# PD-1 inhibitors combined with tyrosine kinase inhibitor-associated hypothyroidism is a predictive factor for antitumor effectiveness in male patients with hepatocellular carcinoma

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**Abstract.** Tyrosine kinase inhibitors (TKIs) and programmed cell death protein-1 (PD-1) inhibitors are well-established first-line systemic therapies for hepatocellular carcinoma (HCC), with hypothyroidism as a prominent immune-related adverse event. However, the female sex is a known risk factor for thyroid dysfunction associated with TKIs. The present study aimed to determine whether PD-1 inhibitor plus TKIs-induced hypothyroidism predicts treatment efficacy in male patients with HCC. A retrospective cohort study enrolled 77 male patients with confirmed HCC who received PD-1 inhibitor-TKIs combination therapy at Nanjing Second Hospital (Nanjing, China). According to the evaluation of thyroid function, patients were divided into normal thyroid function and hypothyroidism groups, based on serial thyroid function evaluations. The primary endpoint of the present study was progression-free survival (PFS). Baseline serum thyroid-stimulating hormone (TSH) and thyroglobulin antibody (TgAb) levels were significantly higher in the hypothyroidism group compared with the group with normal thyroid function [TSH, 4.2 mIU/l; 95% confidence interval (CI), 2.0-4.6 mIU/l vs. 1.7 mIU/l (95% CI, 1.2-3.1 mIU/l); P<0.001; TgAb, 12.4 IU/ml (95% CI, 2.1-18.2 IU/ml) vs. 1.4 IU/ml (95% CI, 0.8-5.0 IU/ml); P=0.007]. The group with hypothyroidism had a median PFS of 8.0 months, which was longer compared with the group with normal thyroid function (6.1 months; P<0.001). Cox regression analysis demonstrated that PD-1 inhibitors plus TKIs-induced hypothyroidism [hazard ratio (HR)=0.320; 95% CI, 0.160-0.641; P=0.001] and baseline serum α-fetoprotein >200 ng/ml (HR=2.354; 95% CI, 1.277-4.340; P=0.008) were independent prognostic factors of PFS in male patients with HCC. In conclusion, *de novo* hypothyroidism during PD-1 inhibitors combined with TKIs is associated with prolonged PFS in male patients with HCC.

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*Abbreviations:* HCC, hepatocellular carcinoma; PD-1, programmed cell death protein-1; TKIs, tyrosine kinase inhibitors; ICI, immune checkpoint inhibitor; OS, overall survival; PFS, progression-free survival; CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease; CI, confidence interval; TSH, thyroid-stimulating hormone; AFP, α-fetoprotein; TgAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; FT3, free triiodothyronine; FT4, free thyroxine; TD, thyroid dysfunction

*Key words:* HCC, PD-1 inhibitors, TKIs, thyroid function, progress

## Introduction

Hepatocellular carcinoma (HCC) ranks third among the leading causes of cancer-related mortality worldwide (1,2). It has been reported that >50% of patients with HCC are diagnosed at an advanced stage due to the asymptomatic nature of early disease, thereby missing the optimal window for radical surgery intervention. Patients with advanced-stage HCC are now generally treated with systemic therapy, which combines immune checkpoint inhibitor (ICI) with molecularly targeted drugs. According to the findings of IMbravel150, ORIENT-32 and CARES-310, programmed cell death protein-1 (PD-1) inhibitors, programmed death-ligand 1 (PD-L1) inhibitors and cytotoxic T lymphocyte-associated antigen-4 inhibitors are the three main classes of ICIs (3-6). Molecularly targeted drugs include tyrosine kinase inhibitors (TKIs) and vascular endothelial growth factor receptor antagonists. PD-1 inhibitors combined with TKIs have markedly improved prognostic outcomes in several advanced malignancies, including HCC, renal cell carcinoma and non-small cell lung cancer. In addition to their antitumor effects, these therapies also have been associated with immune-related adverse events affecting multiple organ systems in the body, particularly the endocrine systems. The most common endocrine adverse event is thyroid dysfunction (TD) (7,8).

