

# Molecular Markers Are Associated with Onset of Radioiodine Refractoriness in Patients with Papillary Thyroid Carcinoma

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The onset of radioiodine-refractory thyroid carcinoma (RR-TC) is a negative predictor of survival and has been linked to the presence of BRAF<sup>V600E</sup> mutations in papillary thyroid cancer. We aimed to identify further genetic alterations associated with RR-TC. **Methods:** We included 38 patients with papillary thyroid cancer who underwent radioiodine imaging and <sup>18</sup>F-FDG PET/CT after total thyroidectomy. The molecular profile was assessed by next-generation sequencing. The time to the onset of RR-TC for different genetic alterations was compared using the log-rank test. **Results:** The median onset to RR-TC was 0.7 and 19.8 mo in patients with and without, respectively, telomerase reverse transcriptase promoter mutations ( $P = 0.02$ ) and 1.7 and 19.8 mo in patients with and without, respectively, a tumor protein 53 mutation ( $P < 0.01$ ). This association was not observed for BRAF<sup>V600E</sup> mutations ( $P = 0.49$ ). **Conclusion:** Our data show a significant association between the onset of RR-TC and mutations in telomerase reverse transcriptase promoter and tumor protein 53, indicating the need for a more extensive diagnostic workup in these patients. Certain genetic changes put patients with thyroid cancer at risk of developing cancer spread that does not respond to radioiodine therapy.

**Key Words:** molecular imaging; radionuclide therapy; molecular alterations; papillary thyroid cancer; radioiodine refractoriness; TP53

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Radioactive iodine therapy has been a cornerstone in the treatment of metastatic differentiated thyroid carcinoma for decades. However, around 5% of differentiated thyroid cancers are or become refractory to radioactive iodine therapy, which is associated with a drastic reduction in overall survival (1). In these patients, the early diagnosis of radioiodine-refractory thyroid carcinoma (RR-TC) based on accurate stratification tools may facilitate the timely implementation of appropriate therapies (2).

So far, the occurrence of RR-TC has been linked to a subset of histopathologic subtypes and genetic alterations. Although the current American Thyroid Association guidelines on differentiated thyroid carcinoma and thyroid nodules do not yet advocate for the

routine use of molecular markers for outcome prediction, they acknowledge that some mutations are associated with more aggressive tumor biology: Prior studies have shown that mutations in BRAF<sup>V600E</sup>, which is the most common oncogene in papillary thyroid cancer (PTC) (1), are associated with reduced overall survival. Furthermore, a mutation of the promoter of the gene for telomerase reverse transcriptase (TERT) and coexisting BRAF<sup>V600E</sup> and TERT-promoter mutations are particularly associated with poor clinical outcomes of PTC (3).

At present, there are no reliable results regarding a correlation between TERT, tumor protein 53 (TP53) mutations, and the occurrence of RR-TC. Our aim was to assess which mutations are associated with the occurrence of RR-TC to enable improved risk stratification of PTC.

## MATERIALS AND METHODS

### Patients and Study Design

We screened our institutional database for postthyroidectomy PTC patients with available <sup>18</sup>F-FDG PET/CT and radioiodine imaging and sufficient histopathologic tissue for a thorough molecular assessment (4). There were 38 evaluable patients (18 men, 20 women) in the final cohort, with a mean age of 44.63 y (range, 13–79 y). This retrospective study was performed in accordance with the principles of the Declaration of Helsinki. The institutional review board (medical faculty, protocol 19-8842-BO) approved this study, and all subjects gave written informed consent.

### Molecular Assessment

A next-generation sequencing panel was performed by use of a previously published methodology using a 26-gene panel for solid tumors that have shown an association with outcome in patients with thyroid carcinoma (4).

The molecular analysis was performed on tissue of the primary tumor in 28 of the 38 patients, on lymph node metastases in 9 patients, and on remote metastases in 1 patient. An overview of all tested genes is provided in Supplemental Table 1 (supplemental materials are available at <http://jnm.snmjournals.org>). Mutations present in only 2 or fewer patients were not used for further analyses to avoid spurious results.

