 2015 to March 2021. The first was the Panmedic Project of Paramedical Imaging Centers (Shanghai), the second being Sun Yat-sen Cancer Center thyroid cancer (Guangzhou, Guangdong Province), the third being the Affiliated Cancer Hospital of Shantou University Medical College (Shantou, Guangdong Province), the fourth being the First Affiliated

Hospital of Zhengzhou University (Zhengzhou, Henan Province). Eligible Patients must have Eastern Cooperative Oncology Group-Performance Score of 0 to 2, have received no systematic regimens of chemotherapy except for sensitizing or bridging regimens, and lesions were measurable by CT or MRI. Key exclusion criteria included the history of bleeding or diathesis, history of the severe cardio-cerebrovascular disease. Patients systematically treated with other types of kinase inhibitors were excluded.

Under the approval of Institutional review boards, included patients were involved in sequential PET/CT scanning protocols, including baseline and follow-up scans, to evaluate parameter changes associated with treatment response. Patients gave written consent before scanning protocols or treatment plans and all patients demanded full privacy of personal data except for research usage. Medical records of age-matched controls, who did not receive tyrosine kinase or immune checkpoint inhibitors, were retrospectively reviewed, and propensity score regression analysis was carried out to achieve head-to-head comparison for each variable of matched cohorts[22]. The diagnosis of PUDC was confirmed by a pathologist at the time of treatment. Treatment continued until the development of unbearable toxicity or disease progression as defined by RECIST v1.1.

Study Design

The primary follow-up endpoints were disease progression, and overall survival was calculated from the time of treatment initiation to death or end of follow-up. Evaluation of volumetric parameter changes was the secondary goal of the study. The parameter on PET/CT scans that indicated bio-aggressiveness were calculated by PET/CT radiologists and doubled checked by the primary investigator, and differences were settled with in-field calculation of regions of interest. As the RECIST criteria are the traditional standard to assess treatment response, the best objective response (BOR) was evaluated, which was defined as the highest improvement of tumor volumes on CT/MRI scans for patients responding well to therapy, or highest deterioration of tumor volumes for patients with disease progression (PD). clinical benefit was defined as stable disease (SD) plus partial response (PR). Patients were followed up every three months since the initiation of therapy until disease progression or death, and pseudo-progression was excluded in PD in the initial phases of treatment. Treatment-related adverse events were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.03)[23].

Dosing and Molecular Testing

The PD-1 blockers (PD-1B) in the study included the following 2 types: toripalimab and sintilimab. Patients received toripalimab 3 mg/kg once every 2 weeks by intravenous infusion and the dosing was reduced to 2.5mg/kg to 2.75mg/kg in occasions of unbearable toxicity. The dosing of sintilimab was 200mg every 3 weeks via intravenous infusion and was reduced to 250mg to 290mg in occasions of unbearable toxicity. The dosing was anlotinib was 12mg/day in 2 weeks and off-treatment for 1 week, and dosing was reduced to 8 to 10mg/day in occasions of unbearable toxicity.

Molecular testing on tumor tissue was performed by three pathologists who were blind to the study data, through either immunohistochemistry or sequencing. Specifically, we evaluated the expression level of the following potential markers associated with angiogenesis, including FGFR, VEGFR, FLT, VEGF. CD31 was used as the scale to delineate microvascular density (MVD) according to Gundersen et al[24]. Markers potentially associated with PD-1 blockade included CD3/CD8 (tumor lymphocyte infiltration, TLI) and CD68 (tumor macrophage infiltration, TMI). The PD-L1 expression level was defined as positive when the staining proportion was over 50%. The TMI or TLI was defined as positive when the staining proportion surpassed 25%.

Volumetric Evaluation with PET/CT Scanning

The protocol of PET/CT in both centers has been discussed elsewhere. Volumetric parameters used in the current study, which have been reported as a quantitative measurement of tumor cells with high metabolic activity, included the metabolic tumor volume (MTV) and total lesion glycolysis (TLG). TLG was defined as the product of MTV and mean standard uptake value (SUV), which was recognized to simultaneously assess the 18-F FDG uptake and tumor volume. In this study, the threshold of 50% was applied to delineate tumor contours.

Statistics

Demographic variables in the study included gender, age, local treatment, I131 treatment, ECOG-PS, and BRAF mutation status. To minimize potential bias in the study, patients in each treatment/control arm were matched by propensity score to achieve head-to-head paired comparison[22 25 26]. The propensity score of each patient was calculated through a multivariate conditional logistic regression model with a caliper width of 0.04[25]. variables entering the model included all demographic variables. Specifically, as the baseline bio-aggressiveness of the tumor may have a potential impact on prognosis, pathological staging and pre-therapeutic PET/CT scan parameters also entered the model[25]. Specifically, the staging was according to the 8th version of the AJCC system, and in this study, patients were staged as poorly differentiated and undifferentiated (IVA, IVB, and IVC).

In survival analysis, the survival curve of each group was plotted with Kaplan-Meier Method and the log-rank test was used to test the difference. Paired Student t-test was applied to test the differences in continuous variables, and categorical variables were compared with the chi-square test. In each arm, significant variables in univariate analysis entered multivariate survival analysis and independent risk factors could thus be found. Hazard ratios were calculated through Cox proportional hazard regression.

