

Thyroid Differentiation Score Discordance in BRAF-like Papillary Thyroid Carcinoma: Clinicopathologic Associations and Progression-Free Interval in TCGA-THCA

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Research Article

Keywords: Papillary thyroid carcinoma, Thyroid differentiation score, BRAF, Gene fusions, TCGA, Prognosis

Posted Date: June 24th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9612802/v1>

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Abstract

Purpose

BRAF-like papillary thyroid carcinomas (PTCs) typically exhibit low thyroid differentiation scores (TDS). However, the clinical significance of individual-level variation within this subtype remains unclear. We investigated whether TDS–molecular subtype discordance identifies a distinct subgroup within BRAF-like PTC and evaluated TDS component-gene concordance in independent expression cohorts.

Methods

We analyzed 390 PTCs from the TCGA-THCA cohort classified as concordant or discordant based on TDS relative to the overall cohort median. Progression-free interval (PFI) was assessed using Kaplan-Meier and multivariable Cox regression. TDS was correlated with thyroglobulin (TG) and sodium-iodide symporter (SLC5A5) expression in TCGA and two independent PTC cohorts (GSE54958, GSE58545).

Results

81 of 272 BRAF-like PTCs (29.8%) retained higher TDS and were classified as Discordant-BRAF. Compared with Concordant-BRAF tumors ($n = 191$), Discordant-BRAF tumors were enriched for oncogenic fusions (35.8% vs 5.2%), had fewer BRAF V600E mutations (61.7% vs 93.7%), fewer TERT promoter mutations (2.5% vs 13.6%), and higher Kaplan-Meier-estimated 5-year PFI (95.5% vs 81.3%; all $p \leq 0.008$). On multivariable analysis, continuous TDS was inversely associated with progression hazard within BRAF-like tumors (adjusted HR 0.56 per unit TDS, 95% CI 0.35–0.90, $p = 0.016$; $n = 256$, 28 events), and the dichotomous Discordant-BRAF classification was also associated with lower progression hazard, although this estimate was based on only two events (adjusted HR 0.20, 95% CI 0.05–0.83, $p = 0.027$; based on 2 events in the Discordant-BRAF group). TDS correlated with TG expression in TCGA ($\rho = 0.75$, $p < 0.001$) and in BRAF-mutant samples from one of two external cohorts (GSE58545, $n = 18$, $\rho = 0.78$, $p < 0.001$; GSE54958, $n = 14$, $\rho = 0.45$, $p = 0.11$). SLC5A5 correlation was consistent in TCGA ($\rho = 0.54$, $p < 0.001$) but variable across smaller external subsets.

Conclusion

Nearly one-third of BRAF-like PTCs retain higher thyroid-differentiation gene expression, which is associated with a directionally consistent signal toward improved progression-free interval, though limited by low event counts. TDS may add prognostic information within BRAF-like PTC and warrants evaluation in treatment-stratified cohorts.

Introduction

Papillary thyroid carcinoma (PTC) is the most common endocrine malignancy. The Cancer Genome Atlas (TCGA) identified two dominant signaling subtypes (BRAF-like and RAS-like) based on a continuous BRAF-RAS score (BRS) derived from mRNA expression signatures [1]. This classification correlates with histological subtype, DNA methylation patterns, and clinical behavior.

A complementary metric, the thyroid differentiation score (TDS), quantifies expression of 16 genes essential for thyroid hormone biosynthesis and iodine metabolism (including NIS, TPO, TG, TSHR, PAX8, and FOXE1) [1]. TDS is computed as the mean of standardized (z-scored) expression of these 16 genes across the cohort, yielding values approximately centered on zero (cohort mean ≈ 0 , SD ≈ 1) where positive values indicate relative preservation of thyroid-differentiation gene expression and negative values indicate loss of differentiation; in the TCGA-THCA cohort, observed TDS values range from approximately -4 to $+3$. TDS is inversely correlated with BRS as BRAF-like tumors typically exhibit low TDS (dedifferentiation), while RAS-like tumors maintain high TDS. This relationship has been interpreted as evidence that BRAF-driven MAPK signaling suppresses thyroid-specific gene expression, potentially contributing to radioiodine refractoriness in BRAF V600E-mutant disease [2–4]. Prior studies have demonstrated heterogeneity among BRAF-driven PTCs based on TDS, including BRAF-mutant subgroups defined by preserved versus reduced differentiation-gene expression, yet the clinical and molecular significance of discordance between TDS and BRAF-like transcriptomic classification remains incompletely defined [11].

However, this population-level correlation does not hold universally. Subtype-based classification may obscure clinically important within-group heterogeneity, as some BRAF-like PTCs retain relatively higher thyroid-differentiation gene expression despite their transcriptomic classification [11]. Because TDS is derived from thyroid-function genes, including TG and sodium-iodide symporter (SLC5A5/NIS), we also assessed whether TDS tracked these canonical differentiation markers, particularly given their relevance to iodine-handling biology [12]. Using the TCGA-THCA cohort, with additional expression-level validation in independent public PTC cohorts, we evaluated the prevalence, clinicopathologic features, mutational landscape, progression-free outcomes, and differentiation-gene correlates of this Discordant-BRAF phenotype.

Methods

Study population and discordance definition

We analyzed 390 primary PTCs from the TCGA-THCA project with available TDS and BRS classification, using the original TCGA definitions of BRAF-like (BRS < 0) and RAS-like (BRS > 0) [1]. The cohort comprised 272 BRAF-like and 118 RAS-like tumors. Curated survival endpoints were derived from the Pan-Cancer Clinical Data Resource [5]. This study used publicly available, de-identified data and was exempt from institutional review board approval.

