Gilbert W. Beebe Symposium on 30 Years after the Chernobyl Accident: Current and Future Studies on Radiation Health Effects

Genetic Markers

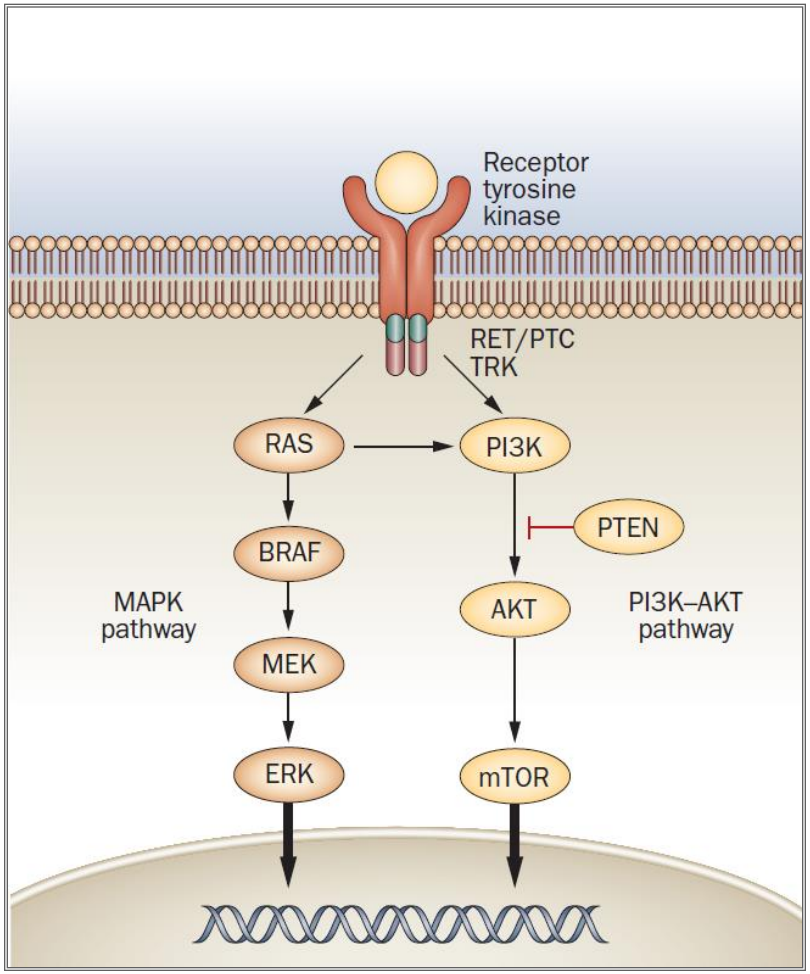
Yuri E. Nikiforov, MD, PhD
Division of Molecular & Genomic Pathology
University of Pittsburgh Medical Center
Pittsburgh, USA

Outline

- Molecular landscape of sporadic PTC
- Evolution of understanding of genetics of post-Chernobyl PTC:
  - Early studies/single gene approaches
  - NGS/genome-wide analyses

Genetic Basis of Thyroid Cancer

Major pathways involved in Thyroid Cancer Development and Progression



TCGA study of PTC (*Cell*, 2014)