Final risk factors in the combined treatment arm (PD-1B plus anlotinib) that significantly affected survival were subjected to pathway analysis to assess crosstalk of pathological biomarkers and PET/CT parameters. Structural equation modeling (SEM) was performed to assess the direct and indirect impacts of each marker on the endpoint events. Survival analysis was performed on SPSS (Chicago, IL, version 24.0), and SEM was performed on Amos (Chicago, IL, version 24.0).

Results

Baseline characteristics

The study recruited a total of 73 consecutive patients in both centers to receive either combined therapy or PI-1 blockers (PD-1B) alone, of whom 4 patients were lost to follow-up and therefore a total of 69 patients (33 males and 36 females, age 57.17 ± 11.27) were included in the final statistical calculation. At the end of follow-up, 26 (37.7%) patients were alive, and 43 (62.3%) patients died from the disease. A total of 45 patients received combined therapy and 24 patients received PD-1 blockers (PD-1B) alone. Twenty-four and 45 patients received toripalimab and sintilimab, respectively. A total of 70 patients were included as negative, background controls. As for pathology types, 43 were diagnosed with poorly differentiated thyroid carcinoma (PDTC), and a total of 96 patients were diagnosed with undifferentiated, or anaplastic, thyroid carcinoma (ATC), in whom 26 patients were staged as IVA, 27 patients as IVB and 43 patients as IVC. Notably, as consulted with pathologists, 24 PDTC specimen has ATC histology, ranging from 4–43%. and in the current study, they were classified as PUDC. A total of 36 patients were BRAF positive. The treatment regimens were relatively well tolerated by the patients, and 9 patients developed hypertension graded as 3 or 4, and other patients reported moderate adverse events during follow-up. The demographic variables and adverse events were listed in Table 1and Supplementary Table 1.

Table 1
Baseline Characteristics of the Enrolled Patients

Factor	Total	PD-1B + Anlotinib	PD-1B alone	Negative Control
Sex, Female / Male	78 / 61	24 / 21	13 / 11	41 / 29
Prior I131, yes / no	46 / 93	15 / 30	6 / 18	25 / 45
Age, Mean (SD)	56.81 (10.51)	56.31 (11.91)	58.79 (9.98)	56.46 (9.76)
Pathology type, IVA / IVB / IVC / PD§	26 / 27 / 43 / 43	8 / 5 / 17 / 15	6 / 4 / 8 / 6	12 / 18 / 18 / 22
Local treatment, Surgery / Radiotherapy / None	44 / 33 / 62	15 / 11 / 19	8 / 4 /12	21 / 18 / 31
ECOG-PS, 2 / 1 / 0	65 / 42 / 32	21 / 13 / 11	13 / 7 / 4	31 / 22 / 17
PD-1B type, tor / sin	24 / 45	15 / 30	9 / 15	—
BRAF mutation, yes / no	36 / 103	11 / 34	7 / 17	18 / 52
TLG, Mean (SD)	426.89 (115.34)	425.58 (111.37)	439.58 (146.95)	423.39 (106.90)
MTV, Mean (SD)	176.70 (68.82)	181.53 (75.58)	173.12 (59.75)	174.81 (67.95)
SUVmax, Mean (SD)	7.79 (2.55)	7.58 (2.60)	7.88 (2.82)	7.90 (2.44)
Estimated Survival Time (95% CI)*	10.45 (9.56–11.34)	16.24 (15.11–17.37)	11.77 (10.37–13.16)	6.49 (5.92–7.05)

PD-1B, PD-1 blockers. tori, toripalimab; sin, sintilimab; SD, standard deviation; CI, confidence interval; PD, poorly-differentiated; ECOG-PS, Eastern Cooperative Oncology Group Performance Score;△, Improvement; TLG, total lesion glycolysis; MTV, metabolic tumor volume; SUVmax, max standardized uptake value. * calculated with Kaplan-Meier method.§ 24 of the PD specimen contained anaplastic thyroid histology.

Unmatched and matched comparison

Regardless of treatment combination, all patients receiving PD-1B were evaluable for objective response, shown in Fig. 1, with a clinical benefit rate of 88.41% (61 / 69) in the entire treatment cohort, and the mean overall survival (OS) was 14.64 (95%CI, 13.58–15.70) months. In the combined treatment group, 8 patients achieved partial response (PR, 17.8%) and 34 patients were rated as stable disease (SD, 75.6%). Three patients were rated as disease progression (PD, 6.7%, Fig. 1A, and 1B). The mean improvement (△) of total lesion glycolysis (TLG) was 120.07 ± 64.82 (29.42% ± 16.75% improved from baseline). The mean improvement of metabolic tumor volume (MTV) was 49.71 ± 53.56 (28.51% ± 29.05%). The mean improvement of max standard uptake value (SUVmax) was − 0.6 ± 2.52 (-13.58% ± 47.54%).

In the PD-1B-only treatment group, 19 patients were rated as stable disease (SD, 79.2%) and 5 patients were rated as disease progression (PD, 20.8%, Fig. 1C, and 1D). The mean △TLG was 72.17 ± 75.10 (18.21% ± 22.82% improved from baseline). The mean △MTV was 70.63 ± 54.23 (39.88% ± 36.69%). The mean △SUVmax was − 0.25 ± 3.52 (23.92% ± 109.33%).