Discordance was defined using the overall cohort median TDS (-0.237) as a prespecified descriptive threshold. An overall cohort median was chosen to preserve a common reference threshold across molecular subtypes and to operationalize discordance descriptively; prognostic analyses were therefore complemented by continuous TDS models within BRAF-like tumors. This cutpoint is an exploratory operational definition, not a biologically validated threshold. Four groups were defined: Concordant-BRAF (BRAF-like, TDS below median), Discordant-BRAF (BRAF-like, TDS at or above median), Concordant-RAS (RAS-like, TDS at or above median), and Discordant-RAS (RAS-like, TDS below median; $n = 4$, excluded). This threshold was selected to provide a clinically interpretable subgrouping, while continuous TDS models serve as the primary inferential framework. Oncogenic fusion status was ascertained from the THCA subtype annotation returned by TCGAAbiolinks::TCGAQuery_subtype("THCA"), specifically the fusionDriverGenes column. To confirm that findings were not artifacts of dichotomization, TDS was also analyzed as a continuous variable to assess whether associations were threshold-dependent, and BRAF-like tumors were stratified into TDS tertiles (T1: -3.163 to -0.874 ; T2: -0.862 to -0.321 ; T3: -0.309 to 2.220).

Biologic validity and external expression cohorts (dup: abstract ?)

To confirm that TDS reflects thyroid-specific biology, we correlated the official TCGA TDS with expression of two canonical thyroid-function genes (TG) and sodium-iodide symporter (SLC5A5/NIS) using Spearman correlation in the TCGA-THCA expression-overlap cohort, within BRAF-like tumors, and within the BRAF V600E subset. As a quality-control step, a 16-gene TDS was recomputed from TCGA RNA-seq and compared with the official score. The 16-gene TDS comprised DIO1, DIO2, DUOX1, DUOX2, FOXE1, GLIS3, NKX2-1, PAX8, SLC26A4, SLC5A5, SLC5A8, TG, THRA, THRB, TPO, and TSHR.

We also analyzed two independent PTC expression cohorts from GEO (GSE54958, 45 total tumor samples; GSE58545, 45 total samples [27 PTC tumors, 18 normal thyroid samples]), deriving a cohort-specific 16-gene differentiation score and assessing its correlation with SLC5A5 and TG. These external cohorts lack TCGA-style BRS and progression annotation, so they validate the differentiation-expression biology rather than the prognostic analysis [13,16]. GSE54958 comprised 45 PTC samples, including 14 BRAF-mutant, 11 BRAF-wild-type, and 20 other or unannotated tumors. GSE58545 comprised 27 PTC samples (18 BRAF-positive, 8 RET-positive, 1 other PTC) and 18 normal thyroid samples, which were excluded from tumor-only analyses. For external cohorts, the same 16-gene panel was used. When multiple microarray probes mapped to the same gene symbol, multiple probes per gene were collapsed using the median expression value, and a cohort-specific differentiation score was calculated as the mean of cohort-internally z-scored expression across the available TDS genes. This score is a cohort-specific approximation of the TCGA TDS construct rather than the identical signature, and is therefore reported separately; Spearman rank correlations are invariant to the difference in scaling but the magnitudes of the score itself are not directly comparable across cohorts.

Statistical analysis

Of 390 tumors, 4 Discordant-RAS tumors were excluded from comparative and survival analyses. Among the remaining 386, 24 (6.2%) lacked both age and sex and were excluded from Cox models, yielding a complete-case Cox dataset of 362 patients (36 of the 38 total PFI events occurred in the complete-case Cox dataset). All Cox models used this complete-case dataset. Excluded patients did not differ significantly from included patients in BRAF-like vs RAS-like classification (16/8 vs 256/106; Fisher's exact, $p = 0.65$) or in the distribution of Concordant-BRAF, Discordant-BRAF, and Concordant-RAS groups (12/4/8 vs 179/77/106; Fisher-Freeman-Halton, $p = 0.82$), consistent with missingness unrelated to the primary exposure.

Clinicopathologic features of Concordant BRAF vs Discordant BRAF were compared exploratorily with Fisher's exact tests and were not adjusted for multiplicity. Additional comparisons including Concordant-RAS are present in Table 1, and were performed with fisher, chi-square, t-test, or Mann-Whitney U as specified. PFI was the primary endpoint analyzed [5]. The multivariable Cox model adjusted was for age, sex, and advanced stage (III/IV), while analyses of continuous TDS within BRAF-like tumors were adjusted for age, stage, and ETE. Covariates were limited for parsimony given the event count (6 parameters: 2 group indicators, age, sex, stage, ETE; 36 events, approximately 6 events per variable). The proportional hazards assumption was satisfied (Schoenfeld residuals, all $p > 0.05$). Continuous TDS was evaluated within BRAF-like tumors after adjustment for age, stage, and ETE ($n = 256$, 28 events). All tests were two-sided ($\alpha = 0.05$). Analyses were performed using Python 3.12 and R 4.5.2; NMF used RcppML 0.3.7 and pathway enrichment used fgsea 1.36.2.