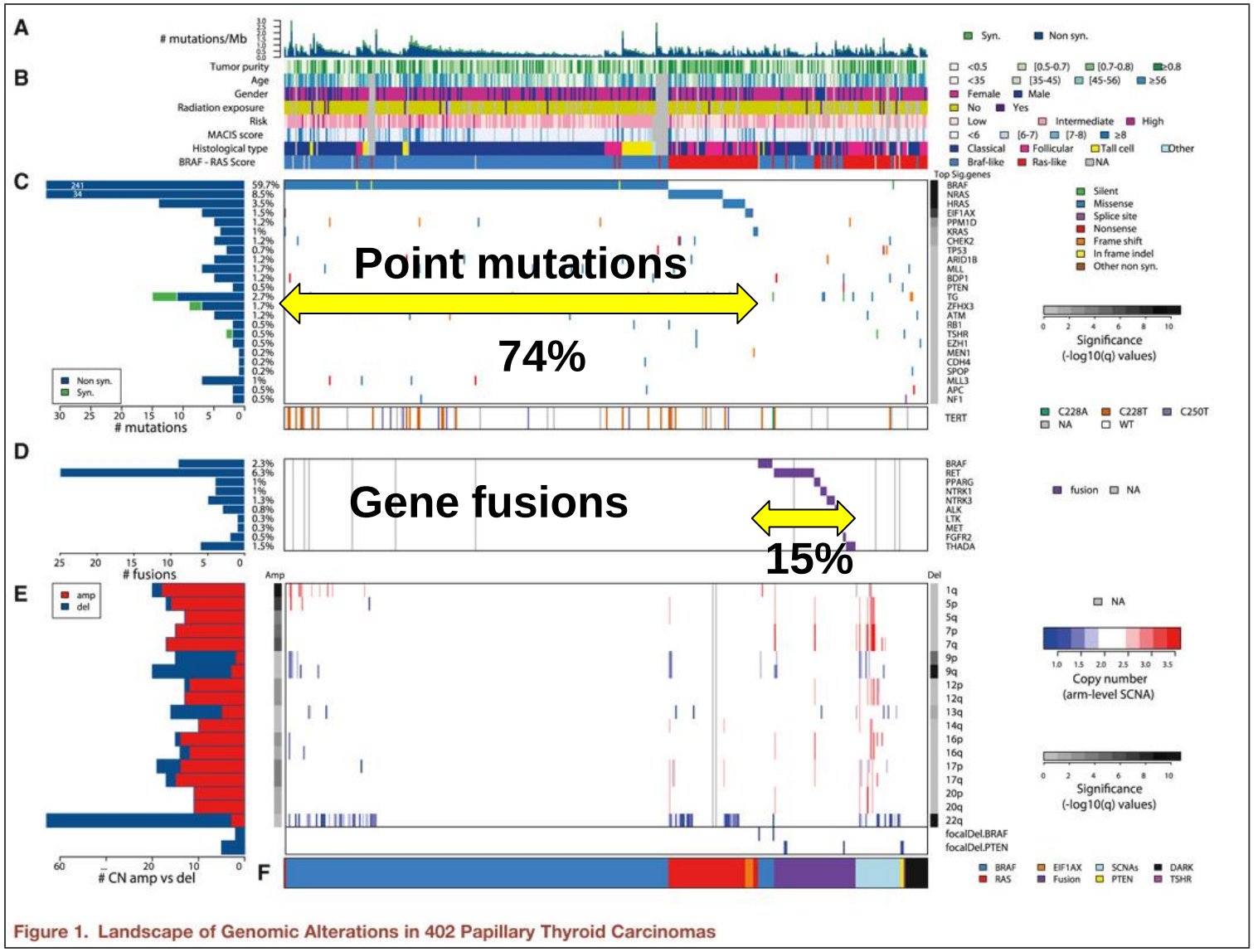


Figure 1. Landscape of Genomic Alterations in 402 Papillary Thyroid Carcinomas

Genetic Alterations in Post-Chernobyl Thyroid Cancer

Lancet. 1994 Jul 23;344(8917):259.

Activated RET oncogene in thyroid cancers of children from areas contaminated by Chernobyl accident.

Ito T, Seyama T, Iwamoto KS, Mizuno T, Tronko ND, Komissarenko IV, Cherstovoy ED, Satow Y, Takeichi N, Dohi K, et al.

Table: **RET oncogene activation in thyroid cancer in children from the contaminated area**

Case	Age		Sex	Histology	Area	RET oncogene activation
	ATA*	ATS†				
1	3 yr	8 yr	F	PC	Minsk	+
2	2 mo	5 yr	F	PC	Kiev	-
3	4 yr	8 yr	M	PC	Minsk	-
4	4 yr	10 yr	M	PC	Gomel	+
5	8 yr	14 yr	M	PC	Gomel	+
6	6 yr	12 yr	F	PC	Gomel	+
7	9 mo	7 yr	F	PC	Gomel	-

PC=papillary carcinoma. *ATA=at the time of the accident. †ATS=at the time of surgery.

Genetic Alterations in Post-Chernobyl Thyroid Cancer

[CANCER RESEARCH 55, 5617–5620, December 1, 1995]

Oncogenic Rearrangements of the *RET* Proto-Oncogene in Papillary Thyroid Carcinomas from Children Exposed to the Chernobyl Nuclear Accident[1]

Laura Fugazzola, Silvana Pilotti, Aldo Pinchera, Tatiana V. Vorontsova, Piera Mondellini, Italia Bongarzone, Angela Greco, Larisa Astakhova, Marta G. Butti, Eugene P. Demidchik, Furio Pacini, and Marco A. Pierotti[2]

Istituto di Endocrinologia, Universita' di Pisa, V.le del Tirreno 64, 56018 Tirrenia Pisa [L. F., A. P., F. P.]; Divisione di Anatomia Patologica e Citologia [S. P.] and Divisione di Oncologia Sperimentale A [P. M., I. B., A. G., M. G. B., M. A. P.], Istituto Nazionale Tumori, Via G. Venezian 1, 20133 Milano, Italy; and Institute of Radiation Medicine [T. V. V., L. A.] and Oncology Center of Thyroid [E. P. D.], Minsk, Byelorussia

RET/PTC
4/6 (67%)

Oncogene. 1995 Dec 21;11(12):2459-67.

High prevalence of RET rearrangement in thyroid tumors of children from Belarus after the Chernobyl reactor accident.

Klugbauer S[1], Lengfelder E, Demidchik EP, Rabes HM.

RET/PTC
8/12 (67%)

[CANCER RESEARCH 57, 1690–1694, May 1, 1997]

Distinct Pattern of *ret* Oncogene Rearrangements in Morphological Variants of Radiation-induced and Sporadic Thyroid Papillary Carcinomas in Children[1]

Yuri E. Nikiforov, Jon M. Rowland, Kevin E. Bove, Hector Monforte-Munoz, and James A. Fagin[2]

Division of Endocrinology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0547 [Y. E. N., J. A. F.]; Department of Pathology, Childrens Hospital Los Angeles, Los Angeles, California 90027 [J. M. R., H. M-M.]; and Department of Pathology, Childrens Hospital Medical Center, Cincinnati, Ohio 45229 [K. E. B.]

Table 3 *Prevalence of ret rearrangements in radiation-induced and sporadic pediatric thyroid tumors*

			TK positive		
	RET/PTC1	*RET/PTC2*	*RET/PTC3*	*Novel RET/PTC?*	Total RET/PTC
Radiation-induced	6 (16%)	1 (3%)	22 (58%)[a]	4 (10%)	33/38 (87%)
Sporadic	8 (47%)[b]	0	3 (18%)	1 (6%)	12/17 (71%)

[a]P = 0.01 in comparison with the prevalence of *ret/PTC3* in sporadic tumors.
[b]P < 0.05 in comparison with the prevalence of *ret/PTC1* in radiation-induced tumors.