A total of 23 patients in the combined treatment arm were matched with patients receiving PD-1B alone, with a mean propensity score of 0.63 ± 0.10. The baseline comparison results were illustrated in Table 2. The objective response rates were significantly different between the two arms ($p = 0.04$), with the combined arm higher than the PD-1B arm. In both cohorts, there was significant improvement of TLG from baseline assessment, with the combined group significantly higher than the other ($p = 0.04$, Fig. 2A, 2B). There was no significant difference between △SUVmax or △MTV. The estimated mean survival time of the combined arm was 15.67 (95% CI 14.28–17.06) months, which was significantly higher than the PD-1B arm ($p < 0.01$, mean survival time, 11.97 months, 95% CI 10.58–13.37). The Kaplan-Meier survival curve of the head-to-head comparison was shown in Fig. 2C.

Table 2
Propensity Score-matched Comparison of Combined and PD-1B Treatment Group

Factor	PD-1B + Anlotinib	PD-1B alone	p
Sex, Female / Male	12 / 11	13 / 10	0.77
BRAF mutation	3 / 20	6 / 17	0.27
Prior I131	6 / 17	6 / 17	1.0
Age, Mean (SD)	58.91(13.75)	58.17(9.73)	0.83
Pathology type, IVA / IVB / IVC / PD	2 / 3 / 11 / 7	5 / 4 / 8 / 6	0.58
Surgery / Radiotherapy / None	10 / 4 / 9	7 / 4 / 12	0.62
ECOG-PS, 2 / 1 / 0	10 / 9 / 4	13 / 6 / 4	0.61
TLG, Mean (SD)	425.91 (108.83)	439.83 (150.25)	0.78
MTV, Mean (SD)	138.91 (58.72)	175.17 (60.23)	0.73
SUVmax, Mean (SD)	8.04 (2.48)	7.83 (2.87)	0.04
Follow-up parameters			
ORR, PD / SD / PR	1 / 18 / 4	5 / 18 / 0	0.04
BOR, Mean (SD)	11.04 (19.31)	2.04 (21.84)	0.20
Follow-up TLG	305.43 (99.92)	366.57 (167.35)	0.11
△TLG	120.48 (65.79)	73.26 (76.59)	0.04
Follow-up MTV	91.57 (56.71)	103.87 (60.17)	0.52
△MTV	47.35 (52.65)	71.30 (55.35)	0.15
Follow-up SUVmax	9.74 (2.45)	8.17 (3.55)	0.11
△SUVmax	-1.70 (2.27)	-0.35 (3.56)	0.17

SD, standard deviation; ECOG-PS, Eastern Cooperative Oncology Group Performance Score;PD, poorly-differentiated; △, Improvement; TLG, total lesion glycolysis; MTV, metabolic tumor volume; SUVmax, max standardized uptake value. ORR, objective response rate; BOR, best objective response.

A total of 44 patients in the combined treatment arm were matched with the negative control group, with a mean propensity score of 0.40 ± 0.05, and 23 patients in the PD-1B treatment arm were matched with the negative control group, with a mean propensity score of 0.28 ± 0.08. The comparison results of baseline characteristics were illustrated in Supplementary Table 2. Both treatment arms showed significant improvement in survival as compared to the control group. The Kaplan-Meier survival curve was shown in Supplementary Fig. 1.

Prognostic Biomarker Identification

Survival analysis by the univariate and subsequent multivariate method was carried out in each arm to identify markers associated with survival. In the combined treatment arm, △TLG (HR = 0.98, 95%CI = 0.96–0.99) was the only marker in PET/CT parameters independently associated with longer survival (Table 3). The estimated survival time was 16.59 (95%CI = 15.38–17.99) months in patients with △TLG > 120, which was significantly higher than patients with △TLG < 120 ($p < 0.01$, Fig. 2D). This differentiating effect of survival prognosis was not seen in objective response rate (ORR) or clinical benefit ($p = 0.77$ and 0.69, respectively). In all immunohistochemistry (IHC) markers, positive PD-L1 expression (N = 11, HR = 0.11, 95%CI = 0.03–0.45, Fig. 2E) and positive FGFR expression (N = 17, HR = 0.32, 95%CI = 0.11–0.93, Supplementary Fig. 2A) was independently associated with significantly longer survival time. Other biomarkers, including VEGFR, VEGF, and FLT, were significant in univariate analysis but had borderline or lost significance in multivariate analysis (Supplementary Fig. 2B-D).

Table 3
Survival Analysis of Combined Treatment Group

Factor	Mean (SD) or No.	Univariate		Multivariate	
		HR (95%CI)	P	HR (95%CI)	P
PD-1B type, toripalimab / sintilimab	15 / 30	0.14 (0.03–0.62)	< 0.01	0.14 (0.03–0.62)	< 0.01
PET / CT Parameters					
Follow-up TLG	305.51 (116.23)	1.00 (1.00–1.00)	0.58	—	—
△TLG	120.07 (64.82)	0.98 (0.96–0.99)	< 0.01	0.98 (0.96–0.99)	0.01
Follow-up MTV	131.82 (78.64)	1.01 (1.00–1.02)	0.02	1.00 (0.99–1.01)	0.90
△MTV	49.71 (53.56)	0.99 (0.98–1.00)	0.05	0.99 (0.99–1.00)	0.25
Follow-up SUVmax	8.18 (3.08)	1.01 (0.89–1.14)	0.88	—	—
△SUVmax	-0.60 (2.52)	0.98 (0.82–1.17)	0.83	—	—
Pathological Biomarkers					
FGFR, +/-	17 / 28	0.21 (0.08–0.58)	< 0.01	0.32 (0.11–0.93)	0.04
Pathology type, IVA / IVB / IVC / PD§	8 / 5 / 17 / 15	0.82 (0.54–1.26)	0.36	—	—
FLT, +/-	16 / 29	0.38 (0.15–0.94)	0.04	0.35 (0.10–1.26)	0.11
MVD, high / low	20 / 25	0.95 (0.29–3.14)	0.93	—	—
PDGFR, +/-	24 / 21	0.89 (0.38–2.06)	0.78	—	—
VEGF, +/-	31 / 14	3.16 (1.14–8.77)	0.03	2.22 (0.60–8.33)	0.23
TIL, high / low	19 / 26	1.29 (0.59–2.85)	0.53	—	—
TIM, high / low	16 / 29	1.03 (0.40–2.85)	0.95	—	—
VEGFR, +/-	17 / 28	0.25 (0.09–0.66)	< 0.01	0.24 (0.06–1.01)	0.05