Table 1

Clinicopathologic features by TDS–molecular subtype discordance group *P* values are pairwise Concordant-BRAF vs Discordant-BRAF using Fisher exact or chi-square tests for categorical variables, t-tests for normally distributed continuous variables, and Mann-Whitney U tests for non-normally distributed continuous variables, as noted

Characteristic	Concordant-BRAF (n = 191)	Discordant-BRAF (n = 81)	Concordant-RAS (n = 114)	<i>p</i>
Age, mean ± SD [‡]	47.0 ± 15.6	43.9 ± 14.4	49.0 ± 15.7	0.128 [‡]
Female sex, n/N (%) [‡]	136 (76.0)	56 (72.7)	78 (73.6)	0.694
Classical variant, n (%)	146 (76.4)	61 (75.3)	42 (36.8)	0.877
Follicular variant, n (%)	8 (4.2)	9 (11.1)	63 (55.3)	0.051
Tall cell variant, n (%)	23 (12.0)	5 (6.2)	0 (0)	0.191
Stage III/IV, n (%)	69 (36.1)	21 (25.9)	20 (17.5)	0.135
Any ETE, n (%)	74 (38.7)	20 (24.7)	10 (8.8)	0.027
ATA low risk, n (%)	40 (20.9)	31 (38.3)	70 (61.4)	0.004
ATA high risk, n (%)	14 (7.3)	0 (0)	3 (2.6)	0.013
BRAF V600E, n (%)	179 (93.7)	50 (61.7)	0 (0)	< 0.001
Gene fusion, n (%)	10 (5.2)	29 (35.8)	12 (10.5)	< 0.001
TERT promoter mut, n (%)	26 (13.6)	2 (2.5)	8 (7.0)	0.004
Mutation count, median (IQR)	12 (8–17)	9 (6–14)	12 (8–15)	0.002 [‡]
TDS, mean ± SD	−0.958 ± 0.502	0.451 ± 0.507	1.304 ± 0.586	< 0.001 [‡]
BRS, mean ± SD	−0.827 ± 0.145	−0.591 ± 0.245	0.762 ± 0.231	< 0.001 [‡]
Discordant-RAS (n = 4) excluded. Concordant-RAS shown for reference. [‡] Age and sex data available for 179/191 Concordant-BRAF, 77/81 Discordant-BRAF, and 106/114 Concordant-RAS patients; percentages use available denominators [‡] t-test. [‡] Mann-Whitney U. ATA American Thyroid Association, BRS BRAF-RAS score, ETE extrathyroidal extension, IQR interquartile range, TDS thyroid differentiation score, TERT telomerase reverse transcriptase				

Results

Cohort characteristics and discordance prevalence

Among 390 PTCs, 272 (69.7%) were BRAF-like and 118 (30.3%) were RAS-like. Eighty-one of 272 BRAF-like tumors (29.8%) were classified as Discordant-BRAF (Fig. 1). Only 4 RAS-like tumors (3.4%) showed discordant dedifferentiation and were excluded.

Clinicopathologic features

Discordant-BRAF tumors had a distinct clinicopathologic profile versus Concordant-BRAF (Table 1). Discordant-BRAF tumors showed lower BRAF V600E prevalence (61.7% vs 93.7%, $p < 0.001$) and higher fusion prevalence (35.8% vs 5.2%, $p < 0.001$): predominantly RET rearrangements ($n = 16$), NTRK fusions ($n = 6$), BRAF fusions ($n = 3$), ALK fusions ($n = 2$), MET fusions and LTK fusions (both $n = 1$). Discordant-BRAF tumors exhibited fewer TERT promoter mutations (2.5% vs 13.6%, $p = 0.004$), less ETE (24.7% vs 38.7%, $p = 0.027$), lower mutation burden (median 9 vs 12, $p = 0.002$), no ATA high-risk patients (0% vs 7.3%, $p = 0.013$), and more ATA low-risk classification (38.3% vs 20.9%, $p = 0.004$). The follicular variant was numerically more common (11.1% vs 4.2%, $p = 0.051$) and the tall cell variant numerically less common (6.2% vs 12.0%, $p = 0.191$); neither reached significance (Fig. 3)

Biologic validity and external expression cohorts

In TCGA, the official TDS correlated strongly with TG ($\rho = 0.75$, $p < 1 \times 10^{-72}$) and moderately with SLC5A5 ($\rho = 0.54$, $p < 1 \times 10^{-29}$; $n = 389$). These associations persisted within BRAF-like tumors (TG $\rho = 0.57$; SLC5A5 $\rho = 0.61$) and within the BRAF V600E subset (TG $\rho = 0.56$; SLC5A5 $\rho = 0.54$; all $p < 1 \times 10^{-17}$; Table 2). A recomputed 16-gene TDS closely tracked the official score ($\rho = 0.93$).

Table 2
TCGA TDS correlations with canonical thyroid-function genes

Subgroup	n	SLC5A5 ρ	SLC5A5 p	TG ρ	TG p
All eligible THCA	389	0.54	2.5×10^{-30}	0.75	1.5×10^{-72}
BRAF-like	271	0.61	1.2×10^{-28}	0.57	6.8×10^{-25}
BRAF V600E	227	0.54	3.2×10^{-18}	0.56	9.6×10^{-20}
<i>Spearman rank correlation between the official TCGA TDS and gene expression. n reflects samples with both TDS and expression data available</i>					

In two independent PTC expression cohorts, TG correlated positively with a cohort-specific differentiation score among BRAF-mutant or BRAF-positive samples: GSE54958 $\rho = 0.45$ ($n = 14$, $p = 0.106$) and GSE58545 $\rho = 0.78$ ($n = 18$, $p < 0.001$). Overall survival events were too few ($n = 14$) to support multivariable modeling. In GSE58545, the RET-positive subset also showed strong TG-TDS correlation ($\rho = 0.81$, $p = 0.015$). The SLC5A5 relationship was less consistent across external microarray subsets (Table 3), likely reflecting smaller sample sizes and platform-specific limitations.

