





# Risk Assessment for Distant Metastasis in Differentiated Thyroid Cancer Using Molecular Profiling: A Matched Case-Control Study

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**BACKGROUND:** Risk stratification for patients with differentiated thyroid cancer (DTC) is based primarily on pathologic tumor characteristics. Accurate preoperative prognostication could allow for more informed initial surgical recommendations, particularly among patients at a higher risk for distant metastasis (DM). The objective of this study was to characterize the genetic profile of DTC with DM and to validate a molecular-based risk stratification. **METHODS:** A case-control study design was used to analyze patients who had DTC with DM ( $n = 62$ ) and a propensity matched cohort of patients who had DTC without DM after at least 5 years of follow-up using the ThyroSeq version 3 targeted next-generation sequencing assay. The results were classified into high-risk, intermediate-risk, and low-risk of aggressive disease. **RESULTS:** Most patients who had DTC with DM (66%) had a late-hit mutation in *TERT*, *TP53*, or *PIK3CA*. After propensity matching by age, tumor size, and sex, the high-risk molecular profile had strong association with DM (high-risk vs intermediate-risk: odds ratio, 25.1; 95% CI, 3.07-204.4;  $P < .001$ ; high-risk vs low-risk: odds ratio, 122.5; 95% CI, 14.5-1038.4;  $P < .001$ ). Overall, molecular risk categories were associated with DM risk, with a concordance index of 0.836 (95% CI, 0.759-0.913), which remained consistent after internal validation. Within the range of 5% to 10% of DM observed in DTC, the expected probability of DM would be 0.2% to 0.4% for the low-risk molecular profile, 4.7% to 9.4% for the intermediate-risk molecular profile, and 19.3% to 33.5% for the high-risk molecular profile. **CONCLUSIONS:** In this matched case-control study, genetic profiling using an available molecular assay provided accurate and robust risk stratification for DM in patients with DTC. The availability of preoperative prognostication may allow tailoring treatment for patients with DTC. *Cancer* 2021;127:1779-1787. © 2021 The Authors. *Cancer* published by Wiley Periodicals LLC on behalf of American Cancer Society. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

**KEYWORDS:** distant metastasis, molecular genetics, molecular profile, thyroid cancer.

## INTRODUCTION

More than 50,000 new cases of differentiated thyroid cancer (DTC) are diagnosed yearly in the United States, but most new cases will not result in disease-specific mortality.<sup>1</sup> Current management guidelines use algorithms that allow for treatment and surveillance de-escalation; however, such guidelines rely on accurate identification of the 5% to 10% of cases that can be associated with aggressive disease, either at presentation or diagnosed at subsequent follow-up.<sup>2,3</sup> Histologic features are prognostically useful. However, if risk assessment of disease aggressiveness could occur preoperatively, then clinicians could provide precise recommendations for the appropriate extent of initial surgery while triaging selected patients to active surveillance protocols.<sup>4</sup>

It is known that the initiation of thyroid cancer involves several early or primary driver mutations, of which the most common are *BRAF* V600E and *RAS*. *BRAF* V600E mutations typically occur in the classic or tall cell variant of papillary thyroid carcinoma (PTC); they were initially described as being associated with lymph node metastasis and recurrence but lack specificity on their own as an accurate prognostic marker.<sup>5,6</sup> Mutations in the *RAS* family of genes (including the *HRAS*, *KRAS*, and *NRAS* genes), commonly found in the follicular variant of PTC and in follicular thyroid carcinoma (FTC), are typically associated with more indolent disease.<sup>7,8</sup> A subset of thyroid cancers have fusions involving *RET*, *NTRK*, *ALK*, *BRAF*, *PPARG*, and other oncogenes as primary driver events, which in fact activate signaling pathways similar to those activated by either *BRAF* V600E or *RAS* mutations.<sup>6</sup> In Hurthle

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cell cancers, chromosomal copy number alterations are the most common finding and may represent a primary driver event.<sup>9</sup>