### Image Acquisition

Continuous data are presented as mean values and range. To assess the presence of iodine-positive lesions, 31 of the 38 patients (81.6%) underwent <sup>124</sup>I-PET/CT and 37 (97.4%) underwent radioactive iodine therapy or additional radioactive iodine therapy with subsequent <sup>131</sup>I whole-body scintigraphy.

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$^{124}\text{I}$ -PET/CT was performed about 20 and 120 h after the ingestion of 22.6 MBq (range, 20–39.8 MBq) of  $^{124}\text{I}$ ; the initial  $^{131}\text{I}$  whole-body scintigraphy was performed 7.3 d (range, 2–10 d) after the administration of 4.1 GBq (range, 0.35–10.0 GBq) of  $^{131}\text{I}$  pursuant to European Association of Nuclear Medicine guidelines (5).

Every patient of our cohort underwent  $^{18}\text{F}$ -FDG PET/CT at least once.  $^{18}\text{F}$ -FDG PET/CT was acquired 60.9 min (range, 51–100 min) after the administration of 278.1 MBq (range, 170–460 MBq) of  $^{18}\text{F}$ -FDG to identify and further characterize possible iodine-negative lesions (6).

### Image Analysis

The images were evaluated visually by 2 physicians aware of patient history and previous reports, as per the clinical standard. In accordance with prior publications, RR-TC was defined as the presence of at least one tumor lesion without iodine uptake on either imaging modality, tumor progression after radioactive iodine therapy within 1 y, or structural persistence of disease after radioactive iodine therapy with a cumulative activity of 22.0 GBq or more. Lesions were considered  $^{18}\text{F}$ -FDG PET-positive if they exhibited focal uptake higher than liver uptake.

### Statistical Analysis

The statistical analysis was performed using SPSS version 27.0 (IBM) for Mac (Apple). The Fisher exact test was performed to compare the occurrence of RR-TC dependent on genotype. Time from initial diagnosis until onset of radioiodine-refractory disease was compared among patients with BRAF<sup>V600E</sup> mutation versus wild type, TERT-promoter mutation versus wild type, and TP53 mutation versus wild type using the log-rank test. Results were plotted using Kaplan–Meier curves.

## RESULTS

### Patient Cohort

Twelve patients developed radioiodine-refractory disease during follow-up ( $n = 11$ ) or presented with radioiodine-refractory lesions from the initial diagnosis ( $n = 1$ ) after a mean of 4.3 y (range, 0–19.8 y). These lesions were cervical or mediastinal lymph nodes in 8 of the 12 (66.7%) patients and pulmonary metastases in 5 (41.7%). During the observation period, 37 of the 38 (97.4%) patients underwent radioactive iodine therapy or repeated radioactive iodine therapy with a cumulative activity of 9.44 GBq (range, 0.35–49.95 GBq) of  $^{131}\text{I}$ . An overview of patient characteristics is provided in Table 1.

### Next-Generation Sequencing Results

A BRAF<sup>V600E</sup> mutation was found in 24 of 38 (63.2%) tumor samples, a TERT-promoter mutation in 6 (15.8%), and a TP53 mutation in 8 (21.1%). An overview of the frequency of each respective mutation is provided in Table 2.

### Association of Time to Radioiodine-Refractory Disease and Mutation Status

The Fisher exact test revealed no significant differences in the prevalence of RR-TC for patients with a BRAF<sup>V600E</sup> mutation (8/24 patients, 33%) versus BRAF<sup>V600E</sup> wild type (3/14 patients, 21%;  $P = 0.49$ ). Similarly, the median time to RR-TC did not differ significantly among patients with a BRAF<sup>V600E</sup> mutation versus BRAF wild type (11.1 vs. 6.2 mo;  $P = 0.7$ ).