Table 3
External cohort differentiation-expression validation

Cohort	Group	n	SLC5A5 ρ	SLC5A5 p	TG ρ	TG p
GSE54958	BRAF_mut	14	-0.31	0.274	0.45	0.106
GSE54958	BRAF_wt	11	0.72	0.013	0.77	0.005
GSE58545	BRAF_pos	18	0.23	0.350	0.78	< 0.001
GSE58545	RET_pos	8	-0.16	0.713	0.81	0.015
<i>Spearman correlation between cohort-specific 16-gene differentiation score and gene expression. External cohorts lack TCGA-style BRS and progression annotation; analyses validate differentiation-expression biology</i>						

Survival analysis

Over a median follow-up of 2.5 years, 38 PFI events occurred among 386 patients. Discordant-BRAF tumors had 2 events (2.5%) versus 27 (14.1%) for Concordant-BRAF. Five-year PFI (95% Greenwood CIs) was 95.5% (81.7–99.0) for Discordant-BRAF, 81.3% (73.2–87.2) for Concordant-BRAF, and 85.3% (71.2–92.8) for Concordant-RAS (3-group log-rank $p = 0.025$; pairwise Concordant-BRAF vs Discordant-BRAF $p = 0.008$; Fig. 2a).

On univariable Cox regression ($n = 362$, 36 events), Discordant-BRAF phenotype was associated with lower progression hazard (HR 0.22, 95% CI 0.05–0.90, $p = 0.035$). This persisted after multivariable adjustment (HR 0.20, 95% CI 0.05–0.83, $p = 0.027$). Advanced stage was the only other significant covariate (HR 3.18, 95% CI 1.24–8.18, $p = 0.016$; Table 4).

Table 4
Cox proportional hazards regression for progression-free interval

Variable	Univariable HR (95% CI)	<i>p</i>	Multivariable HR (95% CI)	<i>p</i>
Discordant-BRAF	0.22 (0.05–0.90)	0.035	0.20 (0.05–0.83)	0.027
Concordant-RAS	0.76 (0.35–1.67)	0.491	0.81 (0.35–1.86)	0.623
Age (per year)	1.02 (1.00–1.04)	0.069	0.99 (0.96–1.02)	0.493
Female sex	0.56 (0.29–1.12)	0.100	0.55 (0.27–1.11)	0.093
Stage III/IV	3.72 (1.92–7.22)	< 0.001	3.18 (1.24–8.18)	0.016
Any ETE	2.69 (1.40–5.18)	0.003	1.67 (0.79–3.56)	0.182
<i>Complete-case analysis: n = 362, 36 events. Reference: Concordant-BRAF</i>				
<i>Covariates limited for parsimony (6 covariates, 36 events). PH assumption satisfied (Schoenfeld, all <i>p</i> > 0.05). CI confidence interval, ETE extrathyroidal extension, HR hazard ratio</i>				

Continuous TDS analysis confirmed the association within BRAF-like tumors: univariable HR 0.44 (95% CI 0.28–0.71, *p* < 0.001) per 1-unit TDS, equivalent to HR 0.51 (95% CI 0.35–0.76) per 1-SD increase (1 SD = 0.82 within BRAF-like). Adjusted HR was 0.56 (95% CI 0.35–0.90, *p* = 0.016; *n* = 256, 28 events) per 1-unit, equivalent to HR 0.62 (95% CI 0.43–0.92) per 1-SD.

TDS tertile analysis showed a concordant gradient (log-rank *p* = 0.021; events: T1 15/92, T2 11/89, T3 3/91; Fig. 2b). To test whether the prognostic signal of TDS within BRAF-like tumors was independent of mutation class, the multivariable continuous-TDS Cox model (*n* = 255, 28 events) was additionally adjusted for BRAF V600E status and for oncogenic fusion status. Per 1-SD increase in TDS, the adjusted hazard ratio was 0.61 (95% CI 0.41–0.93, *p* = 0.020) when adjusted for V600E and 0.62 (95% CI 0.41–0.94, *p* = 0.023) when adjusted for fusion status, compared with the unadjusted-for-mutation-class baseline of 0.60 (95% CI 0.40–0.88, *p* = 0.009). The TDS effect therefore persisted after accounting for either mutation class, indicating that within BRAF-like disease, transcriptomic differentiation conveys prognostic information beyond the canonical mutation/fusion dichotomy. OS events were too few (*n* = 14) for multivariable modeling.

In a sensitivity analysis using the threshold applied by Boucai et al. (TDS = 0) [11], 67 of 272 BRAF-like tumors (24.6%) were reclassified as Discordant-BRAF, with high concordance with the median-based classification (Cohen's κ = 0.87). Feature distributions were preserved at the alternative threshold (V600E 58.2% Discordant-BRAF vs 92.7% Concordant-BRAF; fusion 38.8% vs 6.3%), and Discordant-BRAF tumors retained superior 5-year PFI (94.7%, 95% CI 78.6–98.8 vs 82.4%, 95% CI 74.6–88.0; pairwise log-rank *p* = 0.030). On multivariable Cox regression (Discordant-BRAF, age, sex, advanced stage), the Boucai-threshold hazard ratio for progression was 0.27 (95% CI 0.07–1.13, *p* = 0.073), directionally consistent with the median-based primary analysis but with reduced precision reflecting fewer events in the smaller reclassified group (Supplementary Table 2).

Discussion

This study extends prior observations of heterogeneity within BRAF-driven papillary thyroid carcinoma by characterizing discordance between thyroid differentiation score (TDS) and BRAF-like transcriptomic classification within the TCGA cohort [11]. Our principal finding is that nearly one-third of BRAF-like PTCs retain higher TDS and exhibit a more favorable clinicopathologic and prognostic profile. BRAF V600E is associated with adverse clinicopathologic features, and is considered alongside other factors in molecular risk assessment [2, 6]. Our data suggest that a subset of BRAF-like tumors retain the gene expression machinery for iodine uptake and organification. However, survival findings should be interpreted as supportive given the limited number of events. Whether this translates to retained clinical radioiodine avidity cannot be determined from TCGA data; however, the relative preservation of thyroid-differentiation signaling provides biologic rationale for investigating this question in clinically annotated postoperative cohorts with treatment-response data.

