Association Between BRAF V600E and Redifferentiation Therapy in Patients with Radioiodine-refractory Papillary Thyroid Cancer

Tissue samples were provided by the tissue bank of the National Center for Tumor Diseases (NCT, Heidelberg, Germany) in accordance with the regulations of the tissue bank and the approval of the ethics committee of the University of Heidelberg.

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Summary

Papillary thyroid cancer (PTC) is the most common neoplasia in the thyroid gland [1,2]. The combination of surgery, followed by radioiodine therapy (RIT) and thyroid-stimulating hormone (TSH) suppressive therapy is usually a curative option for differentiated thyroid cancer (DTC). Although DTC has a good prognosis generally, it is problematic when dedifferentiation is suspected and radioiodine refractoriness presumed. One possible therapy option for redifferentiation is the pretreatment with retinoids. From 2008 to 2014 there were 13 patients with PTC who were treated with retinoids after thyroidectomy before a further course of radioiodine. A recent study has shown that the efficacy of Selumetinib, another option for redifferentiation depends on the mutational status of the treated patient. In this retrospective study *Groener and Gelen et al.* investigated whether there is a similar association between BRAF V600E and redifferentiation therapy with retinoids. As retinoids have fewer side effects compared to TKI, it is worth performing studies to assess the importance of genetic marker for the response and to estimate the chances of this specific patient collective. BRAF V600E seems to be associated with better long-term response after redifferentiation therapy with 13-cis RA in RAI-R PTC. Therefore, evaluation of BRAF mutational status prior to redifferentiation therapy could be beneficial for predicting response.

1. Participant Flow

Study design

The investigators performed a retrospective analysis studying the association between BRAF V600E mutation and the response to retinoids at Heidelberg University Hospital. DNA sequencing was performed in formalin-fixed paraffin-embedded tissue (FFPE), using commercially available kits. Tissue samples were provided by the tissue bank of the National Center for Tumor Diseases (NCT, Heidelberg, Germany) in accordance with the regulations of the tissue bank and the approval of the ethics committee of the University of Heidelberg (local ethics vote number S-206/2005).

Pre-assignment Details

Eligibility criteria for radioiodine-refractory (RAI-R) patients included:

- PTC
- no response to former RIT
- decrease or loss of initial sufficient RI-Uptake
- redifferentiation therapy with 13-cis-RA
- available FFPE tissue.

Eligibility criteria for radioiodine-sensitive (RAI-S) patients included:

- PTC
- cured after a maximum of two RIT
- no redifferentiation therapy necessary
- available FFPE tissue.

Exclusion criteria included:

- DTC other than PTC
- patients lost to follow-up
- other redifferentiation therapy than retinoids
- anaplastic or medullary thyroid cancer
- benign thyroid disease, no available FFPE tissue
- more than two RITs in the control group
- insufficient clinical information.

<u>Group Information:</u>

Although almost all (one of 13 patients could not be evaluated) PTC patients treated with 13-cis RA at the University Hospital of Heidelberg between 2008 and 2014 were included in our

study, the most important limitation is the small sample size. Different authors indicate that there is only a small group of patients with primary or secondary dedifferentiated tumors, who are palliative or have a lethal outcome due to refractoriness to standard therapy [3]. Due to this relative rarity, there are no large randomized studies including a comparable patient cohort [4]. Twelve patients with radioiodine-refractory papillary thyroid cancer (RAI-R PTC) were treated with 13-cis retinoic acid (Isotretinoin, Roaccutan®) for up to two months before another course of radioiodine-therapy was applied. Their mutational status was determined to find possible associations between BRAF V600E mutation and response to retinoids compared to BRAF wild type. Furthermore, their mutational status was compared to a radioiodine-sensitive control group of twelve PTC (RAI-S PTC) patients.

- RAI-R PTC group: Thirteen patients with radioiodine refractory papillary thyroid cancer who received redifferentiation therapy with 13-cis retinoic acid (RA), followed by a further course of iodine-131 treatment (RIT).

- RAI-S PTC group: This control group consisted of twelve patients who were considered cured after a maximum of two RIT and did not need any redifferentiation therapy.

Periods

Study was divided into three parts:

- Period one: Selection of patients with available FFPE tissue and fulfilling of eligibility criteria.
- Period two: Determination of mutational status by DNA extraction and sequencing.
- Period three: Analysis of findings. For assessment of clinical outcome of 13-cis retinoic acid treatment three parameters, tumor size, thyroglobulin levels, and radioiodine uptake were considered in a graduated model according to existing literature. One patient of RAI-R PTC group has to be excluded due to a lack of follow-up data.

