

Anaplastic lymphoma kinase (*ALK*) gene rearrangements in radiation-related human papillary thyroid carcinoma after the Chernobyl accident

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Abstract

Childhood radiation exposure has been associated with increased papillary thyroid carcinoma (PTC) risk. The role of anaplastic lymphoma kinase (*ALK*) gene rearrangements in radiation-related PTC remains unclear, but *STRN-ALK* fusions have recently been detected in PTCs from radiation exposed persons after Chernobyl using targeted next-generation sequencing and RNA-seq. We investigated *ALK* and *RET* gene rearrangements as well as known driver point mutations in PTC tumours from 77 radiation-exposed patients (mean age at surgery 22.4 years) and PTC tumours from 19 non-exposed individuals after the Chernobyl accident. *ALK* rearrangements were detected by fluorescence *in situ* hybridisation (FISH) and confirmed with immunohistochemistry (IHC); point mutations in the *BRAF* and *RAS* genes were detected by DNA pyrosequencing. Among the 77 tumours from exposed persons, we identified 7 *ALK* rearrangements and none in the unexposed group. When combining *ALK* and *RET* rearrangements, we found 24 in the exposed (31.2%) compared to two (10.5%) in the unexposed group. Odds ratios increased significantly in a dose-dependent manner up to 6.2 (95%CI: 1.1, 34.7; $p = 0.039$) at Iodine-131 thyroid doses >500 mGy. In total, 27 cases carried point mutations of *BRAF* or *RAS* genes, yet logistic regression analysis failed to identify significant dose association. To our knowledge we are the first to describe *ALK* rearrangements in post-Chernobyl PTC samples using routine methods such as FISH and IHC. Our findings further support the hypothesis that gene rearrangements, but not oncogenic driver mutations, are associated with ionising radiation-related tumour risk. IHC may represent an effective method for *ALK*-screening in PTCs with known radiation aetiology, which is of clinical value since oncogenic *ALK* activation might represent a valuable target for small molecule inhibitors.

Keywords: Chernobyl; papillary thyroid carcinoma; ionising radiation; *ALK*; *RET*; *BRAF*

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Introduction

The risk of papillary thyroid carcinoma (PTC) has been clearly associated with a history of ionizing radiation exposure, especially at young ages [1]. The higher metabolic activity makes the gland especially vulnerable to the carcinogenic effects of radioactive

Iodine-131 during childhood and adolescence. Follow-up after the Chernobyl nuclear accident in 1986 revealed a striking increase in the incidence of childhood PTC among the population in the highly I-131 contaminated areas of Belarus and Ukraine. A strong relation between I-131 thyroid dose and the risk of PTC has been shown [2,3]. Additionally,

long-term cohort studies substantiated the strong association between the risk of PTC and young age at exposure [2,4,5].

Investigations of the molecular mechanisms possibly underlying PTC revealed mutually exclusive genetic aberrations that cause constitutive activation along the MAPK-signalling pathway [6]. Yet, the molecular profile of PTC appears also to be strongly influenced by age at diagnosis [7]. While the prevalence of point mutations in the *BRAF* and *RAS* genes found in sporadic PTC cases rises simultaneously with increasing age at diagnosis, chromosomal rearrangements of the *RET/PTC* and *TRK* genes are more common in childhood and adolescent PTC cases than in adult onset PTC [7–13].

As radiation-induced DNA double strand breaks can cause chromosomal rearrangements, it was hypothesized that PTC cases diagnosed after radiation exposure could show a high prevalence of such fusion events. Indeed, some studies of post-Chernobyl PTC cases revealed an association of *RET/PTC* and *NTRK* rearrangements with individual I-131 thyroid doses, but up to one-third of radiation-related tumours harboured none of the known mutations [14]. Efforts have continued to discover other targets, resulting in the identification of new gene rearrangements such as *PAX8/PPAR γ* , *AKAP9/BRAF*, or *TPR/NTRK1* [14–18]. Recently, rearrangements of the anaplastic lymphoma kinase (*ALK*) gene were identified in PTCs of atomic bomb survivors and individuals exposed in the Chernobyl accident, suggesting a possible role of *ALK* rearrangements in radiation-related PTC [16,19].