The correlation between TDS and canonical thyroid-function genes (particularly TG) was robust across TCGA subgroups and replicated in an independent BRAF-mutant cohort (GSE58545, $p = 0.78$). This supports that TDS captures thyroid-differentiation biology rather than behaving as an uninterpretable composite expression metric. The SLC5A5 relationship, while consistent in TCGA, was less stable in small external microarray subsets, likely reflecting probe-level variability and the known regulation of NIS at the post-transcriptional and membrane-trafficking levels rather than transcription alone [4, 10]. Our findings complement those of Boucai et al., who subdivided BRAF-mutant TCGA tumors using a TDS cutoff of 0 and identified a smaller BRAF-TDS_{hi} subgroup with fewer adverse features and better response-to-therapy outcomes. In contrast, we analyzed transcriptomic BRAF-like tumors rather than mutation-defined BRAF-mutant tumors, thereby capturing a fusion-enriched subgroup, and used curated TCGA progression-free interval rather than institutional response-to-therapy as the primary endpoint.

The most notable mechanistic observation is the enrichment of oncogenic fusions in Discordant-BRAF tumors (35.8% vs 5.2%, $p < 0.001$), predominantly RET, NTRK, BRAF, and ALK rearrangements. These kinase fusions may produce a transcriptional state more permissive of preserved thyroid-differentiation gene expression than canonical BRAF V600E-driven tumors [14,15]. The marked enrichment of oncogenic fusions suggests that Discordant-BRAF tumors may represent a biologically distinct MAPK-activated but differentiation-permissive subgroup. The near-absence of TERT promoter mutations (2.5%) further supports a less aggressive phenotype [7, 8]. Current guidelines increasingly incorporate molecular data into risk stratification [6, 9]; our findings suggest TDS may add further granularity within BRAF-like disease. Of the 13 clinicopathologic comparisons in Table 1, the BRAF V600E, fusion, mutation count, TDS, and BRS contrasts remain significant after Bonferroni correction ($\alpha = 0.05/13 \approx 0.0038$); the TERT promoter ($p = 0.004$) and ATA low-risk ($p = 0.004$) comparisons fall just outside this threshold; ETE, ATA high-risk, and follicular variant do not survive correction. These secondary contrasts are descriptive; primary inference rests on the pre-specified survival endpoint.

The continuous TDS analysis (which avoids arbitrary dichotomization and is the more robust biologic finding) confirms a dose-response relationship (adjusted HR 0.56, $p = 0.016$). The dichotomous discordance phenotype is useful for clinical description and communication, whereas the continuous model demonstrates that the association is not purely threshold-driven. Together, these analyses suggest TDS may add prognostic information within BRAF-like disease. Whether retained differentiation-gene expression translates to clinical radioiodine avidity cannot be determined from TCGA, but provides biologic rationale for investigation in treatment-stratified cohorts.

Ultimately, these findings should be considered hypothesis-generating. The TCGA-THCA cohort is predominantly early-stage with short follow-up and few events and should be interpreted as supportive; hazard ratio confidence intervals are wide. The median-based cutpoint is exploratory. External cohorts validated differentiation-expression relationships but did not permit independent prognostic validation, which remains an important next step. Clinicopathologic comparisons were unadjusted for multiplicity. Twenty-four patients with missing demographics were excluded from Cox models. External validation in clinically annotated cohorts with long-term follow-up and RAI response data is warranted.

In conclusion, TDS—molecular subtype discordance identifies a biologically and clinically informative subgroup within BRAF-like PTC associated with oncogenic fusion enrichment, fewer adverse molecular features, and more favorable progression-free outcomes. External expression data support the biologic validity of TDS. These findings warrant prospective evaluation in treatment-stratified cohorts.

Declarations

Competing Interests:

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

Ethics Approval:

This study used publicly available, de-identified data from The Cancer Genome Atlas. No institutional review board approval was required.

Funding:

No funding was received for conducting this study.

Author Contributions:

DZA: Conceptualization, methodology, formal analysis, writing. AH, RJK, JJK, KJ: Critical revision for intellectual content, data interpretation. JOR, MES: Supervision, critical revision for intellectual content.

All authors reviewed and approved the final manuscript.

Acknowledgments

The results shown here are in whole or part based upon data generated by the TCGA Research Network (<https://www.cancer.gov/tcga>).

Data Availability:

All data analyzed in this study are publicly available from The Cancer Genome Atlas (<https://portal.gdc.cancer.gov>, project TCGA-THCA), the Pan-Cancer Clinical Data Resource [5], and the Gene Expression Omnibus (GSE54958, GSE58545). The analytic dataset is available from the corresponding author on reasonable request.