The development of more aggressive and dedifferentiated thyroid tumors is believed to be associated with additional late-hit driver mutations.<sup>6,10</sup> The most common and well studied of those are *TERT* promoter mutations, which have been associated with recurrence and mortality, particularly when they co-occur with a primary *BRAF* or *RAS* mutation.<sup>11-13</sup> *TERT* promoter mutations may be a prognostic marker even among patients with thyroid cancer who are considered to already have disease at high risk for recurrence according to American Thyroid Association (ATA) stratification.<sup>13</sup> Additional late mutations that may also serve as prognostic markers include mutations in *TP53* and genes in the PI3K/AKT/mTOR pathway, such as *PIK3CA* and *AKT1*.<sup>14-16</sup> More recently, *PLEKHS1* promoter mutations have also been identified with distant metastasis (DM) in some thyroid cancers.<sup>17</sup> However, many molecular studies have included poorly differentiated or anaplastic cancers, which by definition are aggressive histologic variants, and other studies have limited molecular analysis to only a few known genetic markers of poor prognosis.<sup>18,19</sup>

Aggressive DTC is challenging to define. Although tumor recurrence in the neck is an important prognostic metric, whether recurrence is caused by tumor biology or incomplete initial treatment is sometimes unclear. However, when DTC is associated with DM, 5-year relative survival decreases from 99.9% to 55%.<sup>1</sup> In addition, DTC at high risk or with known DM should be treated with initial total thyroidectomy and radioactive iodine, as recommended by current guidelines.<sup>2,3</sup> The objective of our current study was to characterize the genetic profile of DTC associated with DM and validate the molecular-based risk stratification of DTC using a matched case-control study design.

## MATERIALS AND METHODS

### *Patient Cohorts*

After we obtained Institutional Review Board approval, unselected patients with DTC and DM (cases) who were seen for multidisciplinary follow-up after January 2008 at a single institution were identified, and clinical, radiographic, and pathologic data were retrieved from existing medical records. Histopathology slides were reviewed to confirm a diagnosis of metastatic DTC. All patients who had poorly differentiated or anaplastic thyroid cancer

were excluded. Patients underwent thyroidectomy with or without lymphadenectomy and received radioactive iodine ablation according to standard management algorithms.<sup>2</sup> DM required pathologic confirmation and/or characteristic extracervical avidity on a whole-body scan after the administration of iodine-131. For the matched cohort (controls), consecutive patients were identified as those who had DTC and  $\geq 5$  years of follow-up without evidence of DM.

### *Molecular Analysis and Risk Groups*

Nucleic acids were extracted from formalin-fixed, paraffin-embedded tissues obtained from surgically excised tumor samples and were tested using the ThyroSeq version 3 (TSv3) targeted next-generation sequencing assay (University of Pittsburgh Medical Center), as previously described.<sup>20</sup> The test analyzes 112 genes for point mutations, fusions, DNA copy number alterations (CNAs), and gene expression alterations of either *BRAF* V600E type or *RAS* type. Detected molecular alterations were classified into 5 groups (high-risk, *BRAF*-like, *RAS*-like, CNAs, and gene expression alterations), as reported by Steward et al,<sup>21</sup> and were further categorized into low-risk, intermediate-risk, and high-risk of aggressive disease molecular risk groups (MRGs). The risk stratification was based on associations between molecular alterations and the probability of disease aggressiveness reported by us and others.<sup>2,5,7,13,22</sup> Specifically, the low-risk MRG included *RAS* and *RAS*-like alterations present as the only event. The intermediate-risk MRG included *BRAF* V600E, other *BRAF*-like alterations, and CNA. The high-risk profile included the presence of an early mutation and a late-hit mutation, including *TERT*, *TP53*, *AKT1*, and *PIK3CA*.<sup>21</sup>

### *Statistical Analysis*

Propensity matching was used for the case-control analysis and was based on age at diagnosis, tumor size, and sex, 3 factors known preoperatively that influence thyroid cancer prognosis. Ten caliper-matched (ie, with similar variables) controls (DTC without DM) were selected at random, from which 1 match was selected using the minimum Mahalanobis distance. The process was repeated until each case had a propensity-matched control. For each individual risk factor, odds ratios were computed with 95% CIs. A multivariate logistic regression model for the risk of DM was constructed using Akaike's information criteria for variable selection. Candidate variables included age, sex, nodule size, individual molecular alteration, and the MRG. The