ALK encodes for a membrane tyrosine kinase receptor, which is physiologically expressed in fetal neuronal progenitor cells and plays a key role in cell proliferation, survival, and differentiation [17]. Aberrant non-neuronal expression of *ALK* caused by rearrangement events has been shown to drive the carcinogenesis of various malignancies, including anaplastic large cell lymphoma, a subset of non-small-cell lung cancer (NSCLC) and, more recently, aggressive forms of thyroid cancer [15,17,18]. Targeted therapy with *ALK* inhibitors like Crizotinib is in clinical use for the treatment of *ALK*-rearranged NSCLC and offers a promising therapeutic option for other *ALK*-driven tumours [20]. The diagnosis of *ALK* rearrangements is required prior to *ALK*-inhibiting therapy and can be routinely performed using immunohistochemistry (IHC) or fluorescence *in situ* hybridisation (FISH).

In this study, we screened young Ukrainian PTC patients exposed to I-131 fallout after the Chernobyl

nuclear accident for common driver mutations and gene rearrangements, including *ALK*, by DNA pyrosequencing, IHC and FISH to investigate whether a molecular radiation signature can be established using routine diagnostic techniques.

Materials and methods

DNA and tissue samples

We studied tissue samples from 100 Ukrainian patients who were diagnosed with PTC and underwent surgery between 1999 and 2013. Of these, 80 patients were exposed to I-131 fallout during the Chernobyl accident. Twenty were born after 31 March 1987 and, due to the isotope's physical half-life of approximately 8 days, were considered unexposed. Pathological diagnoses were performed by the Institute of Endocrinology and Metabolism (Kiev, Ukraine) and were reviewed by the International Pathology Panel of the Chernobyl Tissue Bank (CTB). Preparation of tissue microarrays (TMA) by arranging formalin-fixed paraffin-embedded (FFPE) archive tissue samples, and isolation of corresponding tumour DNA, were carried out by the CTB (CTB project number 002/14). We obtained a total of nine TMA slides consisting of three different TMAs containing cores of varying areas of the respective tumour.

Sample inclusion and exclusion criteria

We excluded four samples from the study because tumour tissues from three persons were not present across all analysed TMA slides and, for one person, no thyroid dose estimate was available. Pyrosequencing analysis of point mutations in *BRAF* or *RAS* genes was performed on 95 samples, because an additional sample lacked a DNA sample. FISH analysis of *RET* gene rearrangements was performed using a single TMA slide and included a total of 69 samples due to missing cores or unrepresentative tissue. Results for IHC-testing are based on the analysis of 93 PTCs, representing the number of samples present on two TMA slides stained by IHC.

One sample could not be analysed by FISH as a consequence of high background fluorescence. This sample was included in the study and regarded as *ALK*-non-rearranged due to negative IHC staining.

I-131 thyroid dose estimates

Individual thyroid doses from I-131 fallout exposure of persons diagnosed with PTC were estimated by

the Ukrainian Institute of Radiation Protection (Kiev, Ukraine) as previously described [21,22]. In brief, for each CTB donor, 1000 stochastic dose estimates were calculated while randomly changing values of uncertain parameters and the resulting arithmetic mean was used as a single thyroid dose value for statistical analysis.

Pyrosequencing

Sequence analysis of mutational hot-spot regions in *KRAS*, *NRAS*, and *BRAF* oncogenes was performed using the PyroMark Q24 platform and the In Vitro Diagnostic marked therascreen *KRAS*, *RAS* extension, and *BRAF* pyro kits according to the instructions of the manufacturer (Qiagen, Hilden, Germany). In brief, the respective genetic regions of interest were amplified using biotin-labelled primers and 10 ng of patient DNA per PCR approach. After denaturation and purification steps, the single stranded PCR products were used as templates for sequence analysis.

FISH and IHC analyses

FISH analysis was performed on TMAs using the ZytoLight[®]SPEC *ALK/EML4* TriCheck[™]Probe or the ZytoLight[®]SPEC *RET* Dual Colour Break Apart Probe. Pre-treatment, denaturation, hybridization as well as washing steps were performed as recommended by the manufacturer (Zytomed Systems, Berlin, Germany). For evaluation, at least 50 tumour cells were taken into account per core. Cases were considered positive when ≥ 15 cells showed break-apart signals.