Previous research has indicated that elevated TSH receptor expression is associated with poor prognosis in patients with HCC (9). Thyroid hormones serve a key role in regulating the

proliferation and differentiation of HCC (10). Thyroxine facilitates the proliferation of cancer stem-like cells and enhances resistance to *in vitro* treatments, thereby accelerating tumor growth in patients with HCC (11). Previous studies have reported that, compared with patients without TD, patients with solid tumors such as melanoma and non-small cell lung cancer, who developed TD after ICI therapy, exhibit prolonged overall survival (OS) and progression-free survival (PFS) (12-14). A 2022 study that enrolled 74 patients with HCC undergoing PD-1 inhibitor-based combination therapy set PFS as the primary outcome. The results demonstrated that patients with hypothyroidism had a longer PFS (7.44 months) compared with patients without hypothyroidism (5.68 months). However, this study did not further explore the association between sex and PD-1 inhibitor-related hypothyroidism (15) The female sex is a well-recognized risk factor for the occurrence of autoimmune thyroid disorders, such as Graves' and Hashimoto's thyroiditis with a female-to-male ratio of 8:1 (16-19). The association of the female sex with TKIs-associated hypothyroidism suggests an underlying immune mechanism. Previous studies have also indicated that TD is more prevalent in female patients with cancer treated with TKIs (20,21). Thus, female patients with HCC were excluded from the present study to minimize the influence of potential confounding factors.

The aim of the present study was to assess whether hypothyroidism induced by TKIs and PD-1 inhibitors are associated with favorable clinical outcomes in male patients with HCC.

## Patients and methods

*Study population*. A retrospective analysis was conducted on male patients with HCC who received PD-1 inhibitors combined with TKI therapy at Nanjing Second Hospital (Nanjing, China) from July 2020 to August 2024. The inclusion criteria were as follows: i) Male patients confirmed with HCC who received anti-PD-1 drugs combined with TKI drugs; ii) completion of at least two cycles of standard anti-PD-1 therapy combined with TKI drugs; and iii) normal thyroid function prior to treatment. Exclusion criteria were as follows: i) Metastatic liver cancer or malignant bile duct tumors; ii) incomplete imaging data or lack of thyroid function records before and after treatment; and iii) female patients with HCC. PD-1 inhibitors included sintilimab (cat. no. S20180016; Innovent Biologics, Inc.) and tislelizumab (cat. no. S20190045; BeiGene, Ltd.), both administered intravenously at a fixed dose of 200 mg every 3 weeks. For TKIs, sorafenib (cat. no. H20160201; Bayer Schering Pharma; Bayer AG) was administered orally at a dosage of 400 mg daily, while lenvatinib (cat. no. H20180052; Eisai Co., Ltd.) was administered orally dosed at 8-12 mg/day based on the body weight of the patient. The present study was approved by the Ethics Committee of Nanjing Second Hospital (approval no. 2024-LS-ky-098; Nanjing, China).

*Data collection*. Baseline clinical data were collected from enrolled patients, including age, body mass index (BMI), Child-Pugh classification (22) and history of previous therapy. Furthermore, the present study collected relevant indicators such as peripheral blood biochemistry, complete blood count, α-fetoprotein (AFP) and thyroid-related hormones.

*Diagnostic criteria*. Chemiluminescent immunoassay was used to measure thyroid function parameters. Free triiodothyronine (FT3) and free thyroxine (FT4) have normal ranges of 2.43-6.01 pmol/l and 9.01-19.05 pmol/l, respectively. The normal range for thyroid-stimulating hormone (TSH) is 0.35-4.94 mIU/l. The normal range for thyroglobulin antibody (TgAb) is 0-150 IU/ml. The normal range for thyroid peroxidase antibody (TPOAb) is 0-34 IU/ml. TD was classified into four subtypes based on the recommendation of the American Thyroid Association (23): i) Overt hyperthyroidism: Elevated FT4 or FT3 with decreased TSH; ii) subclinical hyperthyroidism: Normal FT4 and FT3 levels with decreased TSH; iii) overt hypothyroidism: Decreased FT4 or FT3 with elevated TSH; and iv) subclinical hypothyroidism: Normal FT4 and FT3 levels with elevated TSH.