**TABLE 1.** Patient Demographics and Molecular Characteristics of Differentiated Thyroid Cancer With Distant Metastasis

Variable	No. (%)			P
	All	Synchronous Metastasis	Metachronous Metastasis	
No. of patients	62 (100)	33 (53)	29 (47)	
Age at initial surgery: Mean $\pm$ SD, y	60.5 $\pm$ 16.1	62.2 $\pm$ 16.3	58.7 $\pm$ 15.9	.4
No. of men	34 (55)	22 (67)	12 (41)	.07
Cancer type				
Papillary	54 (87)	29 (88)	25 (86)	1.0
Follicular	2 (3)	2 (6)	0 (0)	
Oncocytic	6 (10)	2 (6)	4 (14)	
Tumor size: Mean $\pm$ SD, cm	3.6 $\pm$ 2.4	3.4 $\pm$ 2.5	3.9 $\pm$ 2.2	.4
Metastatic location				
Bone	8 (13)	5 (15)	3 (10)	.5
Lung	39 (63)	22 (67)	17 (59)	
>1	12 (19)	4 (12)	8 (28)	
Other	3 (5)	2 (6)	1 (3)	
Survival: Median [IQR], mo	92 [82]	77 [31]	151 [160]	—
No. of deaths	31 (50)	18 (55)	13 (45)	.6
Primary molecular alteration				.8
<i>BRAF</i> V600E	27 (44)	16 (48)	11 (38)	
<i>RAS</i>	8 (13)	5 (15)	3 (10)	
<i>NTRK</i> fusion	5 (8)	2 (6)	3 (10)	
<i>RET</i> fusion	7 (11)	4 (12)	3 (10)	
Copy number alterations	12 (19)	4 (12)	8 (28)	
Other	3 (5)	2 (6)	1 (3)	
Late secondary mutation	41 (66)	21 (64)	20 (69)	.8
Molecular risk group				
Low	1 (1.6)	0 (0)	1 (3)	.9
Intermediate	20 (32)	11 (33)	8 (28)	
High	41 (66)	21 (64)	20 (69)	

Abbreviation: IQR, interquartile range

final model was presented as the predicted probability of DM and summarized using the concordance index (C-index) to quantify the area under the receiver operating characteristic curve and  $R^2$  to assess the proportion of variation explained by the regression model. Variable selection and the C-index were internally cross-validated with 200 bootstrap samples. To check model calibration, we used bootstrap resampling of another 200 samples to calculate the bias and corrected mean absolute error of prediction. By using the Bayes theorem, the posterior probability of DM was computed to identify the observed proportions of patients with DM in each of the 3 MRG. Predicted probabilities were then computed for selected prevalence scenarios from 0.0 to 0.2 to identify the estimated population-level proportion of patients with DM according to the MRG.

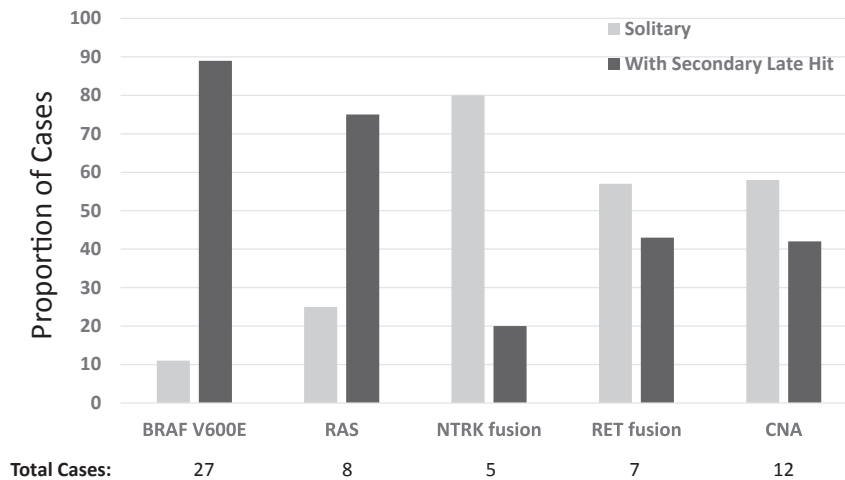
## RESULTS

### Molecular Profiles of DTC With DM

In total, 62 patients who had DTC with DM were identified and, within this cohort, the mean age was  $60.5 \pm 16.1$  years, most patients were men (55%), and the majority

