Supplementary Data

Results

Demographics of the whole cohort including patients with prophylactic and therapeutic LND

A total of 315 patients, including 226 women (72%) and 89 men (28%) with a mean age of 47.5 ± 15.6 years and a mean tumor size of 2.1 ± 1.3 cm were included in this analysis. Two hundred thirty seven patients (75%) underwent prophylactic CLND, and 78 patients (25%) underwent therapeutic CLND. Fifty-two patients (17%) also received therapeutic lateral neck dissection (levels II to V). Multifocality was observed in 140 (44%) cases, involvement of surgical margins in 48 (15%) cases, LVI in 50 (16%) cases, ETE in 88 (28%) cases, and CLNM in 171 (54%) cases. Two hundred sixteen (70%) tumors were positive for a $BRAF^{V600E}$ mutation. In five cases (1.5%) the results were equivocal for at least one of the molecular analyses ($BRAF^{V600E}$ mutation or miR expression); these were therefore excluded from those specific analyses. Two hundred and fifty-six cases had CVPTC (81%), 31 had FVPTC (10%) and 28 had TCVPTC (9%) (Supplementary Table S1).

Predictors of CLNM in patients with prophylactic and therapeutic LND including all PTC subtypes

Univariate logistic regression analyses performed on the whole cohort, including patients with therapeutic and prophylactic neck dissections, demonstrated a significant positive association between miR-146b-3p ($p = <0.01$), miR-146b-5p ($p = 0.02$), miR-221 ($p = 0.02$), miR-222 ($p = <0.01$), tumor size ($p = <0.01$), multifocality ($p = <0.01$), positive surgical margins ($p = <0.01$), LVI ($p = <0.01$), ETE ($p = <0.01$) and advanced AJCC stage ($p = <0.01$). Furthermore, both FVPTC ($p = 0.02$) and percent follicular component ($p = 0.01$) were inversely associated with the presence of CLNM. The $BRAF^{V600E}$ mutation was not found to be associated with CLNM ($p = 0.16$) in the whole patient cohort.

Multivariable logistic regression analysis of the whole cohort (Supplementary Table S2) controlling for each molecular marker, sex, age, size, multifocality, LVI, positive surgical margins, ETE and histological subtypes, found only miR-146b-3p ($p = 0.03$), multifocality ($p = <0.05$), LVI ($p = <0.01$) and ETE ($p = <0.01$) to be independent predictors of CLNM. Notably, multivariate analysis found no significant association between $BRAF^{V600E}$ mutation and CLNM ($p = 0.27$) (Supplementary Table S2).

In another multivariate logistic regression analysis in patients with predictors only available preoperatively (molecular markers, age, sex, and tumor size) miR-146b-3p ($p = <0.01$), miR-146b-5p ($p = 0.04$), miR-221 ($p = 0.03$), miR-222 ($p = 0.01$), and tumor size ≥2 cm ($p = <0.05$) were found to independently predict CLNM. There was no independent association between $BRAF^{V600E}$ mutation and CLNM ($p = 0.14$) (Supplementary Table S3).

SUPPLEMENTARY TABLE S1. PATIENT CHARACTERISTICS

Clinicopathologic and molecular characteristics	n = *315 (%)*
Female	226 (72)
Male	89 (28)
Age at diagnosis (y, mean ± *SD*)	48 ± 16
Tumor size (cm, mean ± *SD*)	2.1 ± 1.4
Histological subtype	
Classical	256 (81)
Follicular	31 (10)
Tall cell	28 (9)
Multifocality	140 (44)
Involvement of surgical margins	49 (16)
Lymphovascular invasion	50 (16)
Extrathyroidal extension	88 (28)
Lymph node metastasis	
Central	171 (54)
Lateral	53 (17)
Total number of CLN dissected	9.1 ± 7.0
Positive number of CLN dissected+	10.9 ± 7.6
Total number of LLN dissected	34.1 ± 25.4
Positive number of LLN dissected+	8.1 ± 7.9
AJCC	
Stage I	185 (59)
Stage II	11 (3)
Stage III	87 (28)
Stage IV	32 (10)
Prophylactic CLND	237 (75)
Therapeutic CLND	78 (25)
BRAF mutation	216 (70)
microRNA	*mean** (SD)
21	−0.51 (1.29)
146b-3p	−7.62 (1.95)
146b-5p	−0.06 (1.78)
204	−7.99 (1.76)
221	−2.22 (1.78)
222	0.13 (1.32)
375	−6.25 (2.18)

*Inverse dCt values relative to miR-16
+Only includes those patients with positive disease
CLND, Central Lymph Node Dissection.