Factor	Mean (SD) or No.	Univariate		Multivariate	
		HR (95%CI)	P	HR (95%CI)	P
PD-L1, +/-	11 / 34	0.24 (0.08–0.70	< 0.01	0.11 (0.03–0.45)	< 0.01

PD-1B, PD-1 blockers. SD, standard deviation; HR, hazard ratio; CI, confidence interval; ECOG-PS, Eastern Cooperative Oncology Group Performance Score;PD, poorly-differentiated; △, Improvement; TLG, total lesion glycolysis; MTV, metabolic tumor volume; SUVmax, max standardized uptake value. TIL, tumor infiltrating lymphocyte; TIM, tumor infiltrating macrophage.§ 10 of the PD specimen contained anaplastic thyroid histology.

In PD-1B-only treatment arm, △TLG (HR = 0.98, 95%CI = 0.96–0.99) was the only marker in PET/CT parameters independently associated with longer survival (Table 4). The estimated survival time was 14.13 (95%CI 12.87–15.38) months in patients with △TLG > 72, which was significantly higher than patients with △TLG < 72 ($p < 0.01$, Fig. 2F). This differentiating effect of survival prognosis was also seen in ORR or clinical benefit ($p < 0.01$ for both). In all IHC markers, positive PD-L1 expression was also independently associated with better survival prognosis (HR = 0.30, 95%CI = 0.09–0.97, $p = 0.04$, Fig. 2G) by multivariate analysis.

Table 4
Survival Analysis of Checkpoint Blockade Treatment Group

Factor	Mean (SD) or No.	Univariate		Multivariate	
		HR (95%CI)	P	HR (95%CI)	P
PD-1B type, toripalimab / sintilimab	9 / 15	0.23 (0.07–0.83)	0.02	—	—
PET / CT Parameters					
Follow-up TLG	367.42 (163.72)	1.00 (1.00–1.00)	0.24	—	—
△TLG	72.17 (75.10)	0.98 (0.98–0.99)	< 0.01	0.98 (0.96–1.00)	0.02
Follow-up MTV	102.50 (59.23)	1.01 (1.00–1.01)	0.09	—	—
△MTV	70.63 (54.23)	0.99 (0.98–1.00)	< 0.01	1.01 (0.99–1.04)	0.37
△SUVmax	-0.25 (3.52)	0.99 (0.87–1.12)	0.86	—	—
Follow-up SUVmax	8.13 (3.48)	1.09 (0.94–1.28)	0.26	—	—
Pathological Biomarkers					
FGFR, +/-	16 / 8	0.62 (0.24–1.58)	0.32	—	—
Pathology type, IVA / IVB / IVC / PD§	6 / 4 / 8 / 6	1.02 (0.65–1.58)	0.95	—	—
FLT, +/-	14 / 10	2.15 (0.76–6.06)	0.15	—	—
MVD, high / low	8 / 14	0.71 (0.2–2.43)	0.71	—	—
PDGFR, +/-	10 / 12	0.50 (0.17–1.50)	0.20	—	—
VEGF, +/-	9 / 15	1.03 (0.36–2.94)	0.96	—	—
TIL, high / low	14 / 10	0.33 (0.12–0.94)	0.04	0.45 (0.15–1.33)	0.15
TIM, high / low	14 / 10	0.37 (0.13–1.06)	0.06	—	—
VEGFR, +/-	15 / 7	2.06 (0.66–6.45)	0.22	—	—

Factor	Mean (SD) or No.	Univariate		Multivariate	
		HR (95%CI)	P	HR (95%CI)	P
PD-L1, +/-	12 / 12	0.25 (0.08–0.76)	0.02	0.30 (0.09–0.97)	0.04

PD-1B, PD-1 blockers. SD, standard deviation; HR, hazard ratio; CI, confidence interval; ECOG-PS, Eastern Cooperative Oncology Group Performance Score;PD, poorly-differentiated; △, Improvement; TLG, total lesion glycolysis; MTV, metabolic tumor volume; SUVmax, max standardized uptake value. TIL, tumor infiltrating lymphocyte; TIM, tumor infiltrating macrophage.§ 3 of the PD specimen contained anaplastic thyroid histology.

In the negative control arm, there were 3 risk factors independently associated with survival, including age, baseline TLG and pathology type (Supplementary Table 3). Older age was found negatively affecting survival (HR = 1.05, 95%CI = 1.02–1.08), higher baseline TLG was found negatively affecting survival (HR = 1.00, 95%CI = 1.00–1.01) and anaplastic histology associated with poorer prognosis (HR = 1.96, 95%CI = 1.07–3.62).