had papillary thyroid cancer (PTC) (87%) (Table 1). Synchronous metastasis (DM diagnosed within 6 months of initial diagnosis) was identified in 53% of patients, and the remaining patients had metachronous metastasis diagnosed at a median of 35 months (interquartile range, 103 months). There were no significant differences in age at diagnosis, sex, histologic thyroid cancer type, mean tumor size, or location of metastasis between patients with synchronous versus metachronous metastasis (Table 1). Median survival was longer for patients who had metachronous metastasis (151 vs 77 months).

The most common primary molecular alteration in these tumors was *BRAF* V600E, which was identified in 44% of DTCs with DM (Table 1). CNAs and *RAS* mutations were also frequent and were identified in 19% and 13% of patients, respectively. Gene fusions were identified in 14 patients (23%), and the most common were *RET* (11%) and *NTRK* (8%) fusions. The distribution of primary molecular alterations did not differ between patients who had DTC with synchronous versus metachronous metastasis. The majority of DTC with DM (66%) had late-hit mutations in *TERT*, *TP53*, or *PIK3CA* (Table 1). Although most *BRAF* V600E-mutated and



**Figure 1.** The proportions of patients who had differentiated thyroid cancer with distant metastasis and late secondary hits are illustrated. CNA indicates copy number alterations.

**TABLE 2.** Propensity Matching of Patients Who Had Differentiated Thyroid Cancer With and Without Distant Metastasis

Prematch	DTC With DM, N = 62	DTC Without DM, N = 225	P
Age at diagnosis: Mean ± SD, y	60.5 ± 16.1	48.8 ± 15.2	<.001
Tumor size: Mean ± SD, cm	3.6 ± 2.4	2.1 ± 1.6	<.001
No. of men (%)	34 (55.0)	48 (21.0)	<.001
Postmatch	DTC With DM, N = 53	DTC Without DM, N = 55	P
Age at diagnosis: Mean ± SD, y	60.4 ± 15.8	56.7 ± 14.1	.20
Tumor size: Mean ± SD, cm	3.6 ± 2.4	3.2 ± 2.0	.34
No. of men (%)	29 (55.0)	29 (53.0)	1.00

Abbreviations: DM, distant metastasis; DTC, differentiated thyroid cancer.

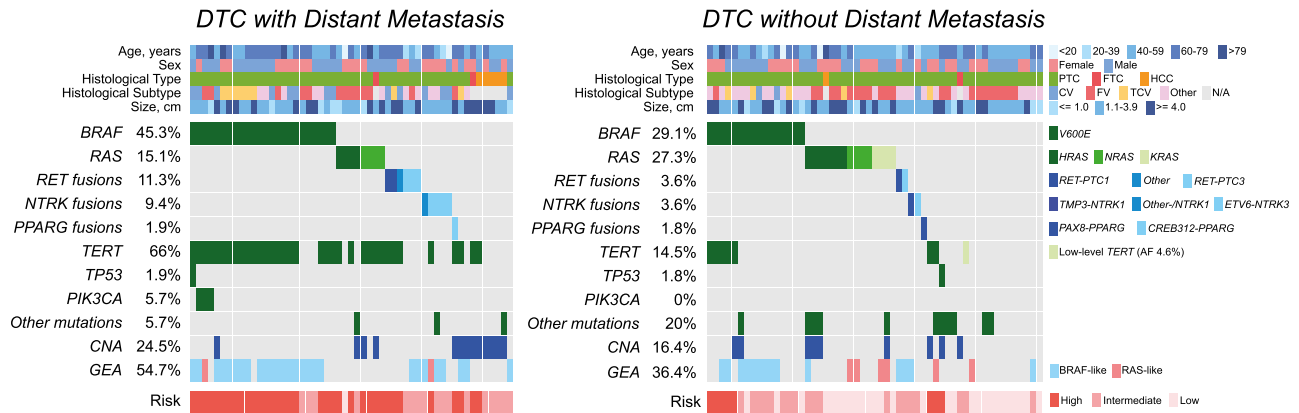
*RAS*-mutated tumors had late-hit mutations (89% and 75%, respectively), these were identified in smaller proportions of metastatic DTCs with *NTRK* (20%) and *RET* (43%) fusions (Fig. 1). Overall, 41 patients (66%) who had DTC with DM were in the high-risk MRG, 20 (32%) were in the intermediate-risk MRG, and only 1 (1.6%) had a low-risk molecular profile. The latter was a patient who had an isolated *NRAS* Q61K mutation in a 2-cm, encapsulated follicular variant PTC and iodine-avid lung metastasis.