In contrast, radioiodine-refractory disease was observed more frequently in patients with a TERT-promoter mutation (4/6 patients, 67%) than in those with TERT-promoter wild type (7/32 patients, 22%;  $P < 0.05$ ). Additionally, the median time to

**TABLE 1**  
Patient Characteristics

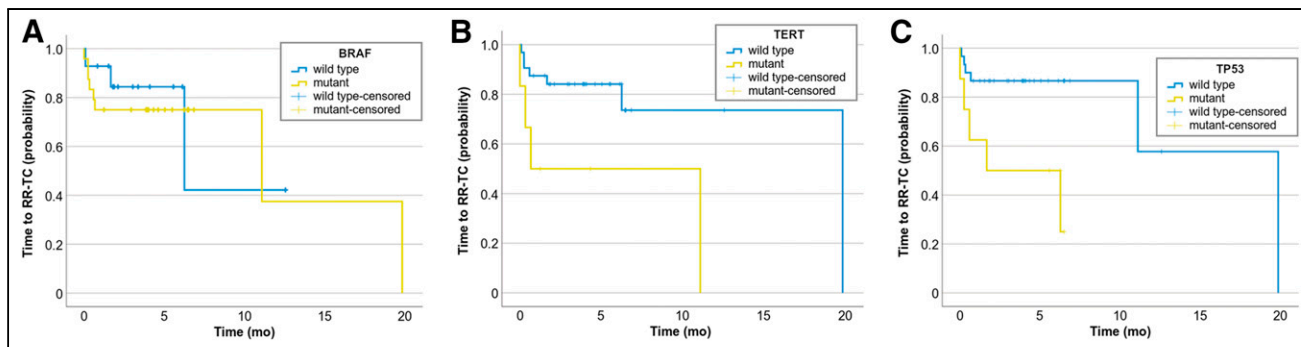
Characteristic	Data
Sex	
Male	18 (47%)
Female	20 (53%)
Mean age (y)	44.6 (13–79)
UICC stage	
I	27 (71%)
II	6 (16%)
III	1 (3%)
IV	4 (10%)
Mean $^{131}\text{I}$ activity (GBq)	9.4 (0–50.0)
Mean $^{124}\text{I}$ activity (MBq)	22.6 (0–39.8)
Mean $^{18}\text{F}$ -FDG activity (MBq)	278.1 (170–460)
Occurrence of RR-TC	
No	27 (71%)
Yes	11 (29%)
Median time to RR-TC (y)	4.3 (0–19.8)
Histopathologic subtype	
Classic	19 (50%)
FVPTC	10 (26%)
Tall cell	3 (8%)
Mixed classic/FVPTC	6 (16%)
Initial mean Tg level (ng/mL)	113.71 (<0.03–1,236)

UICC = Union for International Cancer Control; FVPTC = follicular variant of PTC; Tg = thyroglobulin.

Qualitative data are number and percentage; continuous data are mean or median and range.

**TABLE 2**  
Prevalence of Genetic Alterations in Patient Cohort

Mutation	Occurrence in 38 samples (n)
BRAF <sup>V600E</sup>	24 (63%)
TERT promotor	6 (16%)
TP53	8 (21%)
APC	2 (5%)
KRAS	0 (0%)
HRAS	0 (0%)
NRAS	0 (0%)
RET	1 (3%)
DICER	1 (3%)
PTEN	1 (3%)
PDGFA	1 (3%)
ERBB2	0 (0%)
NF1	1 (3%)
SCLC16A2	0 (0%)
MET	0 (0%)



**FIGURE 1.** Kaplan-Meier curves showing time to RR-TC based on presence of BRAF<sup>V600E</sup> (A), TERT promoter (B), and TP53 (C) mutations.

RR-TC was significantly shorter in patients with a TERT-promoter mutation than in those without (0.7 vs. 19.8 mo;  $P = 0.02$ ). In patients with a TP53 mutation, RR-TC was observed more frequently (5/8, 68%) than in those without a TP53 mutation (6/30 patients, 20%;  $P = 0.03$ ). Similarly, the presence of a TP53 mutation was associated with a shorter median time to RR-TC (1.7 vs. 19.8 mo;  $P < 0.01$ ). Figure 1 gives an overview of time to RR-TC according to genotype.