RET/PTC
33/38 (87%)
12/17 (71%)

Genetic Alterations in Post-Chernobyl Thyroid Cancer

Vol. 6, 1093–1103, March 2000 Clinical Cancer Research **1093**

Pattern of Radiation-induced *RET* and *NTRK1* Rearrangements in 191 Post-Chernobyl Papillary Thyroid Carcinomas: Biological, Phenotypic, and Clinical Implications[1]

Hartmut M. Rabes,[2] Evgenij P. Demidchik, Juri D. Sidorow, Edmund Lengfelder, Claudia Beimfohr, Dieter Hoelzel, and Sabine Klugbauer

***ELE1/RET* is related to the solid variant of PTC, *H4/RET* more frequently to typical papillary structures. The genotype/phenotype evaluation of post-Chernobyl PTCs reveals a characteristic spectrum of gene rearrangements that lead to typical phenotypes with important biological and clinical**

Table 3 Changes in the prevalence and type of *RET* and *NTRK1* rearrangements in 191 PTCs of children after the Chernobyl reactor accident on April 26, 1986, as a function of the tumor latency period (interval between exposure and diagnosis/thyroidectomy)

		Rearrangement positive		Rearrangement negative		PTC1		PTC3		PTC5,6,7,X		*NTRK1*	
Latency period	Total	*n*	%	*n*	%	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]
Total (7–11.7 years)	191	100	52.4	91	47.6	48	48.0	38	38.0	8	8.0	6	6.0
≤10 years	61	40[b]	65.6	21[b]	34.4	9[c]	22.5	24[c]	60.0	5	12.5	2	5.0
>10 years	130	60[b]	46.2	70[b]	53.8	39[c]	65.0	14[c]	23.3	3	5.0	4	6.7

[a] Percentages from total number of rearrangement-positive PTCs in this latency group.
[b] $P = 0.012$.
[c] $P < 0.001$.

JCI The Journal of Clinical Investigation

Oncogenic *AKAP9-BRAF* fusion is a novel mechanism of MAPK pathway activation in thyroid cancer

Raffaele Ciampi,[1] Jeffrey A. Knauf,[2] Roswitha Kerler,[3] Manoj Gandhi,[1] Zhaowen Zhu,[1] Marina N. Nikiforova,[1] Hartmut M. Rabes,[3] James A. Fagin,[2] and Yuri E. Nikiforov[1]

[1]Department of Pathology and Laboratory Medicine and [2]Division of Endocrinology, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA. [3]Institute of Pathology, University of Munich, Munich, Germany.

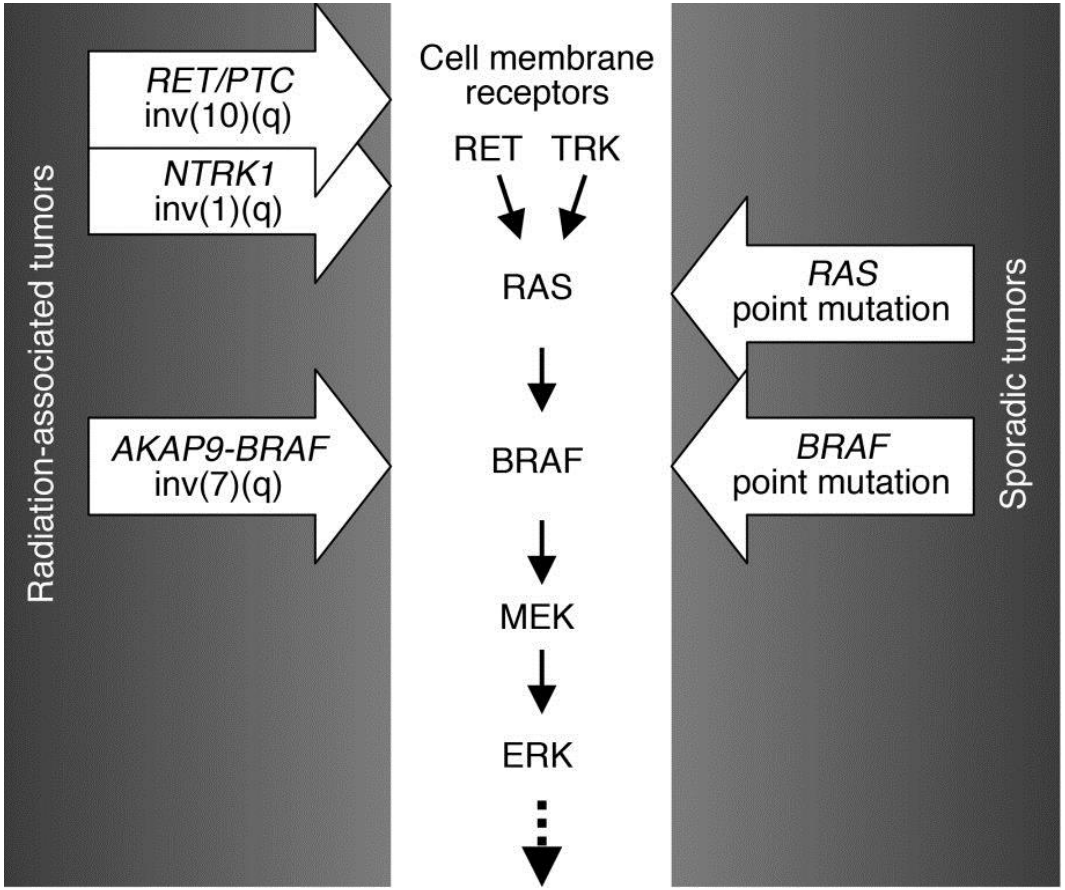
Prevalence of *BRAF* alterations in papillary thyroid carcinomas