Biomarker Crosstalk in Pathway Analysis

Pathway analysis by structural equation modeling was carried out to interrogate the crosstalk among sensitive/resistant biomarkers in the combined treatment arm to demonstrate direct or indirect impacts on value changes of TLG (Fig. 3). There was a direct impact of PD-1 blockade on △TLG affecting survival, in which the regression coefficient for PD-L1 expression (β) was 0.21 on the impact of △TLG ($p = 0.04$). Since there were 3 other biomarkers significant in the univariate survival analysis (VEGFR, VEGF, and FLT), indirect mediating effects were tested for these biomarkers in the pathway between PD-L1 and △TLG to query the reason of insignificance in multivariate analysis (Fig. 3A). The impact of PD-1 blockade on △TLG was positively mediated by VEGFR expression ($\beta = 0.41$ and $\beta = 0.45$, $p < 0.01$ for both) and by FGFR expression ($\beta = 0.41$ and $\beta = 0.18$, $p < 0.01$ and $p = 0.04$, respectively). Also, the impact of PD-1 blockade was negatively mediated by VEGF expression ($\beta = -0.40$ and $\beta = -0.22$, $p < 0.01$ and $p = 0.04$, respectively). FLT expression was not significant all the way through. The critical ratio and unstandardized estimate of each regression pathway were shown in Fig. 3B, and the qualitative △TLG in each group of biomarker expression was shown in Fig. 3C.

Nomogram Development

A nomogram was formulated in the combined treatment arm (PD-1 blockers plus anlotinib) by using the package of rms in R version 2.14.1 (http://www.r-project.org/). The performance of the nomogram was measured by concordance index (C-index) and assessed by comparing nomogram-predicted versus observed Kaplan-Meier estimates of overall survival (OS) probability. Bootstraps with 1,000 resample were used for these activities. During the internal validation of the nomogram, the total points of each patient in the cohort were calculated according to the established nomogram, then Cox regression in this cohort was performed using the total points as a factor, and finally, the C-index and calibration curve was

derived based on the regression analysis. The nomogram was developed and illustrated in Supplementary Fig. 3. The C-index was 0.85 ± 0.08. The internal calibration curves demonstrating 1-year, 15-month, and 18-month survival were shown in Supplementary Fig. 4.

Discussion

Anaplastic and poorly differentiated thyroid carcinoma had poor treatment outcomes with traditional chemotherapy. Although multi-targeting tyrosine kinase inhibitors have shown life-prolonging effects in some reports or clinical trials, the overall outcome has been fluctuating. High expression levels of progression death-1 or its ligand in ATC tissue shed new light on attempts of immunotherapy trials, yet the overall response was less than 30%, with overall survival data still lacking in larger cohorts[17 27]. Combined therapy, which showed promising results in many solid tumors, has been sparsely reported in ATC treatment probably because of low disease incidence. In this real-world, PET-assessed management of ATC/PDTC of 69 patients, we found a total clinical benefit rate of 88.4% regardless of anlotinib combination, with the mean overall survival of 14.64 months. Treatment-related adverse events were well tolerated in the entire cohort. Adding anlotinib can significantly increase the survival prognosis as illustrated in propensity score-matched survival analysis, with a relatively high clinical benefit rate of 93.3%.

While traditional response measures by CT/MRI-based assessment were used in the current study to evaluate patient outcome after PD-1B treatment, PET/CT-based volumetric investigation was added to assess outcome in the early phase after treatment initiation. To date, there have been few studies to evaluate the prognostic value of PET/CT volumetric biomarkers in predicting survival or treatment response of ATC/PTDC, probably on account of relatively low incidence and high mortality of ATC/PDTC. Several studies on differentiated thyroid cancers evidenced that traditional measures, including TNM stages and CT/MRI-based evaluation, are not accurate enough to prognosticate survival or evaluate treatment response[28–30]. We found similar results by comparing CT/MRI-based and PET/CT-based assessment in the evaluation of survival: total lesion glycolysis improvement (△TLG) has a strong association with survival prognosis, both in the combined group and PD-1B group and even in the negative control group the baseline TLG was also independently associated with survival. However, the changes in objective response (OR) was not associated with survival, although the OR rate can have borderline effects in survival prognostication ($p = 0.04$). The reason for this contrast may lie in the principles behind the radiological evaluation of cancers. While traditional measurement only gives information about volume or local circulation, PET/CT measurement can involve both tumor volume and glucose uptake through assessment of Warburg effects[31]. The combined, sophisticated method allows better identification of energy uptake in a three-dimensional manner[32]. Therefore, in contrast to traditional ways, PET/CT volumetric marker TLG may give more information on tumor bio-aggressiveness or bio-behavior.

Not only was PET/CT marker TLG indicate treatment outcome, but also pathological biomarkers were found associated with △TLG via several pathways as seen in patients receiving combined treatment, a

piece of evidence concurrently corroborating that the TLG was associated with tumor bio-aggressiveness (all pathways ended in survival prognosis in the paradigm of pathway analysis)[33]. In the pathway analysis, the PD-L1 expression level was directly associated with △TLG, but the effects were mediated by other factors, including FGFR, VEGFR, and VEGF, which are named here as an indirect pathway. Interestingly, the indirect effects had stronger mediating effects than the direct effects (β = 0.21 for direct effects, while the indirect effects were higher in the VEGFR pathway). In a combined blockade of angiogenesis and immune checkpoints, markers for both medications should be considered. although ATC expresses relatively high levels of PD-L1, the malignancy usually fails to have an adequate response to mono-therapy for several reasons. Among them, the tumor microenvironment plays a key role to impair blockade effects[34 35]. The crosstalk between angiogenesis and photomural immune cells co-activate tumor progression and finally leads to failure of effective T cell infiltration and immune cell apoptosis[36 37]. Thus, by this theory blocking these pathways simultaneously can potentiate both immune checkpoint and angiogenesis inhibition thanks to demolishment of the vicious cycle[38 39]. Indeed, in the combined therapy group, blocking VEGFR achieved was the main pathway to achieve PD-LE blockade, and a minor pathway by FGFR was also significant. As different from VEGFR, FGFR mainly plays a role in the migration of endothelial cells and peri-cytes to help reorganization of tumor vasculature and thus a whole structure could be formed[40].