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Figures

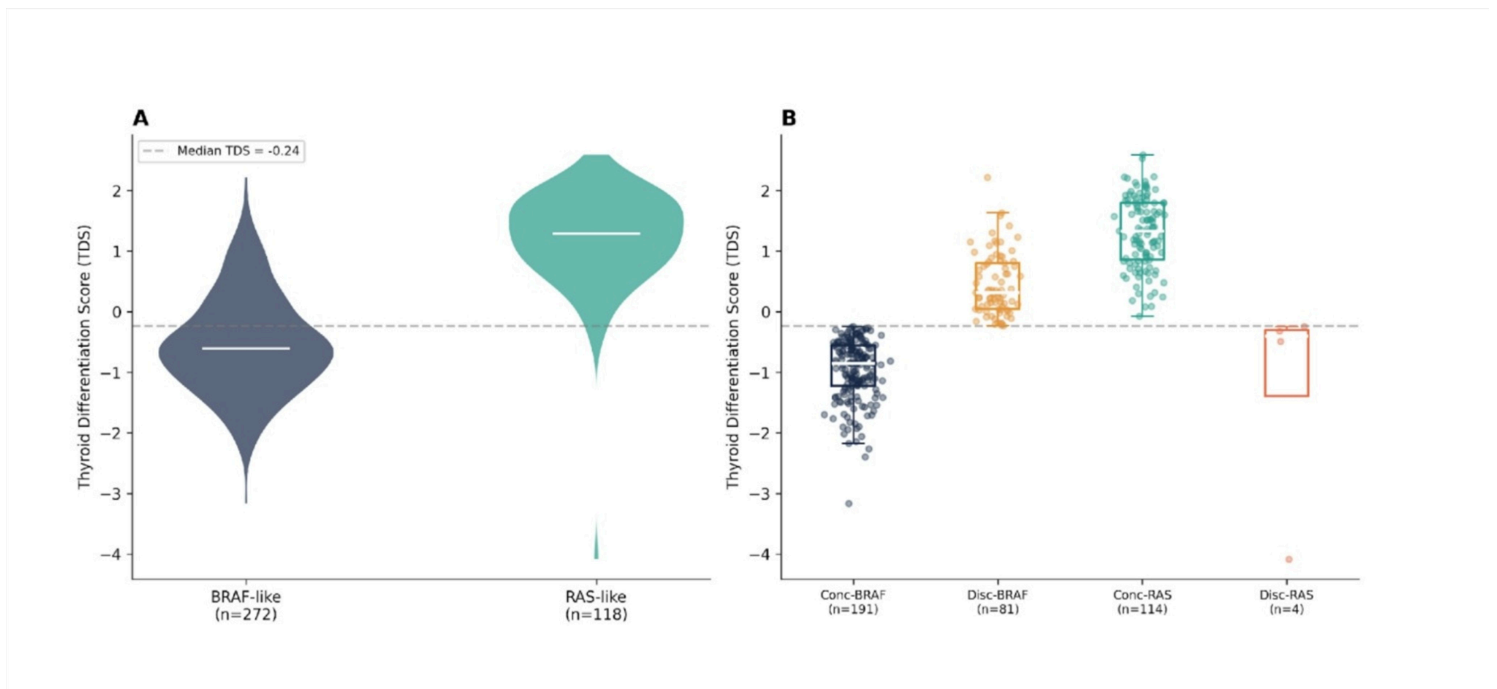


Figure 1

Variation in thyroid differentiation score between (a) BRAF-like, RAS-like PTCs and (b) concordant and discordant phenotypes

(a) Violin plot stratification of thyroid differentiation score (TDS) against tumor phenotypes classified as either RAS-like (n=118, turquoise) or BRAF-like (n=272, navy). Plots are displayed against the cohort median TDS (-0.237, grey dashed line), which serves as the threshold for the discordance classification

(b) Box plot categorization of BRAF-like and RAS-like PTCs based upon concordant or discordant phenotype and further stratified by their thyroid differentiation score. Identified phenotypes are; Concordant-BRAF PTCs (n=191, navy), Discordant-BRAF PTCs (n=81, yellow), Concordant-RAS PTCs (n=114, turquoise), and Discordant-RAS PTCs (n=4, red). As in Fig. 1a, data are displayed against the cohort median TDS (-0.237)