*Patient grouping*. Patients were divided into two groups based on the first occurrence of TD during treatment: The hypothyroidism group (n=36) and the normal thyroid function group (n=41). The hypothyroidism group comprised two subgroups: Overt and subclinical hypothyroidism.

*Statistical analysis*. Data were analyzed using SPSS statistical software (version 27.0; IBM Corp.). Normally distributed data were presented as the mean ± standard deviation (SD) and analyzed using unpaired t-tests, while non-normally distributed data were presented as the median (interquartile range) and analyzed using the Wilcoxon rank-sum test. Bonferroni correction was applied following ANOVA. Count data were presented as n (%). Intergroup comparisons were performed using $\chi^2$ and Fisher's exact tests. Sensitivity analysis was conducted to validate the robustness of the results. Univariate and multivariate Cox proportional hazards regression analyses were performed to identify independent prognostic factors, with PFS as the primary outcome. $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*Baseline characteristics of the two groups*. A total of 77 male patients with HCC were included in the present study, selected from 406 patients with HCC who received anti-PD-1 plus TKI therapy between July 2020 and August 2024 (Table I). The median age was 59.2 years (range, 28-81 years). Prior to treatment initiation, 72 (93.5%), 41 (53.2%) and 6 (7.8%) had received transarterial chemoembolization, microwave ablation or curative surgery, respectively (Table I). Enrolled patients received different PD-1 inhibitors and TKIs, and detailed information is provided in Table I.

36 patients developed hypothyroidism during treatment, among whom 6 initially presented with transient hyperthyroidism following systemic treatment. All 6 patients eventually progressed to hypothyroidism and none received pharmacological treatment. By the end of follow-up (April 2025), no enrolled patients had died from serious drug-related adverse events (such as grade 3 diarrhea, immune-related hepatitis and severe skin toxicity).

*Comparison of baseline thyroid hormone levels between two groups*. No significant differences in baseline FT3 and FT4

levels were observed between the normal thyroid function and the hypothyroidism groups [3.0 pmol/l; 95% confidence interval (CI), 2.5-4.0 pmol/l] vs. 3.5 pmol/l (95% CI, 2.5-4.5 pmol/l); P=0.292] and (12.4±2.1 pmol/l vs. 12.5±1.9 pmol/l; P=0.676), respectively (Table I).

Significant differences in baseline TSH levels were detected between the two groups. Baseline serum TSH levels were significantly higher in the hypothyroidism group [4.2 mIU/l (95% CI, 2.0-4.6 mIU/l)] compared with that in the normal thyroid function group [1.7 mIU/l (95% CI, 1.2-3.1 mIU/l)] (P<0.001; Table I).

Additionally, baseline TgAb levels were significantly higher in the hypothyroidism group [12.4 IU/ml (95% CI, 2.1-18.2 IU/ml)] compared with the normal thyroid function group [1.4 IU/ml (95% CI, 0.8-5.0 IU/ml)] (P=0.007; Table I). For the 45 samples with complete TgAb/TPOAb data, the present study conducted a sensitivity analysis. The baseline characteristics (age, Child-Pugh class, AFP level and PFS) of 45 patients with available data were compared and the results demonstrated that PFS remained the key variable with statistical significance (P=0.004; Table II).

*Comparative prognosis between two groups*. The hypothyroidism group had a significantly longer PFS [8.0 months (95% CI, 6.0-12.1 months)] compared with that of the normal thyroid function group [6.1 months (95% CI, 3.1-6.7 months)] (P<0.001). However, the hypothyroidism group had a longer OS compared with that of the normal thyroid function group [16.3 months (95% CI, 7.8-20.4 months) vs. 11.2 months (95% CI, 6.5-18.7 months); P>0.05] (Table I), the difference was not statistically significant.