Supplementary Table S2. Multivariate Analysis of Individual Molecular Marker with Post-Operative Clinicopathologic Variables in all PTCs ($N=315$)

Statistic of molecular marker				*Significance of clinicopathologic factors* (p-*value only*)								
Molecular Marker	*RR*	*95% CI*	p-*Value*	*Sex*	*Age (≥45 y)*	*Size (>2cm)*	*FVPTC*	*TCVPTC*	*Multifocality*	*LVI*	*ETE*	*Margins*
BRAF^{V600E}	1.14	0.90-1.45	0.27	0.58	0.24	0.35	0.21	0.59	0.02*	<.01*	<.01*	0.19
mir-21	1.01	0.94-1.08	0.88	0.47	0.42	0.29	0.13	0.64	0.01*	<.01*	<.01*	0.15
mir-146-3p	1.07	1.01-1.13	0.03*	0.25	0.26	0.14	0.36	0.72	0.03*	<.01*	<.01*	0.06
mir-146-5p	1.05	0.98-1.13	0.16	0.56	0.14	0.25	0.45	0.65	0.01*	<.01*	<.01*	0.13
mir-204	0.98	0.92-1.05	0.58	0.50	0.16	0.37	0.17	0.97	0.04*	<.01*	<.01*	0.13
mir-221	1.05	0.99-1.10	0.08	0.47	0.11	0.24	0.14	0.78	0.02*	<.01*	<.01*	0.18
mir-222	1.08	0.99-1.17	0.06	0.66	0.18	0.21	0.21	0.90	0.02*	<.01*	<.01*	0.25
mir-375	1.02	0.98-1.07	0.32	0.66	0.18	0.21	0.20	0.59	0.03*	<.01*	<.01*	0.09

Formula: CLNM = Molecular Marker + Sex + Age (≥45y) + Size (>2cm) + FVPTC + TCVPTC + Multifocality + LVI + ETE + Involvement of margins.

Each row represents an independent postoperative predictor model performed for CLNM. In the entire cohort, independent predictors for CLNM were miR-146-3p, multifocality, LVI and ETE.

*Statistically significant.

PTC, papillary thyroid cancer; CLNM, central lymph node metastasis; LVI, lymphovascular invasion; ETE, extrathyroidal extension.

Supplementary Table S3. Multivariate Analysis of Individual Molecular Marker with Pre-Operative Clinicopathologic Variables in all PTCs ($N=315$)

Statistic of molecular marker				*Clinicopathologic factors* (p-*value only*)		
Molecular Marker	*RR*	*95% CI*	p-*Value*	*Sex*	*Age (≥45 y)*	*Size (>2cm)*
BRAF ^{V600E}	1.20	0.94-1.53	0.14	0.72	0.39	<.01*
mir-21	1.04	0.96-1.13	0.31	0.87	0.66	<.01*
mir-146-3p	1.09	1.03-1.15	<.01*	0.70	0.56	<.01*
mir-146-5p	1.08	1.00-1.16	0.04*	0.76	0.28	<.01*
mir-204	0.96	0.90-1.02	0.22	0.76	0.27	0.01
mir-221	1.06	1.01-1.12	0.03*	0.89	0.20	<.01*
mir-222	1.11	1.03-1.21	0.01*	0.65	0.31	<.01*
mir-375	1.03	0.99-1.08	0.18	0.67	0.38	<.01*

Formula: CLNM = Molecular Marker + Sex + Age (≥45y) + Size (>2cm).

Each row represents an independent preoperative predictor model performed for CLNM. In the entire cohort, independent preoperative predictors for CLNM were miR-146-3p, miR-146-5p, miR-221, miR-222 and tumor size.

*Statistically significant.

RR, relative risk; CI, confidential interval; PTC, papillary thyroid cancer; CLNM, central lymph node metastasis; CVPTC, classical variant of PTC.