ALK-IHC was also conducted on TMA slides applying the anti-human ALK monoclonal primary antibody clone 1A4 in a 1:100 dilution (Zytomed Systems). Heat-induced epitope retrieval was done in CC1-buffer for 72 min at 95 °C followed by incubation of anti-ALK antibody for 16 min at 37 °C. Subsequent detection was performed using the Optiview DAB IHC Detection Kit. All steps of the IHC assay were carried out on the Ventana Benchmark XT System (Ventana Medical Systems, Tucson, AZ, USA). Estimation of ALK expression was done by scoring the intensity of cytoplasmic staining in at least 10% of tumour cells assigning scores from 0 to 3+ [23].

Statistics

We examined an association of genetic mutation and rearrangement frequencies with different exposure categories among our case series. Descriptive statistics of continuous (n , mean, standard deviation,

min, max) and categorical variables (frequency distributions) were performed and corresponding P values (t -test/non-parametric rank sum tests, as well as chi-square/Fisher's exact test, where applicable) were calculated for each of the variables. Dose was categorised following this categorisation (0, >0–100, >100–500, >500 mGy) and a categorisation resulting in an even distribution (0, >0–60, >60–300, >300 mGy) of thyroid cancer samples among the dose strata. Frequency distributions of *BRAF/RAS* mutations and *ALK* and *RET* rearrangements separately and combined were examined for both types of dose categorization. Cumulative unconditional logistic regression analysis was performed on dose categories as outcome variable. Summed *BRAF/RAS* mutations and all rearrangements combined were used as explanatory variables in univariate analysis. To these models, we added potential confounders such as gender and age at exposure/surgery in order to adjust for their impact on the association of genetic alterations and dose categories. Odds ratios (ORs), 95% confidence intervals (95% CIs), and corresponding P values (Wald Chi-square) were calculated. Cox-proportional hazard regression models with tumour latency as the time scale was employed on the exposed group using the lowest dose group as the reference. Violation of the proportional hazard assumption was examined for each variable separately. We generated a time-dependent covariate by creating interactions of the predictors and a function of latency time and included them in the model. The model with combined *ALK* and *RET* rearrangements as the explanatory variable was finally adjusted for gender as a potential confounder using multivariate models.

All calculations were performed using SAS (release 9.2, Cary, NC, USA). Data were visualised using Sigma Plot (Version 13, Systat software GmbH, Erkrath, Germany).

Results

Characteristics of the PTC cases, exposure, and biological endpoints

The tumour samples were from 25 male (26%) and 71 female (74%) residents of Ukrainian regions (oblasts; Table 1). The unexposed group included 19 cases (19.8%) with a mean birth date of 5.2 years after the Chernobyl accident. All 77 exposed patients (80.2%) were younger than 18 years at the time of the accident (mean age = 5.5 years).

Table 1. Descriptive characteristics of the radiation unexposed and exposed groups diagnosed with PTC after the Chernobyl accident

Characteristic	Category	Unexposed (n = 19)		Exposed (n = 77)		P value	
		n	%	n	%		
Gender	Female	18	94.7	53	68.8	0.02	
	Male	1	5.3	24	31.2		
Oblast	Chercassy	0	0	3	3.9	0.14	
	Chernigov	1	5.3	11	14.3		
	Kiev	12	63.2	27	35.1		
	Rovno	3	15.8	8	10.4		
	Sumy	2	10.5	7	9.1		
	Zhytomyr	1	5.3	21	27.3		
Age at incidence	n	19		77		NA	
	Mean	-5.2		5.5			
	SD	4.2		5.1			
	Min	-17.5		0.1			
	Max	-0.9		17.7			
Age at surgery	n	19		77		0.46	
	Mean	21.3		22.4			
	SD	4.3		5.5			
	Min	9.4		14			
	Max	26.6		33.7			
Time between exposure and surgery	n	19		77		NA	
	Mean	NA		16.9			
	SD	NA		2.9			
	Min	NA		12			
	Max	NA		25.2			
Variable	Category (mGy)	Exposed (77)					
		n	%	Mean	SD	Min	Max
	all	77	100.0	359.1	480.8	13.2	2559.6
Dose categories	>0-100	29	37.7	41.2	19.0	13.2	95.2
	>100-500	29	37.7	243.8	106.3	103.5	477.2
	>500	19	24.6	1020.2	558.9	501.2	2559.6
	>0-60	24	31.2	34.0	10.2	13.2	58.9
	>60-300	27	35.1	172.8	73.3	62.5	288.5
	>300	26	33.7	852.5	552.2	302.4	2559.6