In the hypothyroidism group (n=36), 9 patients achieved complete response (CR; 25%), 6 achieved partial response (PR; 16.7%), 6 had stable disease (SD; 16.7%) and 15 had progressive disease (PD; 41.6%). In the normal thyroid function group (n=41), 4 patients achieved CR (9.8%), 6 achieved PR (14.6%), 10 had SD (24.4%) and 21 had PD (51.2%) (Fig. 1). The hypothyroidism group exhibited higher objective response rate and disease control rate compared with that in the normal thyroid function group: 41.7 vs. 24.4% and 58.4 vs. 48.8%, respectively (P=0.170 and P=0.402).

*Factors influencing PFS after systemic therapy*. Univariate Cox regression analysis revealed that hypothyroidism [hazard ratios (HR), 0.317; 95% CI, 0.158-0.637; P=0.001] was a favorable prognostic factor for male patients with HCC receiving PD-1 inhibitor plus TKI therapy. By contrast, baseline serum AFP level >200 ng/ml was an independent adverse prognostic factor (HR=2.364; 95% CI, 1.283-4.354; P=0.006). The HRs of developing hypothyroidism after treatment and baseline AFP levels >200 ng/ml were (HR=0.320; 95% CI, 0.160-0.641; P=0.001) and (HR=2.354; 95% CI, 1.277-4.340; P=0.006), respectively, according to the multivariate Cox regression analysis (Table III).

*Comparison of survival curves between two groups*. While there was no statistically significant difference in the median OS, the median PFS in the hypothyroidism group was significantly higher compared with that in the normal thyroid function group (P<0.001). Patients with baseline AFP >200 ng/ml had significantly shorter median PFS and OS compared with patients with baseline AFP ≤200 ng/ml (P=0.004 and P=0.004, respectively; Fig. 2).

## Discussion

The present study data demonstrated that male patients with HCC who developed hypothyroidism following PD-1 inhibitors plus TKI therapy exhibit a longer PFS compared with those with normal thyroid function. Furthermore, a baseline AFP level >200 ng/ml prior to treatment serves as an independent risk factor for both PFS and OS in male patients with HCC.

A previous meta-analysis indicated that TD following PD-1 inhibitors therapy in patients with solid tumors acts as an independent factor influencing PFS and OS, with HR of 0.58 and 0.52, respectively (24). The present study findings were consistent with this observation. Multivariate Cox regression analysis yielded an HR of 0.320 for hypothyroidism, suggesting that hypothyroidism is an independent predictive factor for PFS in patients with HCC. The hypothyroidism group in the present study comprised both overt and subclinical cases. Notably, the majority of patients in this group had mild hypothyroidism, with subclinical hypothyroidism accounting for 66.7% (24/36) of cases, which is consistent with observations from previous studies (25,26). Due to the limited sample size, the present study was unable to compare differences in PFS between these two subgroups. Future prospective multicenter studies are warranted to evaluate prognostic differences between overt and subclinical hypothyroidism in patients with HCC treated with PD-1 inhibitors plus TKIs combination therapy. Although the hypothyroidism group had a longer median OS, the difference was not statistically significant, which was in line with the findings of a previous study (27).

In the IMbrave150 study, the incidence of hypothyroidism among patients with HCC treated with atezolizumab plus bevacizumab was 10.9%, with a median follow-up of 8.9 months (28). By contrast, the present study demonstrated a significantly higher incidence of hypothyroidism (46.8%). More frequent thyroid function monitoring and a relatively longer follow-up duration may account for the higher detection rate of hypothyroidism in the present study. The small sample size is another potential contributing factor. The third contributing factor was that all patients received combined TKI therapy. TKIs induce transient thyrotoxicosis or even permanent hypothyroidism by damaging thyroid blood vessels and inhibiting thyroid iodine uptake (20,29). Previous studies have also confirmed that the use of TKIs in patients with metastatic renal cell carcinoma is associated with an increased risk of hypothyroidism (30-32). Whether thyroid injury induced by TKIs treatment is transient or permanent remains controversial. After TKIs discontinuation, certain patients recover normal thyroid function, whereas others persist with hypothyroidism (29).