	n	Age at surgery	Age at exposure	*AKAP9-BRAF*	*BRAF*^{V600E}
Early radiation–associated tumors (latency period 5–6 yr)	28	11.4 ± 3.6	5.0 ± 3.8	11%[A]	0[B]
Late radiation–associated tumors (latency period 9–12 yr)	64	16.0 ± 5.0	5.4 ± 5.1	0	16%
Sporadic tumors	102	40.0 ± 17.7	–	1%	37%

[A]$P = 0.03$ compared with sporadic tumors and late radiation–associated tumors. [B]$P < 0.0001$ compared with sporadic tumors; $P = 0.03$ compared with late radiation–associated tumors.

Ciampi R et al. *JCI* (2005)

Mechanisms of Mutations in Radiation-Associated and Sporadic Thyroid Cancer

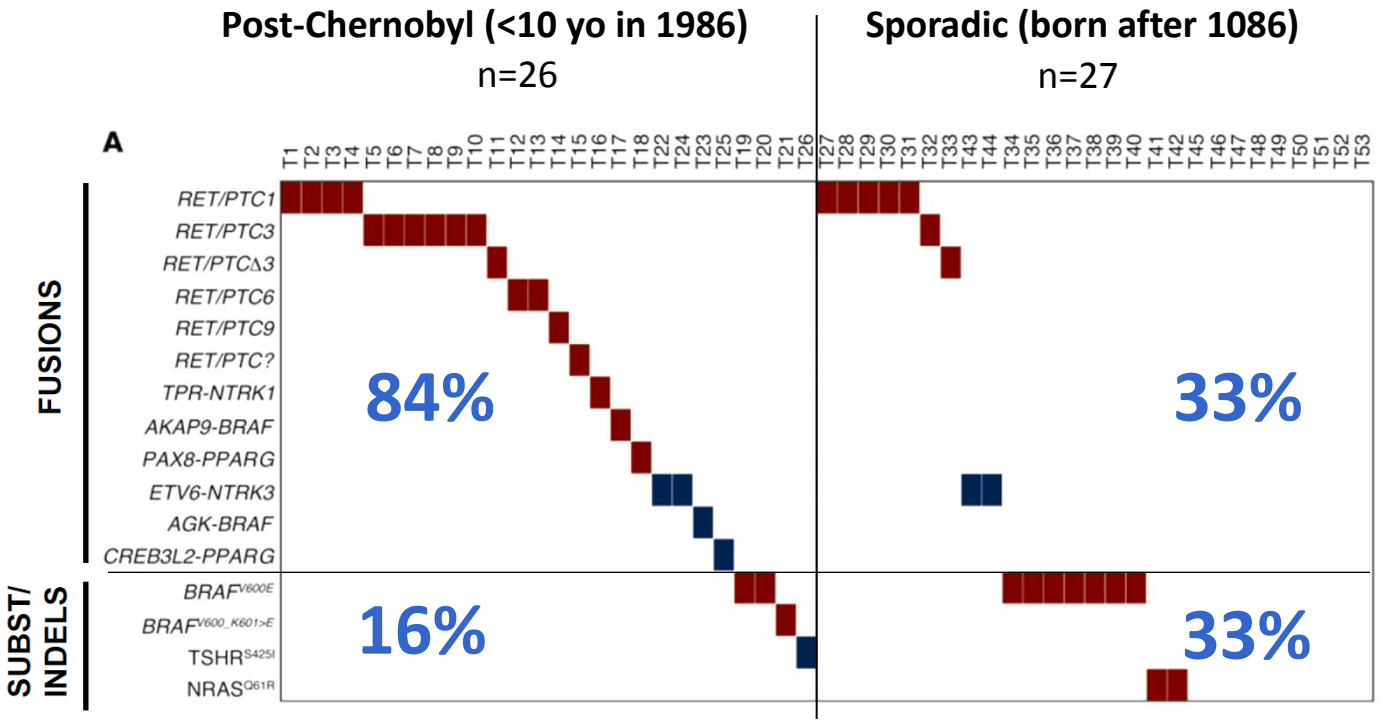


Ciampi R et al. *JCI (*2005)

Identification of kinase fusion oncogenes in post-Chernobyl radiation-induced thyroid cancers

Julio C. Ricarte-Filho,[1] Sheng Li,[2,3] Maria E.R. Garcia-Rendueles,[1] Cristina Montero-Conde,[1] Francesca Voza,[1] Jeffrey A. Knauf,[1,4] Adriana Heguy,[1] Agnes Viale,[5] Tetyana Bogdanova,[6] Geraldine A. Thomas,[7] Christopher E. Mason,[2,3] and James A. Fagin[1,4]

- Ukrainian patients from contaminated areas <10 yo in April 1986 or born after 1986
- Combination of candidate gene approach and RNA-Seq



Ricarte-Filho et al. *JCI* (2013)

Molecular Landscape and Dose Association of Thyroid Cancers in UkrAm Cohort

Institute of Endocrinology and Metabolism of AMS of Ukraine
Mykola Tronko
Tetiana Bogdanova
Liudmyla Zurnadzy
Ilya Likhtarev