To compare treatment response and survival between therapeutic arms, the propensity score was calculated in each arm to achieve a head-to-head, matched comparison. In previous studies, factors entering the propensity score models included mainly included demographic and socioeconomic variables[41 42]. Instead, for ATC/PDTC and other types of deadly malignancies, the potential confounders may mainly come from the bio-aggressiveness of the tumor itself and clinical factors may not be the key role to determine treatment response[43]. Therefore, in this study, the baseline volumetric and glycolysis parameters of PET/CT were included in propensity score calculation to achieve tumor-to-tumor comparison.

Although this research was the first study with a relatively large sample to systematically compare the effectiveness of anlotinib combined with PD-1 blockers and mono-therapy, our work bears limitations. First, although the sample size is adequate in a combined therapy group, the sample size in the mono-therapy of PD-1 blockers was relatively small, resulting in correspondingly limited matched analysis (23 pairs) in the two arms, and the multivariate survival analysis in the mono-therapy can be inaccurate because of the limited size. Second, the current work did not analyze anlotinib mono-therapy, and future research is encouraged to unearth the response to the multi-target medication.

Conclusion

Anlotinib combined with PD-1 blockers was found to have an adequate response in the treatment of anaplastic or poorly-differentiated thyroid carcinoma and could be well tolerated. Anti-angiogenesis and anti-checkpoint may cause synergistic effects in total lesion glycolysis of the tumor in PET/CT scanning.

Declarations

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Authors' contributions

All authors contributed to the study conception and design. Material preparation were performed by Yifei Ma, Youlong Wang and Pengfei Zhu. Data collection and analysis were performed Jiling Zeng, Ying-Ying Hu , Hao Wu and Huazheng Shi. The first draft of the manuscript was written by Wei Fan and Xinjia Wang, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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Figures