The present study findings demonstrated that the baseline TgAb level in the hypothyroidism group was significantly higher compared with that in the normal thyroid function group. A retrospective analysis of 316 patients with cancer demonstrated that 28.48% of TD patients had positive baseline thyroid autoantibodies (including TgAb), compared with 5.70% of euthyroid patients (P<0.001) (33). Another previous study confirmed that abnormal baseline TgAb significantly elevates

Table I. Baseline characteristics of 77 male patients with HCC.

| Characteristics | Normal thyroid function group (n=41) | Hypothyroidism group (n=36) | P-value |
|---|---|---|---|
| Age (years), n (%) | | | 0.726 |
| <60 | 20 (48.8) | 19 (52.8) | |
| ≥60 | 21 (51.2) | 17 (47.2) | |
| BMI, n (%) | | | 0.940 |
| ≤24.9 | 27 (65.9) | 24 (66.7) | |
| >24.9 | 14 (34.1) | 12 (33.3) | |
| Type 2 diabetes, n (%) | | | 0.951 |
| No | 31 (75.6) | 27 (75.0) | |
| Yes | 10 (24.4) | 9 (25.0) | |
| Hypertension, n (%) | | | 0.966 |
| No | 26 (63.4) | 23 (63.9) | |
| Yes | 15 (36.6) | 13 (36.1) | |
| Prior TACE, n (%) | | | 0.364 |
| No | 4 (9.8) | 1 (2.8) | |
| Yes | 37 (90.2) | 35 (97.2) | |
| Prior microwave ablation, n (%) | | | 0.593 |
| No | 18 (43.9) | 18 (50.0) | |
| Yes | 23 (56.1) | 18 (50.0) | |
| Prior operation, n (%) | | | >0.999 |
| No | 38 (92.7) | 33 (91.7) | |
| Yes | 3 (7.3) | 3 (8.3) | |
| Child-Pugh, n (%) | | | 0.508 |
| A | 27 (65.9) | 27 (75.0) | |
| B or C | 14 (34.1) | 9 (25.0) | |
| BCLC, n (%) | | | 0.438 |
| A | 5 (12.2) | 3 (8.3) | |
| B or C | 36 (87.8) | 33 (91.7) | |
| AFP (ng/ml), n (%) | | | 0.683 |
| ≤200 | 28 (68.3) | 23 (63.9) | |
| >200 | 13 (31.7) | 13 (36.1) | |
| PD-1 inhibitors, n (%) | | | 0.321 |
| Nivolumab | 24 (58.5) | 17 (47.2) | |
| Tislelizumab | 17 (41.5) | 19 (52.8) | |
| TKIs, n (%) | | | 0.857 |
| Sorafenib | 27 (65.9) | 23 (63.9) | |
| Lenvatinib | 14 (34.1) | 13 (36.1) | |
| FT3, pmol/l (95% CI) | 3.0 (2.5-4.0) | 3.5 (2.5-4.5) | 0.292 |
| FT4, pmol/l (95% CI) | 12.4±2.1 | 12.5±1.9 | 0.676 |
| TSH, mIU/l (95% CI) | 1.7 (1.2-3.1) | 4.2 (2.0-4.6) | <0.001[a] |
| TPOAb, IU/ml (95% CI) | 0.8 (0.5-5.5) | 7.0 (2.0-22.5) | 0.003[a] |
| TGAb, IU/ml (95% CI) | 1.4 (0.8-5.0) | 12.4 (2.1-18.2) | 0.007[a] |
| PFS, months (95% CI) | 6.1 (3.1-6.7) | 8.0 (6.0-12.1) | <0.001[a] |
| OS, months (95% CI) | 11.2 (6.5-18.7) | 16.3 (7.8-20.4) | 0.168 |

[a]P<0.05. HCC, hepatocellular carcinoma; BCLC, Barcelona Clinic Liver Cancer; PD-1, programmed cell death protein-1; TKIs, tyrosine kinase inhibitors; OS, overall survival; PFS, progression-free survival; CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease; OR, odds ratio; CI, confidence interval; TSH, thyroid stimulating hormone; AFP, α-fetoprotein; TgAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; FT3, free triiodothyronine; FT4, free thyroxine; TACE, transarterial chemoembolization; BMI, body mass index.