## DISCUSSION

Our results indicate an association between the occurrence of RR-TC and the presence of TERT-promoter and TP53 mutations. In our cohort, this association could not be observed for BRAF<sup>V600E</sup> mutations.

Our study contributes to a growing body of evidence for a negative prognostic impact of TP53 and TERT-promoter mutations in PTC (7,8) while being the first study (to our knowledge) to specifically address the impact of TP53 mutations on radioiodine refractoriness, the determination of which has a high impact on patient management. Early detection of radioiodine-refractory lesions enables the early implementation of multimodal approaches with the aim of optimizing tumor control, especially in the stages of limited disease burden. Our findings also confirm a nonnegligible rate of TP53 and TERT-promoter mutations in PTC.

No association between the presence of BRAF<sup>V600E</sup> mutations could be found in our cohort. On this matter, prior studies have shown that a BRAF<sup>V600E</sup> mutation not only is a driver of tumorigenesis but also is associated with a worse prognosis (7–9). The resulting transformation of the microenvironment through mitogen-activated protein kinase pathway alteration increases the risk of multifocality, lymph node metastasis, advanced TNM stage, and recurrence resulting in a more aggressive phenotype (7) in BRAF<sup>V600E</sup> mutant PTC (10).

Furthermore, there is evidence that a BRAF<sup>V600E</sup> mutation inhibits expression of the sodium iodide symporter (11), resulting in the occurrence of radioiodine refractoriness (5,12,13). Nevertheless, BRAF<sup>V600E</sup> mutation is considered a weak independent prognostic factor for risk stratification (14). Accordingly, our finding should not necessarily be seen as contradictory to the current state of research.

A strength of our study is the extensive medical imaging protocol encompassing 2 PET/CT scans, one using <sup>18</sup>F-FDG and one using <sup>124</sup>I -PET/CT in a majority of patients. The latter—while being limited in its availability—has shown advantages with

regard to the detection rate and quantification in comparison with <sup>131</sup>I whole-body scintigraphy because of its decay properties as a positron emitter (15).

Central limitations of this study include the small sample size, possible selection bias mainly due to the unavailability of tumor tissue for next-generation sequencing analyses, and varying follow-up time. Further studies are needed to validate these results.

## CONCLUSION

Our data show a significant association between the onset of RR-TC and mutations in TERT promoter and TP53, indicating the need for a more extensive diagnostic workup in these patients. Certain genetic changes put patients with thyroid cancer at risk of developing cancer spread that does not respond to radioiodine therapy.

## DISCLOSURE

Ken Herrmann reports personal fees from Bayer, Pharma15, Thernagnostics, Aktis Oncology, SIRTEX, ymabs, Novartis, Amgen, GE Healthcare, Siemens Healthineers, Adacap, Endocyte, IPSEN, and Curium; personal fees and other from Sofie Biosciences; nonfinancial support from ABX; and grants and personal fees from BTG outside the submitted work. Wolfgang Fendler reports fees from Sofie Biosciences, Janssen, Calyx, Bayer, Novartis, Telix, GE, and Eczacıbaşı Monrol outside the submitted work. Manuel Weber reports personal fees from Boston Scientific, Terumo, Advanced Accelerator Applications, IPSEN, and Eli Lilly outside the submitted work. No other potential conflict of interest relevant to this article was reported.

## KEY POINTS

**QUESTION:** Are there molecular markers that predict the occurrence of radioiodine-refractory PTC?

**PERTINENT FINDINGS:** Our study shows an association of TP53 and TERT-promoter mutations with radioiodine-refractory disease.

**IMPLICATIONS FOR PATIENT CARE:** Molecular assessment can improve individual patient workup for early detection of radioiodine-refractory lesions and guidance to appropriate therapy.

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