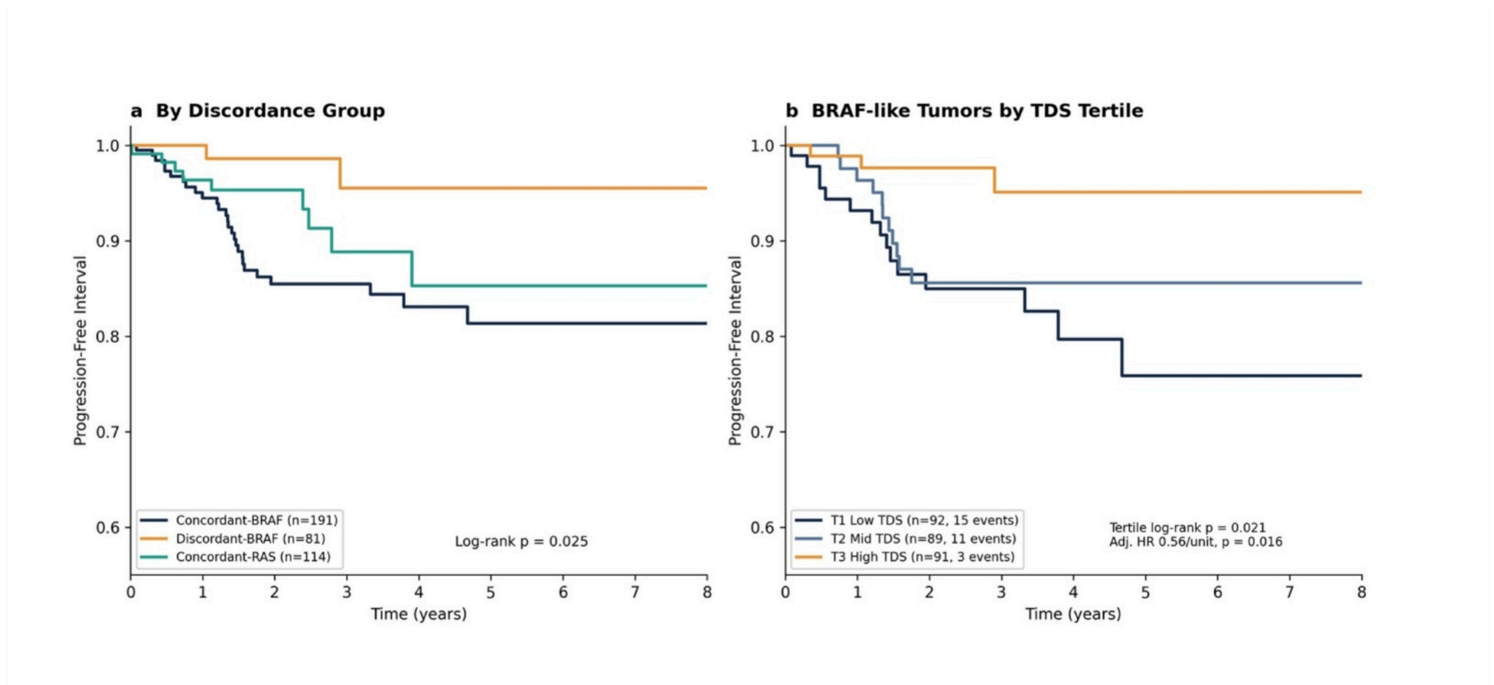


Figure 2

Progression-free interval by TDS–molecular subtype discordance group (a) and BRAF-like tumors by TDS tertile (b)

(a) Kaplan–Meier estimates of progression-free interval for Concordant-BRAF ($n = 191$, 27 events; navy), Discordant-BRAF ($n = 81$, 2 events; yellow), and Concordant-RAS ($n = 114$, 9 events; turquoise). Three-group log-rank $p = 0.025$; pairwise Concordant-BRAF vs Discordant-BRAF $p = 0.008$. Five-year PFI estimates: 95.5% Discordant-BRAF, 81.3% Concordant-BRAF, 85.3% Concordant-RAS. Shaded bands: 95% confidence intervals (Greenwood). Tick marks: censored observations

(b) Kaplan–Meier estimates by TDS tertile within BRAF-like tumors. T1 (TDS -3.163 to -0.874): 15/92 events; T2 (-0.862 to -0.321): 11/89 events; T3 (-0.309 to 2.220): 3/91 events. Log-rank $p = 0.021$. Shaded bands and tick marks as in panel (a)

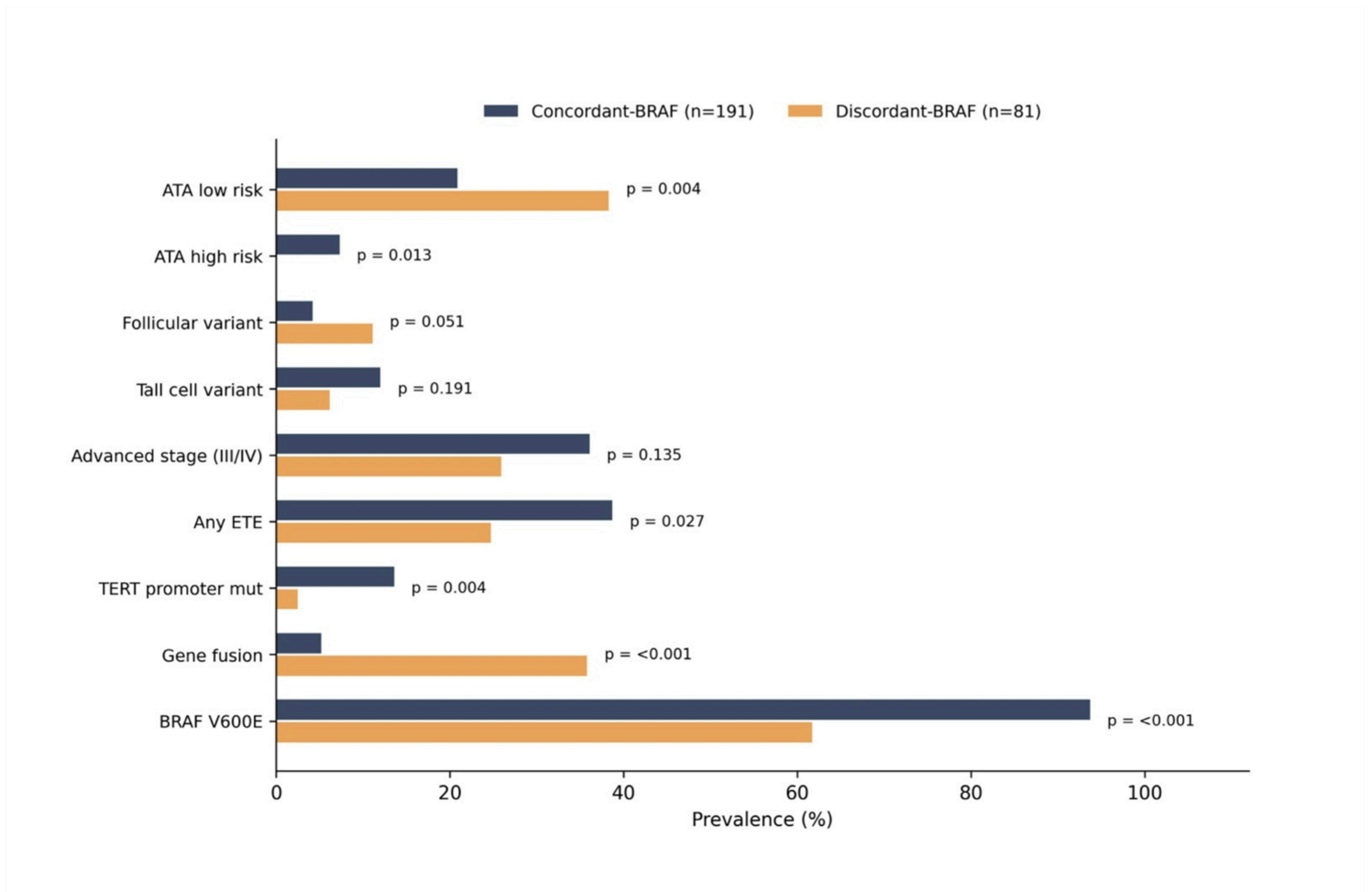


Figure 3

Concordant-BRAF and Discordant-BRAF PTCs display distinct clinicopathologic and molecular features