Table II. Baseline characteristics of 45 male patients with HCC with complete TgAb/TPOAb data.

| Characteristics | Normal thyroid function group (n=23) | Hypothyroidism group (n=22) | P-value |
|---|---|---|---|
| Age (years), n (%) | | | 0.894 |
| <60 | 13 (56.5) | 12 (54.5) | |
| ≥60 | 10 (43.5) | 10 (45.5) | |
| Child-Pugh, n (%) | | | 0.912 |
| A | 15 (65.2) | 14 (63.6) | |
| B or C | 8 (34.8) | 8 (36.4) | |
| AFP (ng/ml), n (%) | | | 0.175 |
| ≤200 | 17 (73.9) | 12 (54.5) | |
| >200 | 6 (26.1) | 10 (45.5) | |
| PFS, months (95% CI) | 6.1 (3.2-6.7) | 8.2 (6.0-12.5) | 0.004[a] |

[a]P<0.05. HCC, hepatocellular carcinoma; TgAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; PFS, progression-free survival; AFP, α-fetoprotein; CI, confidence interval.

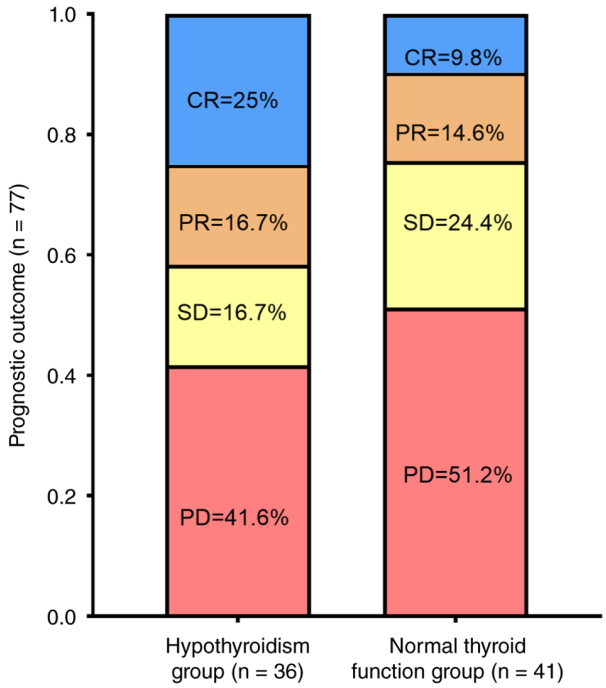


Figure 1. Antitumor effect between two groups of male patients with hepatocellular carcinoma. CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

the risk of TD (34). ICIs further activate TgAb-specific autoreactive CD8+ T cells, which infiltrate the thyroid gland and directly damage thyroid follicular cells, frequently resulting in a biphasic clinical course of TD (namely, transient thyrotoxicosis followed by hypothyroidism). The elevated baseline TSH levels in the hypothyroidism group align with prior findings. In a retrospective study of 560 ICI-treated patients, baseline TSH was identified as a strong independent risk factor for TD (odds ratio, 1.935 per mIU/l; 95% CI, 1.613-2.321; P<0.001) (35). Elevated TSH typically reflects subclinical thyroiditis or impaired hormone synthesis, which reduces thyroid resistance to immune-mediated injury. By activating effector T cells and suppressing regulatory T-cell function, ICIs disrupt immune tolerance; this enhanced immune activation preferentially targets thyroid cells with preexisting structural or functional impairment, accelerating follicular destruction and overt TD. Notably, higher baseline TSH is specifically associated with an increased risk of immune-related hypothyroidism. To ensure early diagnosis and prompt treatment of TD in male patients with HCC receiving PD-1 inhibitors (with or without TKIs), the present study findings highlighted the importance of routine baseline thyroid antibody testing in addition to regular thyroid function monitoring.