National Cancer Institute USA
Alina Brenner
Mark Little
Maureen Hatch
Andre Bouville
Vladimir Drozdovich
Kiyohiko Mabuchi
Stephen Chanock

Department of Pathology University of Pittsburgh USA
Yuri Nikiforov's lab

- Patients from the Ukrainian-American cohort study
- I-131 thyroid doses reconstructed (V. Drozdovich)
- 104 were PTCs diagnosed between 1998 and 2008, diagnosis confirmed by the International Pathology Panel
- Tissue samples collected via Chernobyl Tissue Bank (CTB)
- DNA or RNA isolated at IEM (Kyiv, Ukraine) or Imperial College (London, UK).
- ~70 PTC, 62 had both DNA and RNA available; did not include individuals exposed *in utero*
- Genetic analysis: candidate gene approach, Sanger and targeted NGS, RNA-Seq

RET/PTC and *PAX8/PPARγ* Chromosomal Rearrangements in Post-Chernobyl Thyroid Cancer and Their Association With Iodine-131 Radiation Dose and Other Characteristics

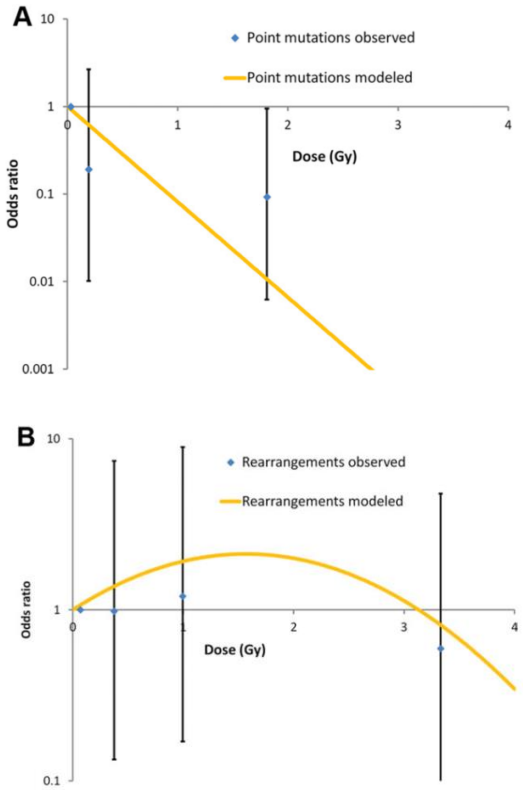
Rebecca J. Leeman-Neill, MD, PhD[1]; Alina V. Brenner, MD, PhD, MPH[2]; Mark P. Little, MA, DPhil[2]; Tetiana I. Bogdanova, PhD[3]; Maureen Hatch, PhD[2]; Liudmyla Y. Zurnadzy, MD, PhD[3]; Kiyohiko Mabuchi, MD, DrPH[2]; Mykola D. Tronko, MD, PhD[3]; and Yuri E. Nikiforov, MD, PhD[1]

- Sanger sequencing for known mutations

Genetic Alteration	Mutation Frequency	^{131}I Dose, Mean (Gy)
RET/PTC1	14 (22%)	1.04
RET/PTC3	8 (13%)	1.54
BRAF	9 (15%)	0.27
RAS[a]	5 (8%)	0.20
PAX8/PPARγ[a]	2 (3%)	0.62
No known mutation	25 (40%)	1.97
Total/overall	62 (100%)	1.27

Point Mutations

Gene Fusions

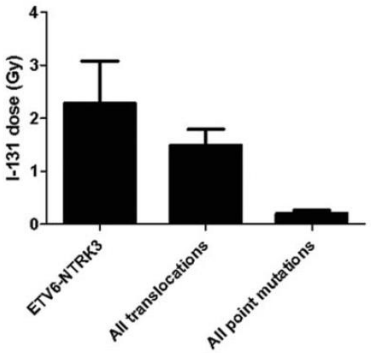


Leeman-Neill et al. *Cancer* (2013)

ETV6-NTRK3 Is a Common Chromosomal Rearrangement in Radiation-Associated Thyroid Cancer

Rebecca J. Leeman-Neill, MD, PhD[1]; Lindsey M. Kelly, BS[1]; Pengyuan Liu, PhD[2]; Alina V. Brenner, MD, PhD, MPH[3]; Mark P. Little, MA, DPhil[3]; Tetiana I. Bogdanova, MD, PhD[4]; Viktoria N. Evdokimova, PhD[1]; Maureen Hatch, PhD[3]; Liudmyla Y. Zurnadzy, MD, PhD[4]; Marina N. Nikiforova, MD[1]; Ning J. Yue, PhD[5]; Miao Zhang, PhD[5]; Kiyohiko Mabuchi, MD, DrPH[3]; Mykola D. Tronko, MD, PhD[4]; and Yuri E. Nikiforov, MD, PhD[1]

- Sanger sequencing and limited RNA-Seq
- ETV6-NTRK3 fusions



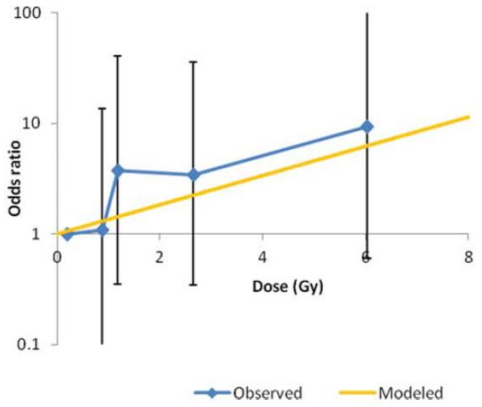


TABLE 2. Dose Response for Different Groups of Mutations in Post-Chernobyl Papillary Thyroid Cancer[a]

