Signature moléculaire de l'étiologie des cancers de la thyroïde

CEA / DSV / IRCM
Sylvie Chevillard

Principe de l'analyse

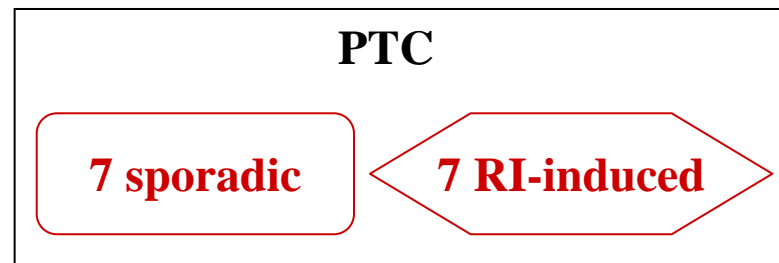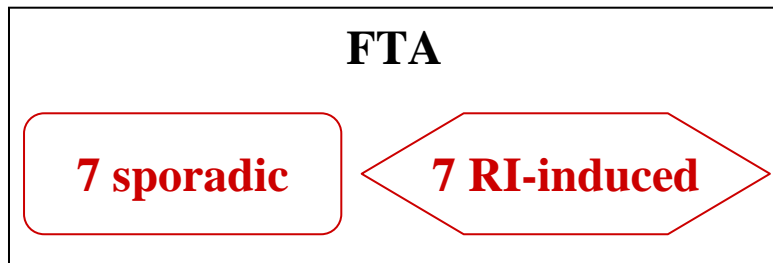**57 tumeurs de la thyroïde induites post-radiothérapie**

hybridations sur puces 25K 50-52mers RNG/MRC collection
Recherche de mutations dans les gènes BRAF, Ras et remaniements RET/PTC

30 adénomes folliculaires FTA
27 carcinomes papillaires PTC

Groupe d'apprentissage

FTA	PTC
7 sporadic 7 RI-induced	7 sporadic 7 RI-induced

Groupe de validation

16 FTC
13 PTC

Clinical data of radiation-induced tumors - learning set

Patient	Histology	Sex	Age at IR	Cause	Age at tumor diagnosis (yr)	Dosimetry (Gy)
RA1	FTA	F	13	Acne	46	20
RA2	FTA	F	3	Hodgkin's disease	36	41.97
RA3	FTA	F	3	Hodgkin's disease	36	41.97
RA4	FTA	M	8	Non-Hodgkin lymphoma	56	43.21
RA5	FTA	M	9	Nasopharynx carcinoma	37	27.73
RA6	FTA	F	11	Hodgkin's disease	29	21.01
RA7	FTA	F	5	Non-Hodgkin lymphoma	25	42.51
			Mean = 7 yr Median = 8 yr		Mean = 38 yr Median = 36 yr	
RP1	PTC	F	6	Acute lymphoblastoid leukemia	20	?
RP2	PTC	F	11	Non-Hodgkin lymphoma	22	41.89
RP3	PTC	M	12	Hodgkin's disease	30	14.94
RP4	PTC	F	10	Lymphoma	40	39.98
RP5	PTC	M	7	Neuroblastoma	22	12
RP6	PTC	F	9	Hodgkin's disease	45	39.78
RP7	PTC	F	14	Hodgkin's disease	48	42.7
			Mean = 10 yr Median = 10 yr		Mean = 32 yr Median = 30 yr	

Clinical data of sporadic tumors - learning set

Patient	Histology	Sex	Age at tumour diagnosis (yr)
SA1	FA	F	59
SA2	FA	M	63
SA3	FA	M	48
SA4	FA	F	22
SA5	FA	M	44
SA6	FA	M	24
SA7	FA	M	21
			Mean = 40 yr
			Median = 44 yr

Mean = 38 yr
Median = 36 yr

Patient	Histology	Sex	Age at tumour diagnosis (yr)
SP1	PTC	F	54
SP2	PTC	F	27
SP3	PTC	F	25
SP4	PTC	F	44
SP5	PTC	F	39
SP6	PTC	F	34
SP7	PTC	F	23
			Mean = 35 yr
			Median = 34 yr