### Results of the Matched Case-Control Study

To estimate the relative risk of DM associated with specific genetic alterations and with the MRG classification, a consecutive cohort of 225 patients with DTC who had ≥5 years of follow-up without DM were identified from a previously characterized group of patients.<sup>22</sup> Patients with DM were older at diagnosis, had larger tumors, and were more likely to be men (Table 2).

To account for this heterogeneity, 53 patients who had DTC with DM were propensity matched to 55 patients who had DTC without DM; and, after matching, the 3 factors that distinguished patients who had DTC with DM from those without DM (age, tumor size, and sex) were no longer significantly different (Table 2). The molecular profiles of the propensity-matched cohorts obtained using the TSv3 targeted next-generation sequencing assay are illustrated in Figure 2. DM was associated with the presence of *TERT* mutation (odds ratio, 11.4; 95% CI, 4.5-29.3;  $P < .001$ ) or a late-hit mutation (odds ratio, 11.4; 95% CI, 4.5-29.3;  $P < .001$ ) (Table 3).

Among the patients who had DTC with DM assessed after propensity matching to those who had DTC without DM, the high-risk MRG had a strong association with DM (odds ratio of high-risk relative to intermediate-risk MRG: 25.1; 95% CI, 3.07-204.4;  $P < .001$ ; odds ratio of high-risk relative to low-risk



**Figure 2.** Molecular profiles and features of differentiated thyroid cancer (DTC) with and without distant metastasis are illustrated in the current matched case-control cohort. CNA indicates copy number alteration; CV, classic variant; FTC, follicular thyroid carcinoma; FV, follicular variant; GEA, gene expression alteration; HCC, Hurthle cell carcinoma; N/A, not applicable; PTC, papillary thyroid carcinoma; TCV, tall cell variant.

**TABLE 3.** Distribution of Molecular Alterations in Patients With Distant Metastasis (Cases) Compared With Propensity-Matched Controls

Molecular Alteration	No. (%)		OR	95% CI	Adjusted <i>P</i>
	Cases, N = 53	Controls, N = 55			
Molecular risk group					
Low	1 (2)	28 (51)	— <sup>a</sup>		
Intermediate	17 (32)	19 (35)	— <sup>b</sup>		
High	35 (66)	8 (15)			
<i>TERT</i>	35 (66)	8 (15)	11.42	4.46-29.27	<.0001
Late secondary hits: <i>TERT</i> , <i>TP53</i> , <i>PIK3CA</i>	35 (66)	8 (15)	11.42	4.46-29.27	<.0001
Gene expression analysis	29 (55)	20 (36)	2.11	0.98-4.57	.12
<i>BRAF</i> V600E	24 (45)	16 (29)	2.02	0.91-4.46	.1454
<i>RAS</i>	8 (15)	15 (27)	0.47	0.18-1.24	.1795
<i>RET</i> fusions	6 (11)	2 (4)	3.38	0.65-17.58	.1795
<i>NTRK</i> fusions	5 (9)	2 (4)	2.76	0.51-14.90	.2416
Copy number alterations	13 (25)	9 (16)	1.66	0.64-4.29	.2914

Abbreviation: OR, odds ratio.

<sup>a</sup>The OR for the high-risk group relative to the intermediate-risk group was 25.1 (95% CI, 3.07-204.4; *P* < .001).

<sup>b</sup>The OR for the high-risk group relative to the low-risk group was 122.5 (95% CI, 14.5-1038.4; *P* < .001).