Genetic Alteration	Risk (95% CI), Gy^{-1}[b]	*P*
Assessment of trend		
ETV6-NTRK3	0.30 (−0.09, 0.74)	.1263[c]
Assessment of heterogeneity: Log-linear dose response		
All translocations	0.09 (−0.24, 0.46)	<.0001[d]
All point mutations: *BRAF*, *NRAS*, *HRAS*	−3.29 (−6.06, −1.38)	

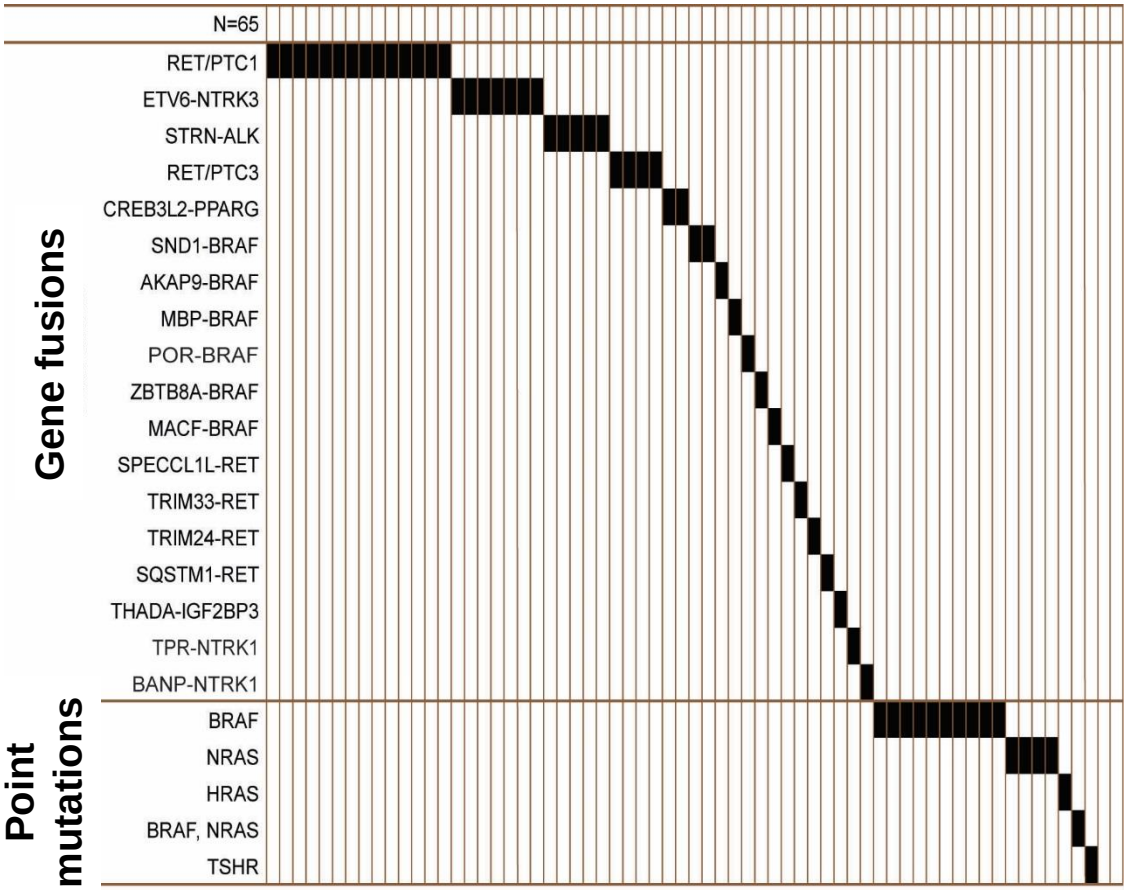
Leeman-Neill et al. *Cancer* (2014)

Genomic Landscape of Post-Chernobyl PTCs from UkrAm Study

- Targeted NGS and whole-transcriptome sequencing (RNA-Seq)
- 63 out of 65 (97%) tumors with driver mutations

71%

26%



Efanov, Brenner, et al. *In preparation*

Genomic Landscape of Post-Chernobyl PTCs from UkrAm Study

Genetic alteration		I-131 thyroid dose, Gy	Age at exposure, yr	Age at surgery, yr	Time since exposure, yr
	N (%)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Point mutation	**17 (26.2)**	**0.2 (0.3)**	**10.9 (3.6)**	**28.4 (4.1)**	**17.6 (2.9)**
BRAF*	11	0.2	10.9	28.0	17.1
RAS	5	0.2	11.0	29.0	18.8
TSHR	1	0.3	10.0	26.6	16.6
Gene fusion	**46 (70.8)**	**1.4 (1.4)**	**7.1 (4.4)**	**23.5 (4.7)**	**16.3 (2.6)**
ALK	5	2.0	6.2	23.2	17.0
BRAF	7	1.8	6.4	23.0	16.6
NTRK	9	1.5	8.4	24.1	15.7
RET	22	1.2	7.1	23.2	16.1
Other gene	3	1.6	7.4	25.3	17.9
Unknown	2 (3.1)	0.3 (0.1)	7.8 (7.6)	22.6 (10.2)	14.8 (2.6)
Total	65 (100)	1.1 (1.3)	8.1 (4.5)	24.7 (5.1)	16.6 (2.7)