Mean = 32 yr
Median = 30 yr

Gene expression analysis

7 sporadic FA

7 sporadic PTC

Learning set

7 RI FA

7 RI PTC

4 tumours

Included at least one tumour of each histology
At least a 50% difference in their tumour compositions (per aetiology)

4 tumours
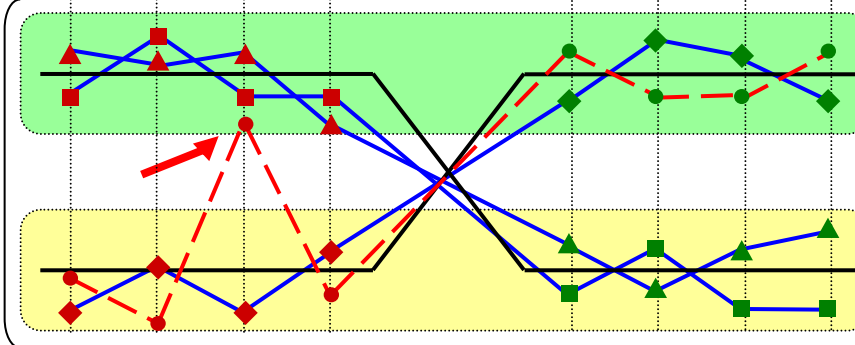
After permutations, 143 combinations have been retained