NA, not applicable; SD, standard deviation.

Most of our unexposed samples were from female patients (18 of 19, 94.7%) compared to 68.8% of female patients in the exposed group ($p = 0.02$; Table 1). About 80–95% of both groups originated either from the Kiev, Rovno, Sumy, or Zhytomyr oblast ($p = 0.1$) and showed a comparable age at surgery (21.3 ± 4.3 versus 22.4 ± 5.5 years in the unexposed versus the exposed group, respectively, Table 1). Surgery was performed with an average of 16.9 years after exposure (± 2.9 ; Table 1).

Individual Iodine-131-thyroid doses ranged from 13 mGy to a maximum value of 2560 mGy. The

mean exposure dose was 359 mGy (± 481 mGy; Table 1). We used a standardised categorisation (0, >0–100, >100–500, >500 mGy) and a categorization resulting in an even distribution (0, >0–60, >60–300, >300 mGy) of the exposed group members so that about 25–28% fell into one of the three dose categories (Table 1).

Among the 96 examined biopsies, we detected 7 *ALK* rearrangements (*ALK*+, break-apart or isolated red signal in FISH analysis) in the exposed group and none in the unexposed group (Figure 1 and Table 2). Detailed patient characteristics of the

Table 2. Frequency of genetic alterations based on FISH and pyrosequencing

Genetic alteration	Category	Unexposed (n = 19)		Exposed (n = 77)		P value
		n	%	n	%	
ALK rearrangement frequency (FISH)						
All	Yes	0	0.0	7	9.1	0.2
	No	19	100.0	70	90.9	
Females	Yes	0	0.0	5	9.4	0.2
	No	18	100.0	48	90.6	
Males	Yes	0	0.0	2	8.3	0.8
	No	1	100.0	22	91.7	
ALK & RET rearrangement frequencies (FISH)						
All	Yes	2	10.5	24	31.2	0.07
	No	17	89.5	53	68.8	
Females	Yes	2	11.1	16	30.2	0.1
	No	16	88.9	37	69.8	
Males	Yes	0	0.0	8	33.3	0.5
	No	1	100.0	16	66.7	
Point mutations (<i>KRAS</i> , <i>NRAS</i> , <i>BRAF</i>)	Category	Unexposed (n = 19)		Exposed (n = 76)		P value
		n	%	n	%	
All	Yes	6	31.6	21	27.6	0.7
	No	13	68.4	55	72.4	
Females	Yes	5	27.8	15	28.9	0.9
	No	13	72.2	37	71.1	
Males	Yes	1	100.0	6	25.0	0.1
	No	0	0.0	18	75.0	
RET rearrangement frequency (FISH)	Category	Unexposed (n = 18)		Exposed (n = 51)		P value
		n	%	n	%	
All	Yes	2	11.1	17	33.3	0.07
	No	16	88.9	34	66.7	
Females	Yes	2	11.1	11	33.3	0.08
	No	16	88.9	22	66.7	
Males	Yes	0	0.0	6	33.3	NA
	No	0	100.0	12	66.7	