MRG: 122.5; 95% CI, 14.5-1038.4; *P* < .001). Although most patients who had DTC with DM (66%) were in the high-risk MRG, there was 1 patient in the low-risk MRG. Conversely, most patients who had DTC without DM (51%) were in the low-risk MRG, with 8 (15%) in the high-risk MRG (Fig. 2, Table 3). Of note, on longer follow-up collected after selecting the control group, 1 of the 8 patients who had DTC without DM in the high-risk MRG developed DM >8 years after initial diagnosis and thus was initially misclassified. Among the 36 patients who had DTC in the intermediate-risk MRG, 47% were associated with DM, and the other 53% were not (Table 3).

#### Estimation of Clinical Probability of DM in MRGs

The C-index demonstrated a strong correlation between MRG and DM (0.836; 95% CI, 0.759-0.913); and, after internal validation with 200 bootstrap samples, the C-index remained high (0.834), with a slight decrease in the *R*<sup>2</sup> from 0.483 to 0.469. Compared with observed values, the mean absolute error estimate from 200 bootstrap samples was 0.023, and the line of agreement between observed and bias-corrected predictive values was essentially coincident with the diagonal. These results suggest that, in a generalized population of patients with DTC, the association of the molecular-based risk stratification to DTC with DM would be

**TABLE 4.** Estimated Associated Risk for Distant Metastasis by Prevalence of Metastasis and Molecular Risk Group

Prevalence of DM, %	Molecular Risk Group, %		
	Low	Intermediate	High
1.0	0.04	0.9	4.4
5.0	0.2	4.7	19.3
10.0	0.4	9.4	33.5
15.0	0.6	14.1	44.9
20.0	0.9	18.8	53.2

Abbreviation: DM, distant metastasis.

expected to be nearly as accurate as that for the analyzed propensity-matched cohorts.

For each MRG, the predicted prevalence of DM in DTC populations with different baseline risks of DM was derived using the probability of DM in the study cohort and the expected population-based prevalence of DM in patients with DTC (Table 4, Fig. 3). Within the 5% to 10% prevalence of DM expected in most populations of patients with DTC,<sup>1</sup> the probability of DM would be from 0.2% to 0.4% in the low-risk MRG, from 4.7% to 9.4% in the intermediate-risk MRG, and from 19.3% to 33.5% in the high-risk MRG (Table 4) (Fig. 3).

## DISCUSSION

DM is an unequivocal marker of aggressive DTC, and the accurate preoperative identification of patients with at-risk disease can affect initial management recommendations. The most common risk stratification system currently used to guide patient management is the ATA risk stratification, which uses primarily histopathologic findings to predict the risk of structural recurrence and incorporates only *TERT* and *BRAF* V600E mutations as molecular risk modifiers. In the current propensity-matched case-control study, we demonstrated that a molecular-based, TSv3-derived classification, which can readily be applied to preoperative biopsy, was able to accurately stratify the risk of DM.

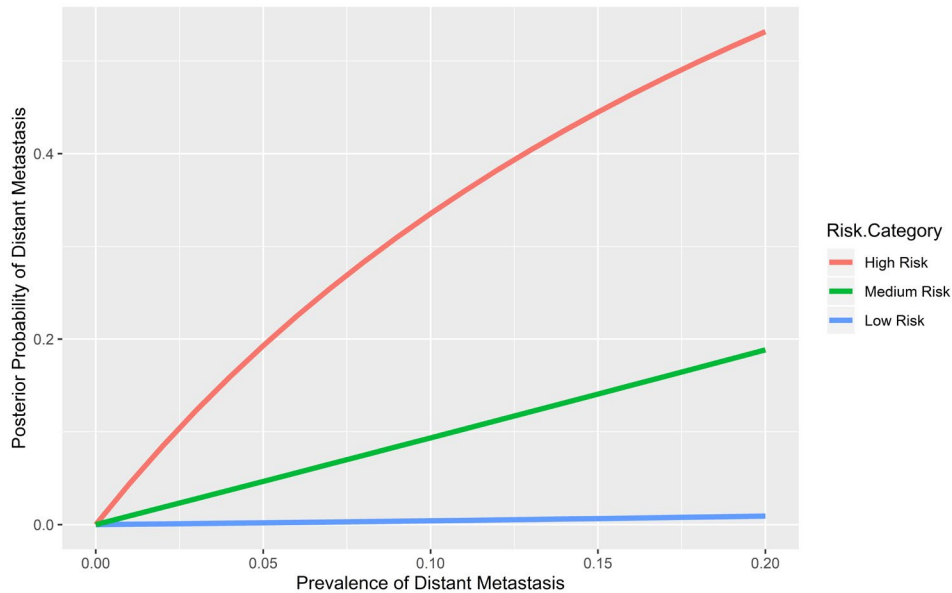
Unlike prior studies describing molecular profiles of aggressive thyroid cancer, we used a commercially available molecular testing panel that is already commonly used for further assessment of cytologically indeterminate nodules.<sup>21</sup> In a consecutive series of 1510 DTCs, we previously demonstrated the prognostic implications associated with a small subset of the genetic changes that were detected in the current study.<sup>22</sup> In short-term follow-up, disease-specific recurrence was associated with 15% of *BRAF* V600E-positive or *RET/PTC* 1 and 3