Sporadic tumours

RI tumours

ST1   ST2   ST3   ST4   RT1   RT2   RT3   RT4

Matrix 1

Not considered

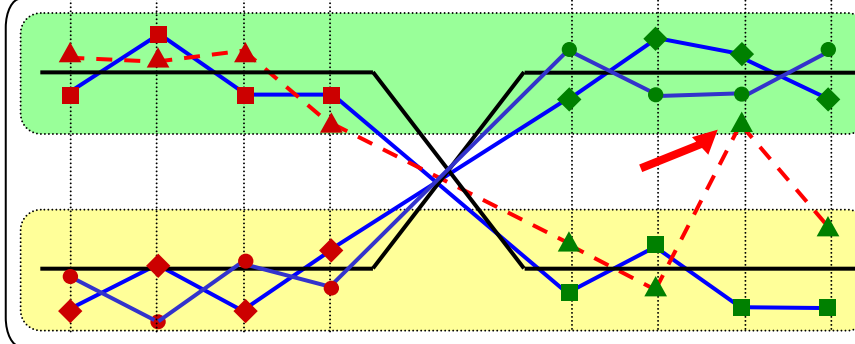Retained gene

**Probability density
to be higher expressed**

**More than 95% of difference between
the two calculated probabilities**

Retained gene

Retained gene

**Probability density
to be lower expressed**

ST2   ST4   ST6   ST7   RT2   RT4   RT6   RT7

Matrix 2

Retained gene

Retained gene

**Genes identified as discriminating
in more than 70% of the
143 RI/S combinations**
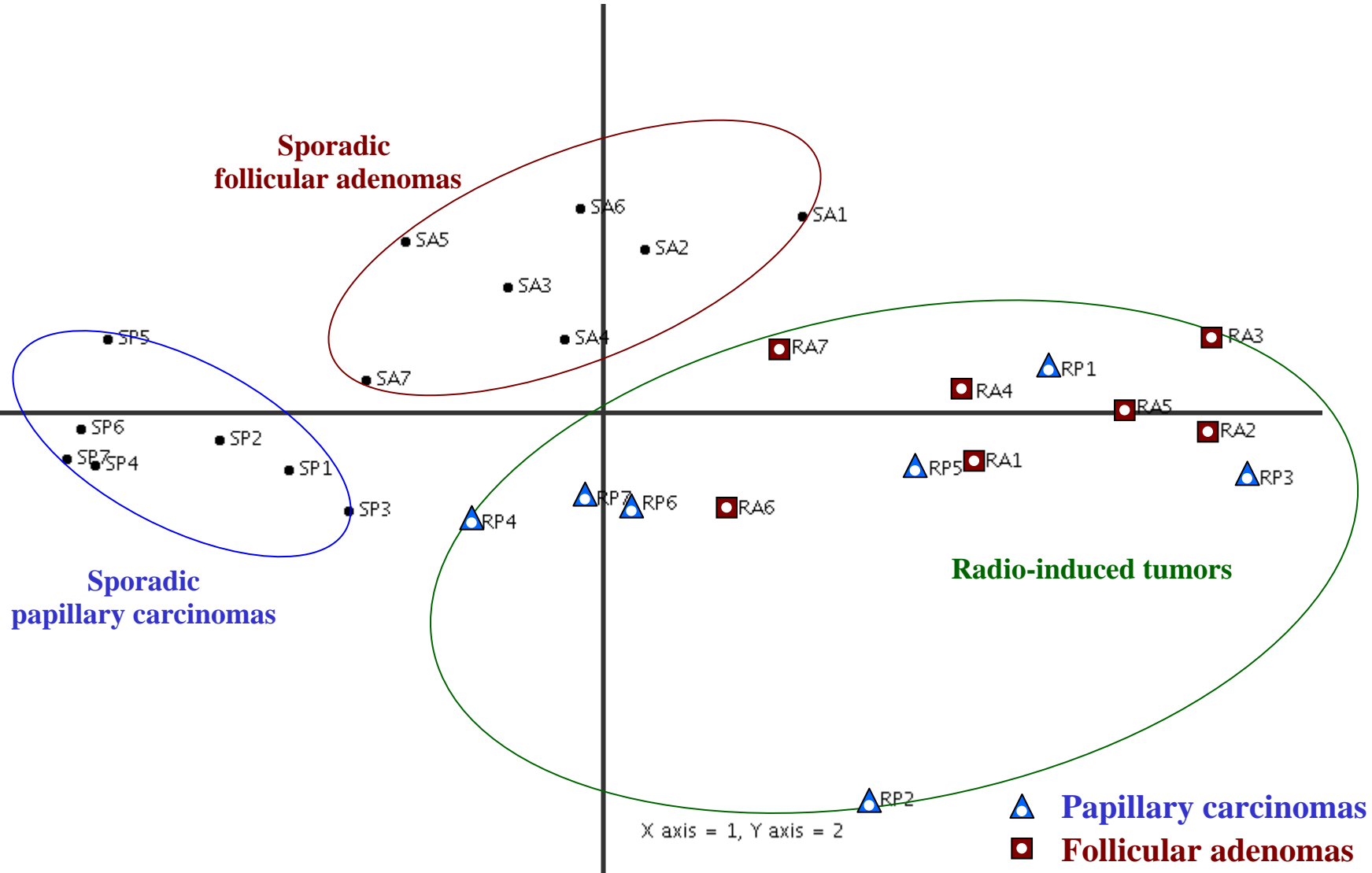
Not considered