seven *ALK* rearranged cases are illustrated in supplementary material, Table S1. The mean ages at incidence of *ALK* translocated patients (4.4 years) and of patients harbouring no *ALK* rearrangement (5.4 years) did not differ significantly ($p = 0.91$). The *ALK* rearrangement was found in about 9.4% of exposed female and 8.3% of exposed male patients. In two of the *ALK*+ cases, nuclear distribution of the *ALK* split and *EML4* probe signals in FISH analysis supported a classification as an *ALK-EML4* gene fusion (Figure 1). Among 69 examined biopsies, we detected 19 *RET* rearrangements in 33.3% of females and males of the exposed group, but only 11.1% of females of the unexposed group ($p = 0.07$ – 0.08 ; Table 2, right panel). These borderline significant differences in

the frequency distributions remained ($p = 0.07$) after combining the *ALK* and *RET* rearrangements resulting in a total number of 26 translocations with a similar distribution (30.2–33.3%) among both genders (Table 2). In 95 examined biopsies, 27 point mutations [1 × *KRAS* (Q61R), 1 × *KRAS* (G13R), 1 × *NRAS* (Q61R), 24 × *BRAF* (V600E)] were detected in about one-third of exposed and unexposed females and males ($p = 0.1$ – 0.9 ; Table 2).

Association of biological endpoints with dose

About 50% of all *BRAF/RAS* mutations were detected in the lowest dose category, and frequencies around 15–20% were observed at higher dose categories and in the unexposed group (Table 3). These differences were of borderline significance ($p = 0.07$ – 0.1 ; Table 3). *ALK* rearrangements showed a non-significant difference in frequency distribution. Almost 60% of the rearrangements were observed in the >100–500 mGy or the >60–300 mGy dose band (Table 3). *RET* rearrangements and combined rearrangements of the *ALK* and *RET* genes were statistically significant ($p = 0.047$) or of borderline significance ($p = 0.06$ – 0.1) as well as increased in frequency with increasing dose. The observed rearrangement frequencies increased about four-fold compared to the unexposed group (Table 3). These borderline significant associations remained in logistic regression analysis with adjustment for gender (but not for *RAS* mutations, data not shown) and ORs increased in a dose dependent manner, up to six-fold, and became significant ($p = 0.04$ – 0.05) after exposure >100 mGy when combining *ALK* and *RET* rearrangement frequencies (Table 4). Neither gender (adjusted or stratified) nor age at exposure or age at surgery contributed significantly to this association (data not shown). Also, these associations were consistent irrespective of the dose cut-point distributions used. Combined *ALK* and *RET* translocations showed a significant ($p = 0.009$) hazard ratio of 2.74 (95% CI: 1.29–5.83), to which gender did not contribute significantly (data not shown).

Immunohistochemistry

All FISH-positive *ALK* rearrangements were confirmed by IHC (strong homogenous *ALK* staining intensity of the tumour cells—score 2+ or 3+). Moreover, IHC staining of two FISH-negative cases highlighted aberrant expression of the *ALK* protein, eliciting a sensitivity of 100% and a specificity of 98% for the *ALK* antibody that was used in this study (clone 1A4). One of the two *ALK*-IHC+/*ALK*-FISH– cases was derived