While hypothyroidism is a known side effect of PD-1 inhibitors (36), not all patients develop this adverse event. Immune-related hypothyroidism may be driven by increased cytokine levels, stimulation of autoantibody formation and enhanced T-cell activation (37,38). Mature CD4+ and CD8+ T cells, B cells and monocytes frequently express PD-1. By preventing PD-1 from binding to PD-L1 or -L2, PD-1 inhibitors exert their antitumor activity (29). Normal thyroid tissue also expresses the PD-1 ligand. Therefore, cytotoxic T cells may target the thyroid gland after PD-1 inhibitor administration, impairing thyroid function. Thyroiditis can also develop when activated effector T cells induce autoimmune responses in thyroid tissues (39). Furthermore, excessive effector T-cell activation increases the risk of TD by promoting the release of cytokines such as interleukin-2, interferon and tumor necrosis factor (40).

Kegasawa *et al* (41) enrolled 178 patients with HCC treated with sorafenib. The study identified that a baseline AFP level >200 ng/ml was an independent risk factor for disease progression and OS in patients with HCC. These findings align with the present study: The present study results suggested that a pretreatment baseline AFP level >200 ng/ml is an independent risk factor for PFS and OS in patients with HCC. Thus, evaluating baseline AFP levels contributes to determining clinical prognosis in patients with HCC. Furthermore, regardless of prior hyperthyroid phase, the present study revealed that all patients with HCC who experienced thyroid adverse events

Table III. Cox regression analysis of factors associated with PFS in male patients with HCC.

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| Factors | HR (95% CI) | P-value | HR (95% CI) | P-value |
| Age (≥60 vs. <60 years) | 1.391 (0.883-2.189) | 0.154 | - | - |
| BMI (>24.9 vs. <24.9) | 0.704 (0.272-1.823) | 0.470 | - | - |
| Prior TACE (yes vs. no) | 1.577 (0.211-11.796) | 0.657 | - | - |
| Prior microwave ablation (yes vs. no) | 0.732 (0.308-1.741) | 0.480 | - | - |
| Child-Pugh (B or C vs. A) | 1.967 (0.818-4.733) | 0.131 | - | - |
| AFP (>200 vs. ≤200 ng/ml) | 2.364 (1.283-4.354) | 0.006[a] | 2.354 (1.277-4.340) | 0.006[a] |
| Hypothyroidism (yes vs. no) | 0.317 (0.158-0.637) | 0.001[a] | 0.320 (0.160-0.641) | 0.001[a] |

[a]P<0.05. HCC, hepatocellular carcinoma; PFS, progression-free survival; AFP, α-fetoprotein TACE, transarterial chemoembolization; CI, confidence interval; HR, hazard ratio; BMI, body mass index.