fusion-positive DTCs but was rarely seen in *RAS*-positive or *PAX8/PPARG*-positive cancers. With the current expanded panel, which included *TERT* and other markers of aggressive thyroid cancer, we were able to validate the molecular profile associated with aggressive DTC and further stratify patients into low-risk, intermediate-risk, and high-risk groups with robust discrimination (C-index, 0.836). Importantly, the probability of DM in each MRG observed in this study was similar to the risk of structural disease recurrence expected for the ATA risk groups.<sup>2</sup>

Although most of the 62 patients who had DTC with DM had either *BRAF* V600E or *RAS* as a primary driver mutation, an associated late-hit mutation, most commonly *TERT*, was also detected in 66%. In meta-analysis, concurrent *BRAF* V600E with a *TERT* mutation was associated with thyroid cancer in older patients, in men, and in cancers at advanced TNM stage (with lymph node metastasis and DM).<sup>18</sup> Even after case matching for age, sex, and tumor size, we observed the association of concurrent *TERT* mutations with DM. However, although a *TERT* mutation alone has been associated with aggressive disease, in our case cohort, we did not observe any isolated *TERT* mutations, and all cases had an additional genetic alteration, including *BRAF* V600E (n = 24), *RAS* (n = 6), and CNA (n = 11). Of note, 8 controls without DM within 5 years of diagnosis also were in the high-risk MRG; however, 1 patient with *BRAF* V600E and a *TERT* mutation developed lung metastasis, which was diagnosed >5 years after initial surgery. Therefore, continued surveillance of patients with a high-risk molecular profile would be prudent.

For tumors in the intermediate-risk MRG, the likelihood of DM had a rate between that of the low-risk and high-risk groups. There were no additional molecular features that further risk stratified this cohort, including the type of primary driver mutation. However, the exclusion of a low-risk MRG is still prognostically valuable and is information that may be used to guide the extent of surgery and surveillance. Further studies to identify clinical or histologic features that may refine risk stratification of tumors with an intermediate-risk molecular profile could be useful. In contrast, only 1 patient with DM in the current study had a low-risk molecular profile with an isolated *RAS* mutation. Analysis in an independent cohort may provide additional confirmation, but our internal validation of the MRGs demonstrated likely excellent reproducibility if applied to a generalized DTC population.

We did not propensity match by histologic subtype; however, histologic subtype is not prognostic information that is known preoperatively and thus cannot



**Figure 3.** Prevalence-adjusted predicted probability of distant metastasis associated with molecular risk group is illustrated.

be used pragmatically in preoperative decision making. Furthermore, studies over the last decade have indicated that the encapsulated follicular variant of papillary carcinoma shares more biologic and clinical characteristics with follicular carcinoma than with classic papillary carcinoma, suggesting that separating these 2 types of DTC may have diminished clinical impact.<sup>6,23,24</sup> There may be differences in the molecular mechanisms of oncocytic carcinoma compared with papillary or follicular cancer, as the former is dominated by widespread chromosomal CNA.<sup>9</sup> Indeed, in the 6 oncocytic carcinomas with DM in the current study, 5 had solitary CNA (considered intermediate-risk), whereas 1 was high-risk with concurrent *TERT* mutation and CNA. However, CNA were identified in all 3 DTC types in this study, pointing toward overlapping genetic mechanisms between Hurthle cell carcinoma and other types of DTC.