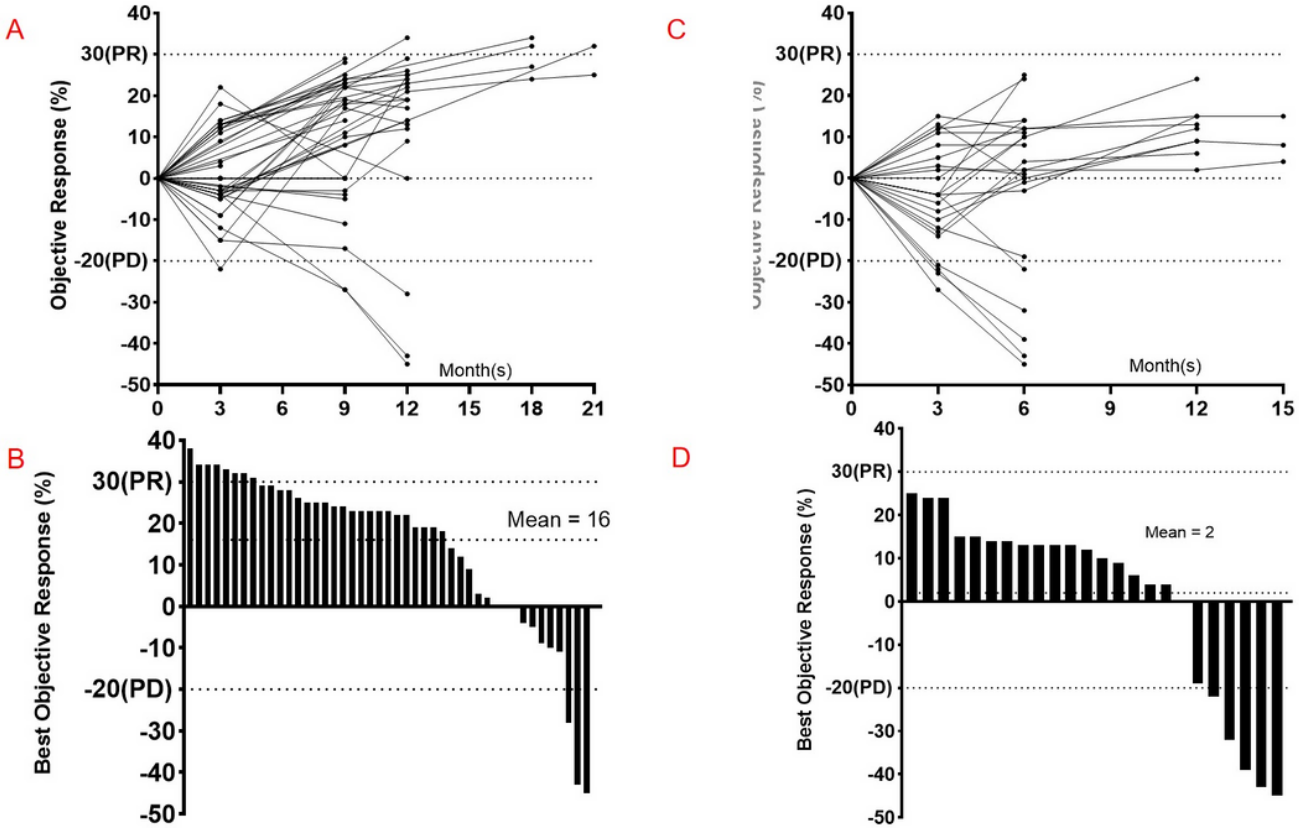


Figure 1

Objective response in combined treatment and PD-1 blocker mono-therapy group. **1A**, the change over time of objective response (%) of each patient in the combined group; **1B**, the best objective response (%) of each patient in the combined group. 1C, the change over time of objective response (%) of each patient in the PD-1 blocker mono-therapy group; 1D, the best objective response (%) of each patient in the PD-1 blocker mono-therapy group.

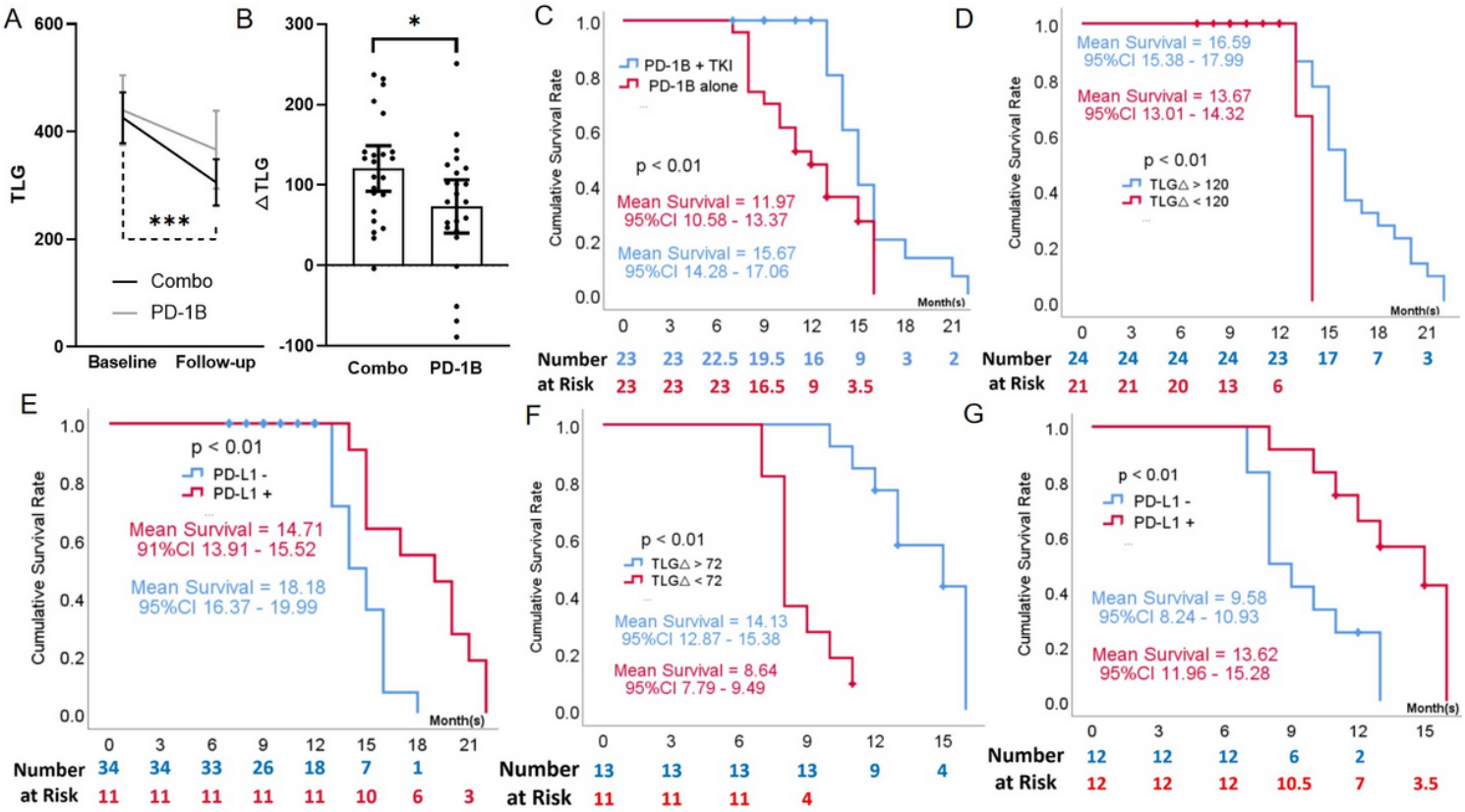
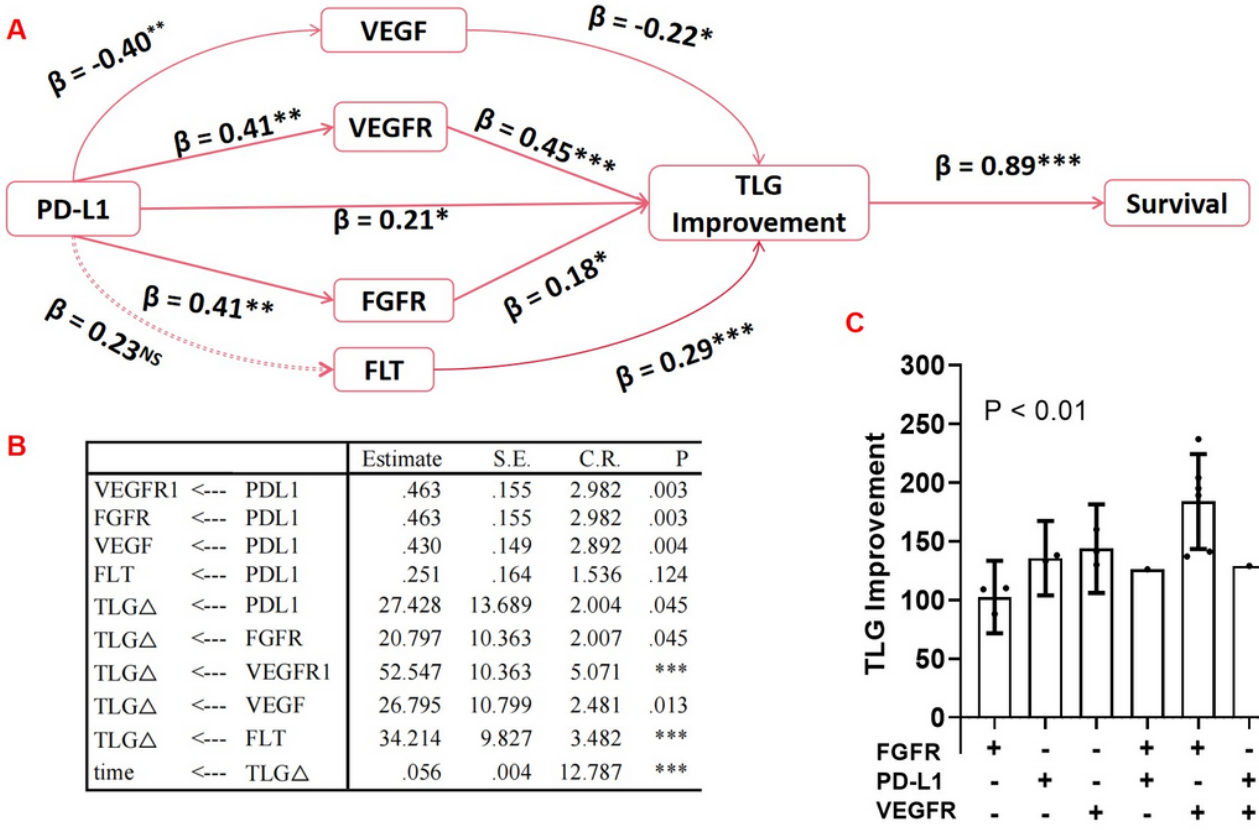