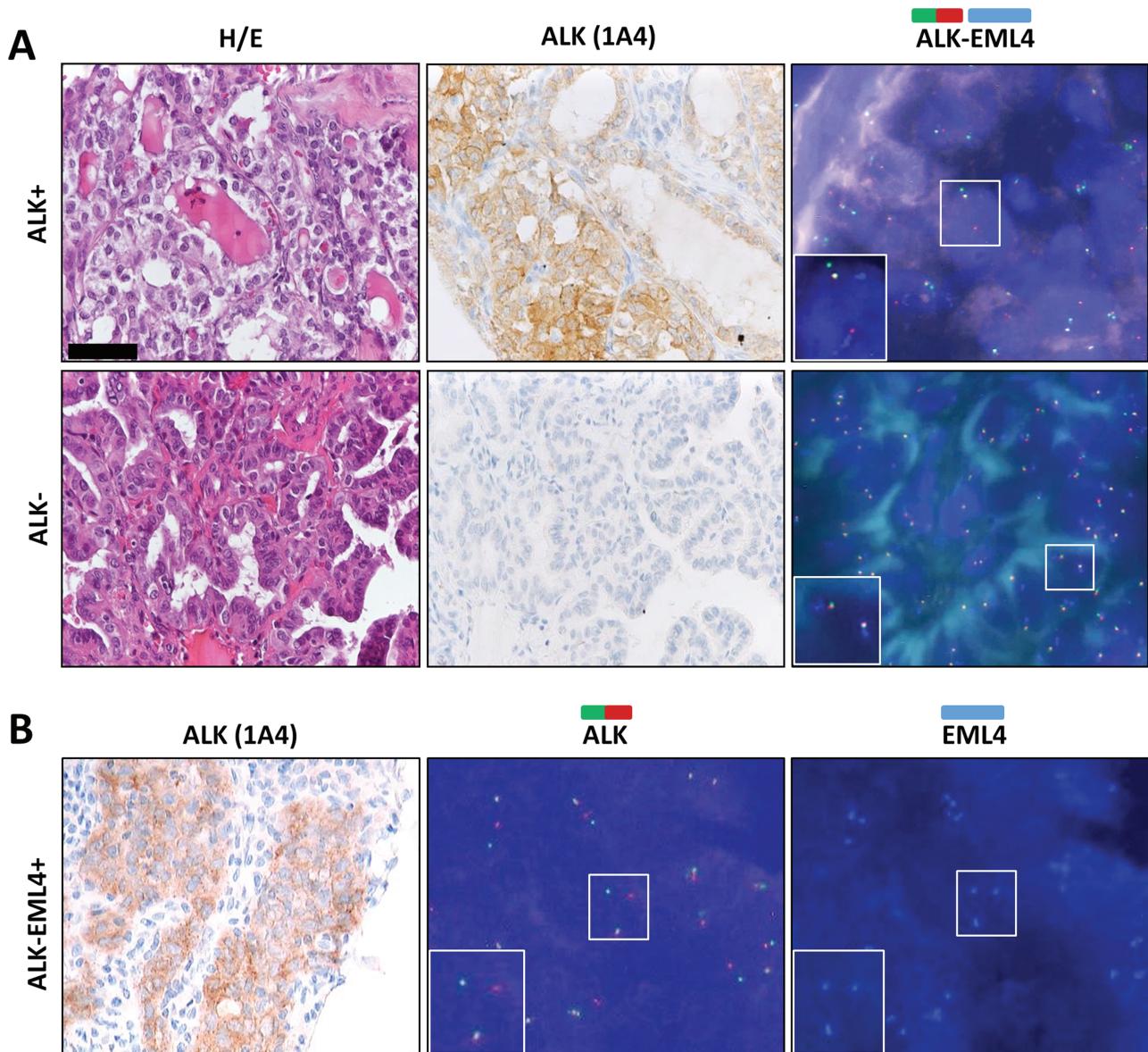


Figure 1. Detection of *ALK* rearrangements using FISH and IHC. (A) Upper panel: An *ALK* rearranged case; lower panel: an *ALK* non-rearranged case (scale bar: 50 μ m). (B) A case exhibiting a FISH signal constellation assigned specifically to an *EML4-ALK*-inversion.

from a patient who received a thyroid dose of 103 mGy and showed heterogeneous and weak staining intensity (score 1+). The second tissue sample, which originated from an unexposed patient, displayed strong but heterogeneous ALK staining of PTC cells (score 2+).

Discussion

In our study, we verified the presence of *ALK* rearrangements in a subset of post-Chernobyl patients who

developed PTC after childhood exposure to radioiodine, but not among unexposed young Ukrainian PTC patients. In combination with the results for *RET* rearrangements, our findings confirm a significant association between calculated thyroid doses and the presence of gene rearrangements in PTCs, supporting results from previous studies [14,16,19,24,25]. On the contrary, point mutations of the *BRAF* and *RAS* genes that are frequently detected among sporadic adult PTCs were not associated with dose.