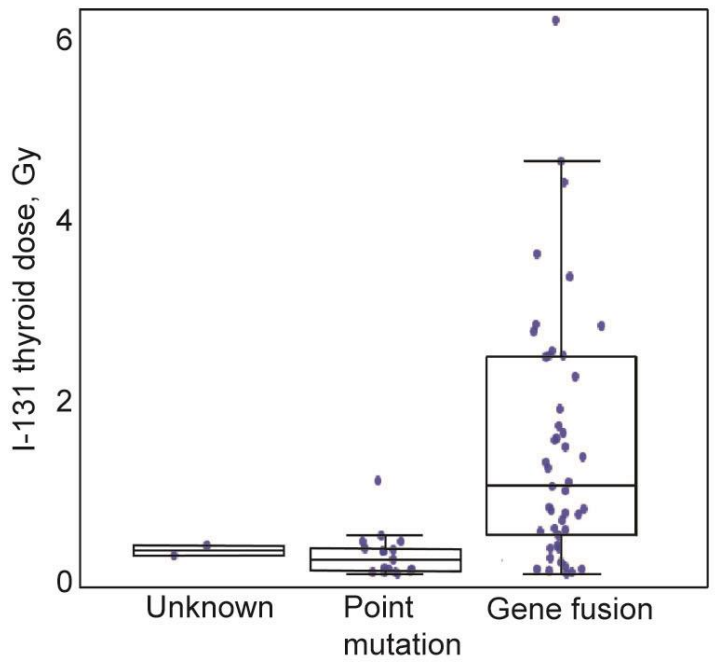
Efanov, Brenner, et al. *In preparation*

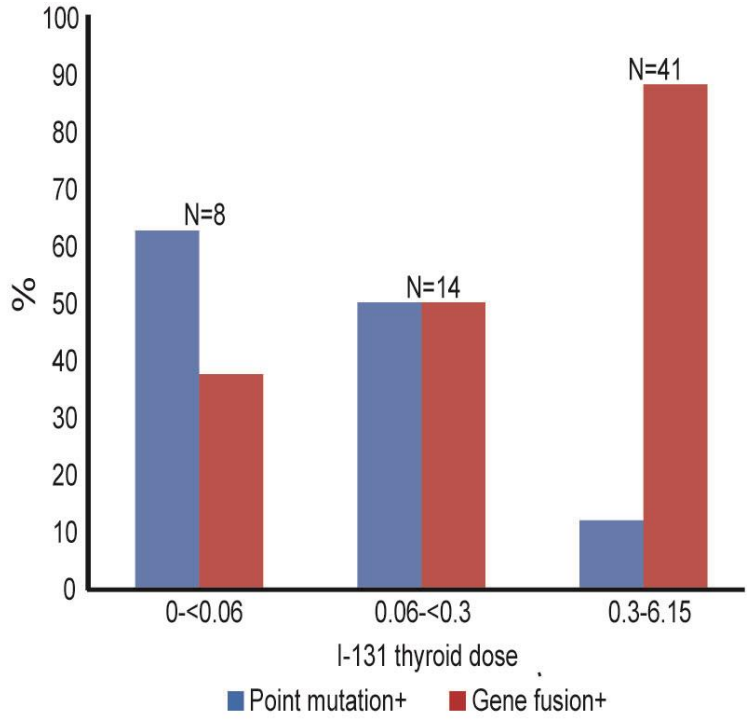
Genomic Landscape of Post-Chernobyl PTCs from UkrAm Study

	Point mutation+	Gene fusion+		
Factor	N (%) or mean (SD)	N (%) or mean (SD)	OR^	95% CI
Sex				
Female	14 (82.4)	23 (50.0)	0.18	0 to 0.75
Male	3 (17.7)	23 (50.0)	1.00	Referent
P*			0.020	
Age at surgery, yr	28.4 (4.1)	23.5 (4.7)	0.74	0.59 to 0.89
P			<0.001	
Oblast of residence in 1986				
Zhytomyr	1 (5.9)	17 (37.0)	1.82	0.81 to Infinity
Kyiv	5 (29.4)	6 (13.0)	1.03	0 to 2.75
Chernihiv	11 (64.7)	23 (50.0)	1.00	Referent
P*			0.164	
I-131 thyroid dose, Gy				
0.009-0.059	5 (29.4)	3 (6.5)	1.00	Referent
0.060-0.299	7 (41.2)	7 (15.2)	1.30	0 to 3.47
0.300-6.154	5 (29.4)	36 (78.3)	2.09	1.07 to Infinity
P*			0.034	
I-131 thyroid dose, Gy	0.2 (0.3)	1.4 (1.4)	20.01#	2.57 to 653.02
P			<0.001	