In addition to patient age, tumor size, and sex, other clinical and histologic factors have been associated with DM in DTC. In a recent National Cancer Database study, lymph node metastasis, minimal or gross extrathyroidal extension, lymphovascular invasion, tumor size, and histology were all risk factors for DM.<sup>25</sup> In another single-institution study, the incidence of lateral lymph node metastasis was higher in patients who had DM compared with those who did not (70% vs 52%).<sup>26</sup> Although lymph node metastasis can be a marker of aggressive disease, in thyroid cancer, its diagnosis often occurs after histologic evaluation, and the presence of lymph node

metastasis lacks specificity for aggressive biologic behavior. For example, the most recent 8th edition of the American Joint Committee on Cancer staging manual downstaged patients with lymph node disease compared with the previous 7th edition<sup>27</sup>; and, in a single-institution, multivariable analysis comparing the 2 editions, the presence of either central or lateral lymph node metastasis indeed was *not* associated with diminished disease-specific survival.<sup>28</sup> Therefore, because lymph node metastases are often diagnosed definitively only after histologic assessment and data on the prognostic impact of lymph node disease in thyroid cancer vary, the presence of lymph node metastasis was not included as a variable in the propensity matching. Future studies to assess whether lymph node status may be of additive value to molecular risk stratification would be important.

The absence of DM after 5 years of follow-up was the inclusion criteria for the matched cohort, yet DM can be identified >10 years after initial diagnosis. The availability and integrity of tissue for molecular testing from original histology >10 years after diagnosis in large part led to this constraint. This limitation likely led to potential misclassification of some metastatic DTCs as control, nonmetastatic cases. Indeed, we observed that 1 of the 8 patients who had DTC with a high-risk molecular profile in the *no-DM* group developed lung metastasis on longer follow-up. Therefore, considering a 10-year window, the high-risk molecular profile of this misclassified case further supports the robustness of the MRG. Regardless, it

is unlikely that this limitation affected the <0.5% risk of DM associated with a low-risk molecular signature expected in DTC with a population-based prevalence of DM at 10%.

Preoperative risk stratification using the tumor's molecular profile could be 1 of the few available clinical parameters that identify DTC with aggressive biology and higher risk for developing DM, which may be useful to determine the extent of initial thyroidectomy. Although lymph node metastasis is another clinical parameter that may be associated with aggressive disease and affects the extent of surgery, it can typically be detected with standard preoperative ultrasound or, occasionally, intraoperatively.<sup>3</sup> Knowing the absolute risk of DM for each MRG may be useful to guide preoperative clinical decisions. For example, in recent Surveillance, Epidemiology, and End Results data, the frequency of DM at presentation in conventional PTC is approximately 3%.<sup>1</sup> If a high-risk profile is detected preoperatively, the DM risk increases to 10%, and initial total thyroidectomy may be a consideration. Conversely, a preoperatively detected low-risk molecular profile would be associated with an exceedingly low 0.12% risk of DM, and thyroid lobectomy would be an oncologically appropriate option. Prospective studies are ongoing to assess whether the molecular profile can accurately guide the extent of initial surgery.

In summary, the results of this matched case-control study demonstrate that the molecular profile can robustly and quite accurately stratify the risk of aggressive DTC, defined as DM. The preoperative detection of DTC at high risk for DM may help to inform the extent of initial surgical treatment and identify patients who may be candidates for more effective therapeutic trials. Furthermore, patients with low-risk DTC may be candidates for de-escalated care. We observed no clinical or molecular features that could distinguish patients with synchronous DM from those with metachronous DM, and extended surveillance would be prudent for all patients who have DTC with a high-risk molecular profile. Molecular testing can further optimize prognostication for patients with DTC and help clinicians to guide clinical management.

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#### CONFLICT OF INTEREST DISCLOSURES

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#### AUTHOR CONTRIBUTIONS

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