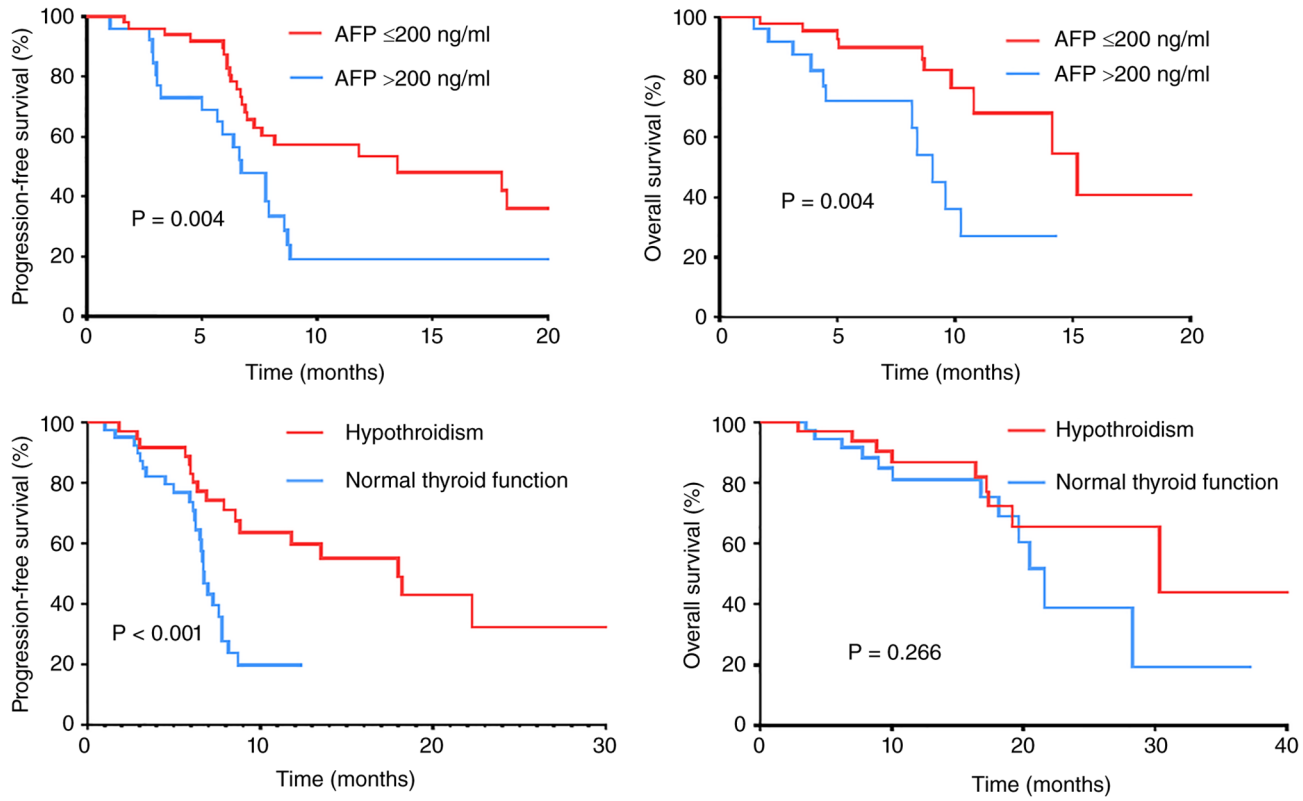


Figure 2. Comparison of PFS and OS between two groups of male patients with hepatocellular carcinoma. OS, overall survival; PFS, progression-free survival; AFP, α-fetoprotein.

ultimately progressed to hypothyroidism. This result was consistent with previous research (25,42).

The present study had several limitations. First, nearly half of the enrolled patients (32/77; 41.6%) lacked baseline thyroid antibody testing data; therefore, the association between the post-treatment hypothyroidism incidence and baseline thyroid antibody levels could not be explored in the present study, which may be investigated in future research. Second, only a small proportion of patients developed hyperthyroidism and the small sample size prevented an analysis of the association between prognosis and hyperthyroidism. Third, the present study acknowledges that excluding female patients restricts the generalizability of the present study findings to women. Accordingly, future studies may recruit female patients with HCC to perform sex-specific subgroup analyses and verify the robustness of the present study findings across sexes. Furthermore, mechanistic investigation is inherently limited in the present retrospective study. To mitigate this key limitation, prospective studies may validate these associations, alongside complementary *in vitro* and *in vivo* experiments aimed at elucidating the underlying molecular mechanisms in the future. Furthermore, since the present study was a retrospective single-center study, larger prospective randomized controlled trials are warranted to confirm the impact of

hypothyroidism on the prognosis in male patients with HCC treated with PD-1 inhibitors plus TKIs combination therapy.

In conclusion, male patients with HCC who developed hypothyroidism during PD-1 inhibitors plus TKI therapy exhibited a more favorable prognosis compared with patients with normal thyroid function. Furthermore, the present study findings identified baseline AFP levels as an independent prognostic factor. Future studies should evaluate baseline serum levels of TSH, TPOAb, TgAb and AFP in patients with HCC prior to initiation of PD-1 inhibitors plus TKIs combination therapy. Close monitoring of thyroid function and antibodies during treatment is key to early prognostic assessment and timely intervention.

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**Availability of data and materials**

The data generated in the present study may be requested from the corresponding author.

**Authors' contributions**

BC wrote the main manuscript text and prepared the figures and tables. PH and HZ conceived and designed the present study. BC and MA collected and analyzed the data. PH and HZ confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

The present study complied with the Declaration of Helsinki. All of the present study procedures were approved by the Ethics Committee of Nanjing Second Hospital (approval no. 2024-LS-ky-098; Nanjing, China). Written informed consent was obtained from all participants included in the present study.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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