Bar graph comparing Concordant-BRAF (n=191; navy) and Discordant-BRAF (n=81; yellow) on clinicopathologic and molecular features, derived from data in Table 1. Words were abbreviated as follows for clarity; “ATA”= American Thyroid Association, “ETE”= Extrathyroidal Extension, “MUT”= mutation. Statistical comparisons between TDS subtypes were pairwise in Concordant-BRAF and Discordant-BRAF and were assessed using Fisher exact tests.

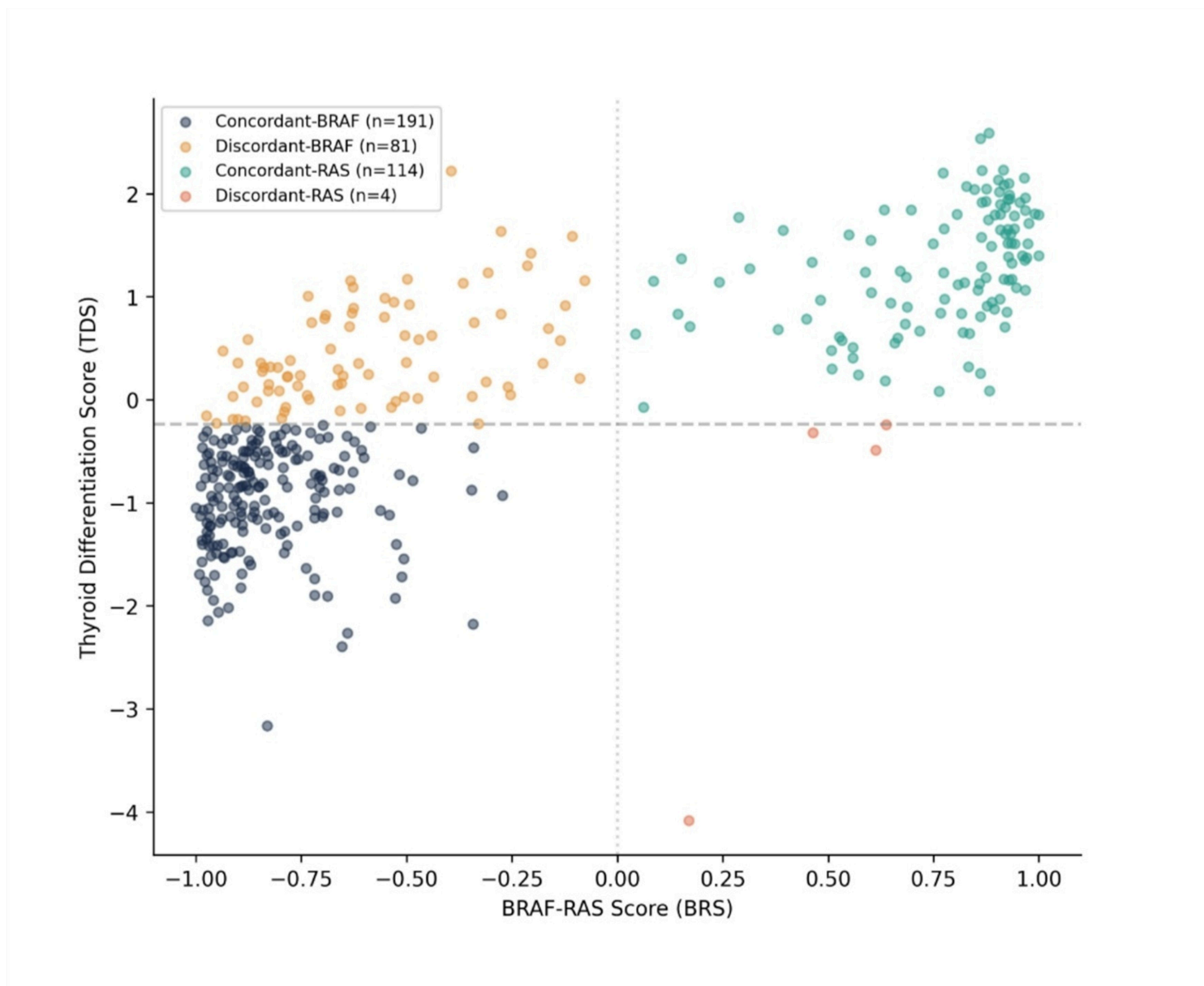


Figure 4

Papillary thyroid cancer reveals a spectrum of distinct concordant and discordant subgroups when stratified by BRAF-RAS score and thyroid differentiation score

Clustered scatterplot depicting relationship between Thyroid Differentiation Score (TDS) and BRAF-RAS Score (BRS). Each point represents $n=1$. Quadrant lines are marked with a vertical line at $BRS = 0$ and horizontal line at the median $TDS = -0.237$ to create four distinct zones; with top left as Discordant-BRAF ($n=81$, yellow), bottom left as Concordant-BRAF ($n=191$, navy), top right as Concordant-RAS ($n=114$, turquoise), and bottom right as Discordant-RAS ($n=4$, red)

Supplementary Files

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