Figure 2

Propensity score-matched head-to-head comparison and biomarker associations. **2A**, improvement of total lesion glycolysis (△TLG) from baseline to follow-up assessment; **2B,** Comparison result of △TLG in combined treatment versus PD-1B treatment; **2C**, Kaplan-Meier survival curve of combined treatment, PD-1 blockers (PD-1B) + tyrosine kinase inhibitor (TKI), versus PD-1B alone; **2D**, △TLG improvement differentiate patient prognosis into shorter (red curve) and longer (blue curve) survival in combined treatment group; **2E**, Kaplan-Meier survival curve of PD-L1 expression in combined treatment group; **2F**, △TLG improvement differentiate patient prognosis into shorter (red curve) and longer (blue curve) survival in PD-1 blocker mono-therapy group; **2G**, Kaplan-Meier survival curve of PD-L1 expression in mono-therapy group.



B

			Estimate	S.E.	C.R.	P
VEGFR1	<---	PDL1	.463	.155	2.982	.003
FGFR	<---	PDL1	.463	.155	2.982	.003
VEGF	<---	PDL1	.430	.149	2.892	.004
FLT	<---	PDL1	.251	.164	1.536	.124
TLG△	<---	PDL1	27.428	13.689	2.004	.045
TLG△	<---	FGFR	20.797	10.363	2.007	.045
TLG△	<---	VEGFR1	52.547	10.363	5.071	***
TLG△	<---	VEGF	26.795	10.799	2.481	.013
TLG△	<---	FLT	34.214	9.827	3.482	***
time	<---	TLG△	.056	.004	12.787	***

Figure 3

3A, structure equation modeling of biomarkers significant in survival analysis of combined treatment arm. * p < 0.05, **p < 0.01, ***p < 0.001, ns, insignificant. Pathway analysis shows PD-1 blockade has a direct impact on TLG improvement (△TLG) affecting survival. The impact of PD-1 blockade on △TLG was positively mediated by VEGFR expression (β = 0.41 and β = 0.45, p < 0.01 for both) and by FGFR expression (β = 0.41 and β = 0.18, p < 0.01 and p = 0.04, respectively). The impact of PD-1 blockade was negatively mediated by VEGF expression (β = -0.40 and β = -0.22, p < 0.01 and p =0.04, respectively); there was significance in regression from FLT to △TLG (β = 0.29, p < 0.01), but there is no significance in regression analysis between FLT and PD-L1. **3B,** The critical ratio and unstandardized estimate of each regression pathway; 3C, △TLG in each subgroups of biomarker expression in combined treatment arm.

Supplementary Files

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- SupplementaryMaterial.docx