Efanov, Brenner, et al. *In preparation*

Genomic Landscape of Post-Chernobyl PTCs from UkrAm Study



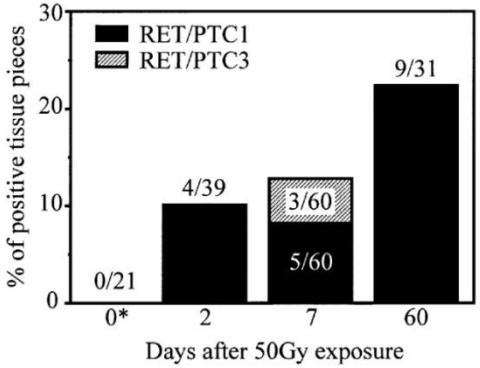


Efanov, Brenner, et al. *In preparation*

Experimental Induction of *RET/PTC* and ETV6/PTRK3 by Radiation in Thyroid Cells

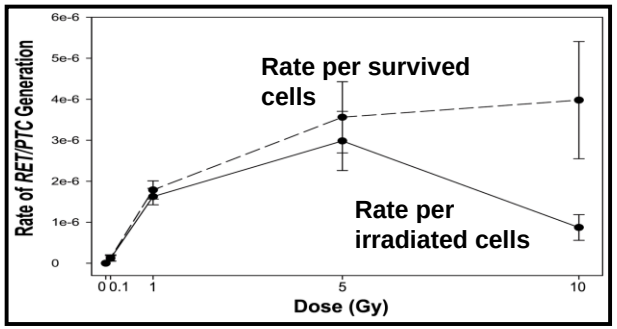
Mizuno et al. Oncogene (2000)

- Fetal human thyroid tissue xenografts in SKID mice
- X ray (50 Gy)

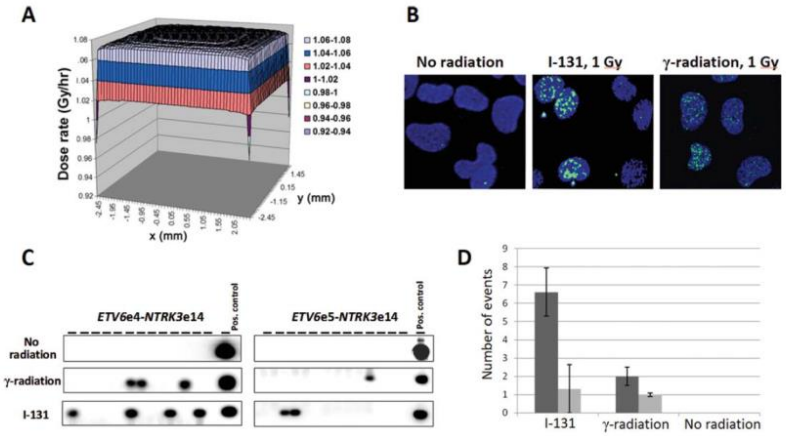


Caudill et al. JCEM (2005)

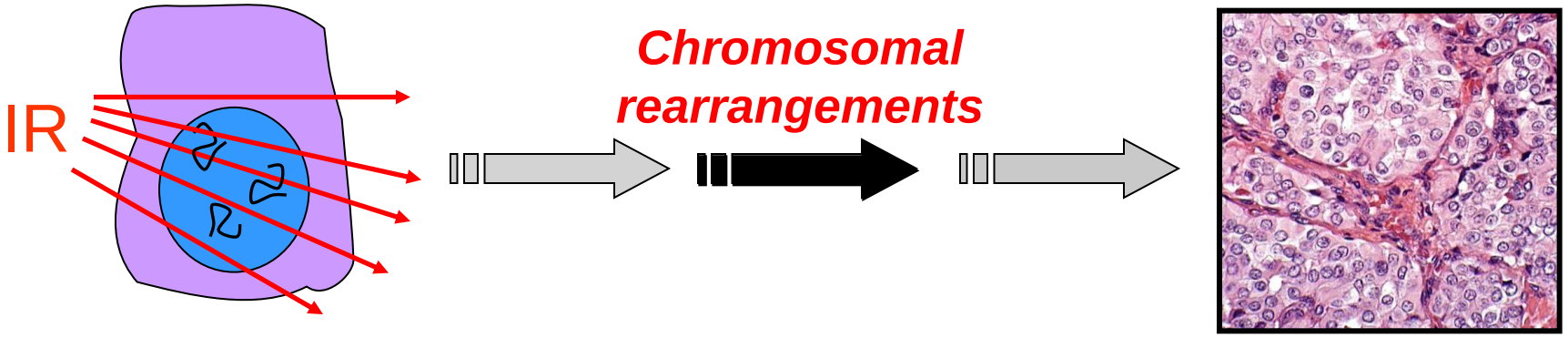
- HTori-3 human thyroid cells
- γ-radiation (0.1-10 Gy)



Leeman-Neill et al. Cancer (2014)



Radiation-Associated Carcinogenesis in Thyroid Cells



DNA Damage

1 Gy

~1,000 SSBs

~30-40 DSBs

~3,000 base damages

Papillary Thyroid Cancer

Future Directions

- Mechanisms of chromosomal rearrangements after radiation exposure: direct, indirect?
- DNA repair mechanisms
- Role of genetic predisposition

- Chromosomal rearrangements - a universal mechanism of radiation-associated cancer? Leukemias, breast cancer, sarcomas

Acknowledgements

Sir Dillwyn Williams
James Fagin
Marina Nikiforova

Kiev, Ukraine
Tetiana Bogdanova
Mykola Tronko
Liudmyla Zurnadzy
Ilya Likhtarev

National Cancer Institute
Alina Brenner
Mark Little
Maureen Hatch
Andre Bouville
Alice Sigurdson
Vladimir Drozdovich
Kiyohiko Mabuchi
Stephen Chanock

CHERNOBYL TISSUE BANK

Gerry Thomas

Munich, Germany
Hartmut Rabes
Roswitha Kerler

Minsk, Belarus
Valentina Drozd
Mikhail Fridman