2. Baseline Characteristics

Table 1 Baseline characteristic of RAI-R PTC and RAI-S PTC

Baseline characteristics	***RAI-R PTC***	***control group***
patients [numbers]	n = 12	n = 12
age at initial diagnosis [years]	57,5 (43-69)	39 (24-69)
sex [m/f] (% of total)	[6/6] (50%/50%)	[5/7] (42%/58%)
sample material [number of patients]		
▪ Primary tumor	6	9

▪ Local recurrence	1	0
▪ Nodal metastases	5	6
▪ Distant metastases	2	0
T-stage [number of patients]		
▪ Tx	0	0
▪ T1 (a and b)	1	8
▪ T2	1	0
▪ T3	8	4
▪ T4	2	0
N-stage [number of patients]		
▪ Nx	3	3
▪ N0	2	5
▪ N1 (a and b)	7	4
M-stage [number of patients]		
▪ Mx	6	6
▪ M0	4	6
▪ M1	2	0
previous RITs [number of patients]		
▪ no RIT	0	10
▪ one RIT	4	2
▪ two RIT	4	n/a
▪ >2 RIT	4	n/a
previous redifferentiation		
▪ no [number of patients]	12	12
▪ yes [number of patients]	0	n/a
▪ duration [days]	59,5 (50-99)	n/a
RIT dose (GBq)		
▪ previous therapy cumulative	13,0 (4,0-24,0)	n/a
▪ current therapy	10,5 (8,0-12,0)	n/a
▪ overall dose cumulative	24,5 (12,0-34,0)	4,0 (4,0-13,7)
TSH-stimulation prior therapy		
▪ endogenous	10	12
▪ endogenous and exogenous	2	0
Serum Tg prior RIT or redifferentiation + RIT [ng/ml]	1,5 (0,8-55980,0)	2,2 (0,8-47,3)

Notes: Tg under not stimulated TSH conditions.

Abbreviations: f female, **GBq** Gigabecquerel, **m** male, **ml** millilitre, **n** number of patients, **ng** nanogram, **n/a** not applicable, **PTC** papillary thyroid cancer, **RAI-R** radioiodine refractory, **RIT** radioiodine therapy, **Tg** thyroglobulin, **TSH** thyroid stimulating hormone

3. Outcome Measures

Primary Outcome Measures:

Response to radioiodine therapy after redifferentiation (Redifferentiation therapy was performed using 13-cis RA (Isotretinoin, Roaccutan®) with a daily dose of orally 1,5mg/kg for up to two months.

For assessment of clinical outcome of 13-cis retinoic acid treatment three parameters, tumor size, thyroglobulin levels and radioiodine uptake were considered in a graduated model.

Secondary Outcome measures

First parameter: Tumor size was evaluated form CT, MRI, or FDG-PET/CT imaging, comparing results before and after redifferentiation and RIT, results were evaluated using Response Evaluation Criteria in Solid Tumors (RECIST).

Second parameter: Non-stimulated serum Tg level (in ng/ml) before redifferentiation therapy was compared with the first Tg level after redifferentiation and RIT. A stable Tg level was defined as ≤10% difference.

Third parameter: Recovery of RI-Uptake was evaluated from the post-therapy whole body scan in comparison to the lesions in CT, MRI, or FDG-PET/CT imaging before redifferentiation. Optimal uptake was defined as intensive accumulation of radioiodine in all tumor lesions. When not all lesions accumulate radioiodine or the signal was weak it was considered as suboptimal uptake.

Statistical Analysis

Categorical variables are presented as numbers and percentages. Continuous variables are presented as arithmetic mean, median and range. Data were analyzed using IBM SPSS Statistics 22 (Ehningen, Germany). Due to the small number of patients in the subgroups, descriptive analysis was performed as described before [5-7].

4. Adverse Event Information

RAI-R PTC group: Nine (75%) patients showed side effects during the period of their redifferentiation therapy. Eight (67%) patients reported problems regarding skin and mucosa, one patient (8%) stated problems with eye dryness, conjunctivitis, and photosensitivity, one patient (8%) suffered from fatigue, and one patient (8%) from coughing.

5. Limitations and Caveats

Although almost all (one of 13 patients could not be evaluated) PTC patients treated with 13-cis RA at the University Hospital of Heidelberg between 2008 and 2014 were included in this study, the most important limitation is the small sample size. Furthermore, it must be considered that there are other therapeutic options besides retinoids, so that the sample size is getting even smaller. A further limitation is the retrospective analysis. The duration of follow-up of both groups was not comparable.

6. Certain Agreements

Yes: The principal investigator is an employee of the sponsor.

7. Results Point of Contact

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References

1. Xing M, Alzahrani AS, Carson KA, et al. Association between BRAF V600E mutation and recurrence of papillary thyroid cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2015;33(1):42-50.

2. Liu J, Dong BF, Wang PS, et al. Silencing NOB1 enhances doxorubicin antitumor activity of the papillary thyroid carcinoma in vitro and in vivo. *Oncol Rep.* 2015;33(3):1551-1559.

3. Mann K, Möller LC, Bockisch A, Quadbeck B, Schmid KW, Janssen OE. Chemotherapie beim Schilddrüsenkarzinom. *Onkologe.* 2005;11(1):78-86.

4. Hussein O, Karen D, Zidan J. Cisplatin based chemotherapy in patients with advanced differentiated thyroid carcinoma refractory to I131 treatment. *Indian J Med Paediatr Oncol.* 2013;34(4):234-237.

5. Fernandez CA, Puig-Domingo M, Lomena F, et al. Effectiveness of retinoic acid

treatment for redifferentiation of thyroid cancer in relation to recovery of radioiodine uptake. *J Endocrinol Invest.* 2009;32(3):228-233.

6. Simon D, Korber C, Krausch M, et al. Clinical impact of retinoids in redifferentiation therapy of advanced thyroid cancer: final results of a pilot study. *European journal of nuclear medicine and molecular imaging.* 2002;29(6):775-782.

7. Ho AL, Grewal RK, Leboeuf R, et al. Selumetinib-enhanced radioiodine uptake in advanced thyroid cancer. *The New England journal of medicine.* 2013;368(7):623-632.