Previously, the prevalence of genetic alterations in PTC has been associated with age at diagnosis or

Table 3. Frequency distribution of mutations and gene rearrangements by dose categories

Variable	Category (mGy)	All BRAF/RAS mutations				Chisq	ALK rearrangements				Chisq
		No (n = 68)		Yes (n = 27)			No (n = 89)		Yes (n = 7)		
		n	%	n	%		n	%	n	%	
Dose categories											
	0	13	19.1	6	22.2		19	21.4	0	0.0	
	>0-100	16	23.5	13	48.2		28	31.5	1	14.3	
	>100-500	24	35.3	4	14.8		25	28.1	4	57.1	
	>500	15	22.1	4	14.8	0.07	17	19.1	2	28.6	0.24
	0	13	19.1	6	22.2		19	21.4	0	0.0	
	>0-60	13	19.1	11	40.7		24	27.0	0	0.0	
	>60-300	22	32.4	4	14.8		23	25.8	4	57.1	
	>300	20	29.4	6	22.2	0.10	23	25.8	3	42.9	0.10
RET rearrangements											
Variable	Category (mGy)	RET rearrangements				Chisq	ALK and RET rearrangements				Chisq
		No (n = 50)		Yes (n = 19)			No (n = 70)		Yes (n = 26)		
		n	%	n	%		n	%	n	%	
Dose categories											
	0	16	32.0	2	10.5		17	24.3	2	7.7	
	>0-100	16	32.0	4	21.1		24	34.3	5	19.2	
	>100-500	10	20.0	7	36.8		18	25.7	11	42.3	
	>500	8	16.0	6	31.6	0.10	11	15.7	8	30.8	0.047
	0	16	32.0	2	10.5		17	24.3	2	7.7	
	>0-60	14	28.0	4	21.1		20	28.6	4	15.4	
	>60-300	9	18.0	7	36.8		16	22.9	11	42.3	
	>300	11	22.0	6	31.6	0.15	17	24.3	9	34.6	0.06

Chisq, Chi-square.

surgery. In young PTC patients, mutually exclusive recombinations of genes like *RET/PTC*, *NTRK1*, *PAX8/PPARγ*, or *AKAP9/BRAF* represent the main oncogenic drivers, whereas *BRAF* or *RAS* point mutations were found in PTC at older age at onset [7]. However, the relevance of *ALK* rearrangements for increased risk of childhood PTC remains unclear. The largest assembly of PTC cases that had been studied so far showed an overall frequency of *ALK* rearrangements of 0.8%, but that study included mainly adults with a mean age of 46 years [8]. Interestingly, a recent study revealed five *ALK* gene alterations in 65 radiation-exposed PTC cases as shown by next-generation sequencing and RNA-seq (7.7%) with younger age at surgery, supporting our hypothesis of higher frequencies of *ALK* rearrangements in PTC among children with radiation aetiology [19].

Pyrosequencing analysis of 95 DNA samples extracted from PTC tissue revealed a total of 27 *BRAF/RAS* mutations (28.4%), of which *BRAF*-V600E mutations were most frequent (n = 24, 88.8%). PTC patients with *BRAF/RAS* mutations were older at the date of surgery (p = 0.01), in line with previous studies that showed a relationship

between point mutations and age at diagnosis of PTC [8,14]. In our study, statistical analysis of a potential association between *BRAF/RAS*-positive tumours and dose failed to reach statistical significance. In summary, our findings strengthen the hypothesis that oncogenic point mutations are involved in the pathogenesis of sporadic PTC, which are frequently diagnosed at older ages, and lack a clear association with radiation-related PTC.

Table 4. Association of rearrangements (*ALK* and *RET*) with radiation dose among PTC cases after the Chernobyl accident

Model	Dose categories (mGy)				P value
	OR	95% CI			
<i>ALK</i> and <i>RET</i> rearrangements	0	1			
	>0-100	1.8	0.3	10.2	0.53
	>100-500	5.2	1.0	26.9	0.050
	>500	6.2	1.1	34.7	0.039
					0.06
<i>ALK</i> and <i>RET</i> rearrangements	0	1			
	>0-60	1.7	0.3	10.4	0.57
	>60-300	5.8	1.1	30.5	0.036
	>300	4.5	0.8	24.0	0.078
					0.08

CI, confidence interval; OR, odds ratio.

RET-FISH revealed 19 *RET* rearrangements out of 69 analysable cases (27.5%). Non-analysable/missing tissue samples were mainly from exposed individuals, so this may be an under-estimate of the effect of ionizing radiation. Nevertheless, our findings align with the expectation that *RET* gene fusions are frequently detected in sporadic and to an even higher extent in radiation-related childhood PTC [7,14,19].

Three-colour FISH analysis of PTC tissue samples revealed *ALK* rearrangements in exposed cases only. Indeed, the 7 identified *ALK*-rearranged cases (out of 77 exposed patients, 9.1%) were observed among those with individual thyroid doses from 62 mGy up to 1085 mGy, for which a borderline significant frequency distribution was seen ($p = 0.10$). Of note, in two of the FISH-positive cases, the *ALK-EML4* split/fusion signals were so distinct that we classified these cases as harbouring an *ALK-EML4* inversion. This type of rearrangement has been previously detected in aggressive *BRAF*-wild type PTC and, interestingly, the patient in that report had undergone radioiodine therapy and external beam radiation [26]. In the other cases, a distinction between *ALK-EML4* or the previously reported *ALK-STRN* rearrangement could not be verified based on FISH analysis alone [15]. This limitation is due to the relatively close proximity of the *EML4* and *STRN* genes on the short arm of chromosome 2. To assess the role of *ALK* IHC in the detection of *ALK* gene rearrangement, we also performed IHC using a commercial *ALK*-antibody (clone 1A4) which is in diagnostic use for routine *ALK* testing in lung cancer [23]. IHC staining of the *ALK* receptor tyrosine kinase confirmed the findings from *ALK*-FISH and provided no false-negative results. However, two FISH-negative cases had to be considered positive for *ALK* staining, represented by one borderline case from an exposed patient and a second clearly *ALK*-positive tissue sample from a patient lacking radiation aetiology. This may not be unexpected since a recent study demonstrated discordant FISH results for NSCLC cases with positive *ALK* IHC staining [27]. This may be due to the fact that aberrant *ALK* protein expression as shown by *ALK* IHC may not solely be ascribed to genetic alterations such as gene fusion or translocation/inversion events, but also to various pathological modifications along mutational, epigenetic, splicing, or transcriptional processes. Interestingly, beneficial *ALK* inhibitor treatment was reported in a FISH negative and IHC positive NSCLC patient, demonstrating the clinical relevance of *ALK* IHC screening [28].

Previous studies have revealed an association of *ALK* rearrangements with aggressive subtypes of thyroid cancer such as poorly differentiated thyroid carcinoma or anaplastic thyroid carcinoma [8,15,29].

Regarding the confirmed association between *ALK* rearrangements and previous exposure to ionizing radiation, our findings suggest *ALK* IHC as a suitable and cost-effective screening method for potential drug targeting of *ALK* rearrangements, particularly among PTC patients with former therapeutic or accidental radiation exposure. Even for sporadic PTC where *ALK* rearrangements have been shown to display low-frequency occurrence, *ALK* IHC testing of thyroid cancer cases which fail to respond to conventional therapy like surgery or I-131 treatment might be advisable.

Our findings support the hypothesis that radiation-related gene fusions trigger the pathogenesis of childhood PTC. In our study of PTC tissue samples after the Chernobyl accident, including a non-exposed group, we confirmed an association between rearrangements of the *ALK* and *RET* genes, but not for *BRAF* or *RAS* point mutations with thyroid gland exposure to I-131. Taking into account that *ALK*-targeted therapy is clinically available, *ALK* IHC may offer a cost-effective screening method especially among PTC patients with known radiation exposure and resistance to conventional therapy.

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Author contributions statement

AA and KS carried out data acquisition and data analysis, helped to conceive the study and helped to draft the manuscript. AR and MP helped to conceive the study and helped to draft the manuscript. MS manufactured the TMA. TB provided pathological diagnosis and marked the pathology sections to make

the TMA. LK calculated thyroid dose estimates. MA helped to draft the manuscript and carried out statistical analysis. SE helped to conceive the study, helped to carry out statistical analysis and drafted the manuscript. The final manuscript was read and approved by all authors.

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SUPPLEMENTARY MATERIAL ONLINE

Table S1. Characteristics of patients harbouring ALK rearrangements