Retained gene

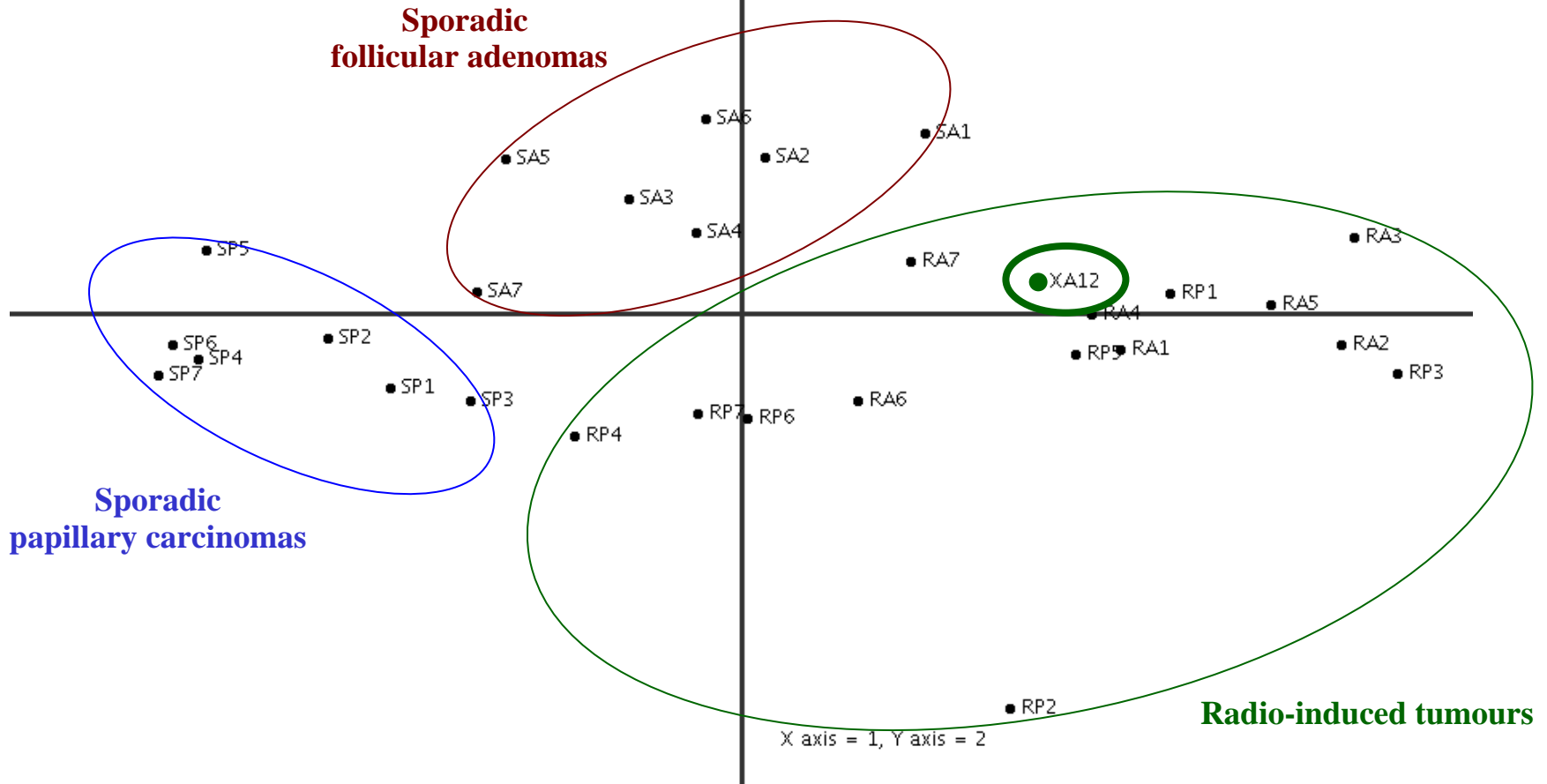## SIGNATURE all RI vs all S
325 genes

Blind validation of the RI/S signature

Validation of the signature by
- **principal component analysis (PCA)**
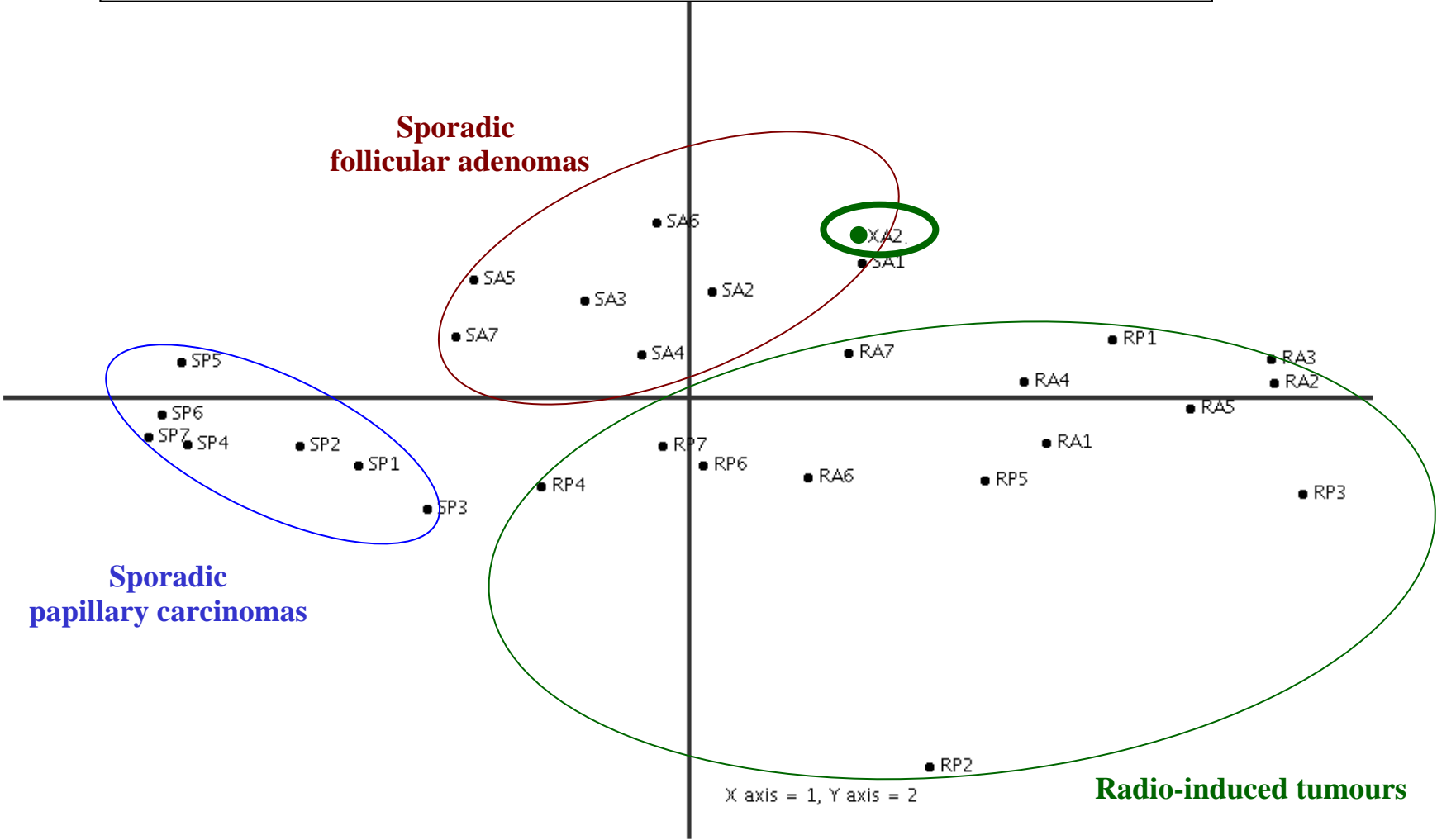- **calculation of the quadratic distance (Root Mean Square (RMS))**

X axis = 1, Y axis = 2

△ **Papillary carcinomas**
▢ **Follicular adenomas**

Patients	Sex	Histology	Signature prediction	Clinical data	Age at IR	Age at tumour diagnosis (yr)	Dosimetry (Gy)
XA12	F	FA	R	R	23	40	42.9

Sporadic follicular adenomas

Sporadic papillary carcinomas

Radio-induced tumours

X axis = 1, Y axis = 2

Patients	Sex	Histology	Signature prediction	Clinical data	Age at tumour diagnosis (yr)
XA2	F	FA	S	S	59

Sporadic follicular adenomas

Sporadic papillary carcinomas

Radio-induced tumours

X axis = 1, Y axis = 2

SA6, SA5, SA3, SA7, SA4, SA2, SA1, XA2

SP5, SP6, SP7, SP4, SP2, SP1, SP3

RP1, RA3, RA7, RA4, RA2, RA5, RA1, RP7, RP6, RA6, RP5, RP3, RP4, RP2

Patients	Sex	Histology	Signature prediction	Clinical data	Age at tumour diagnosis (yr)	Alteration
XP7	F	PTC	S	S	22	/
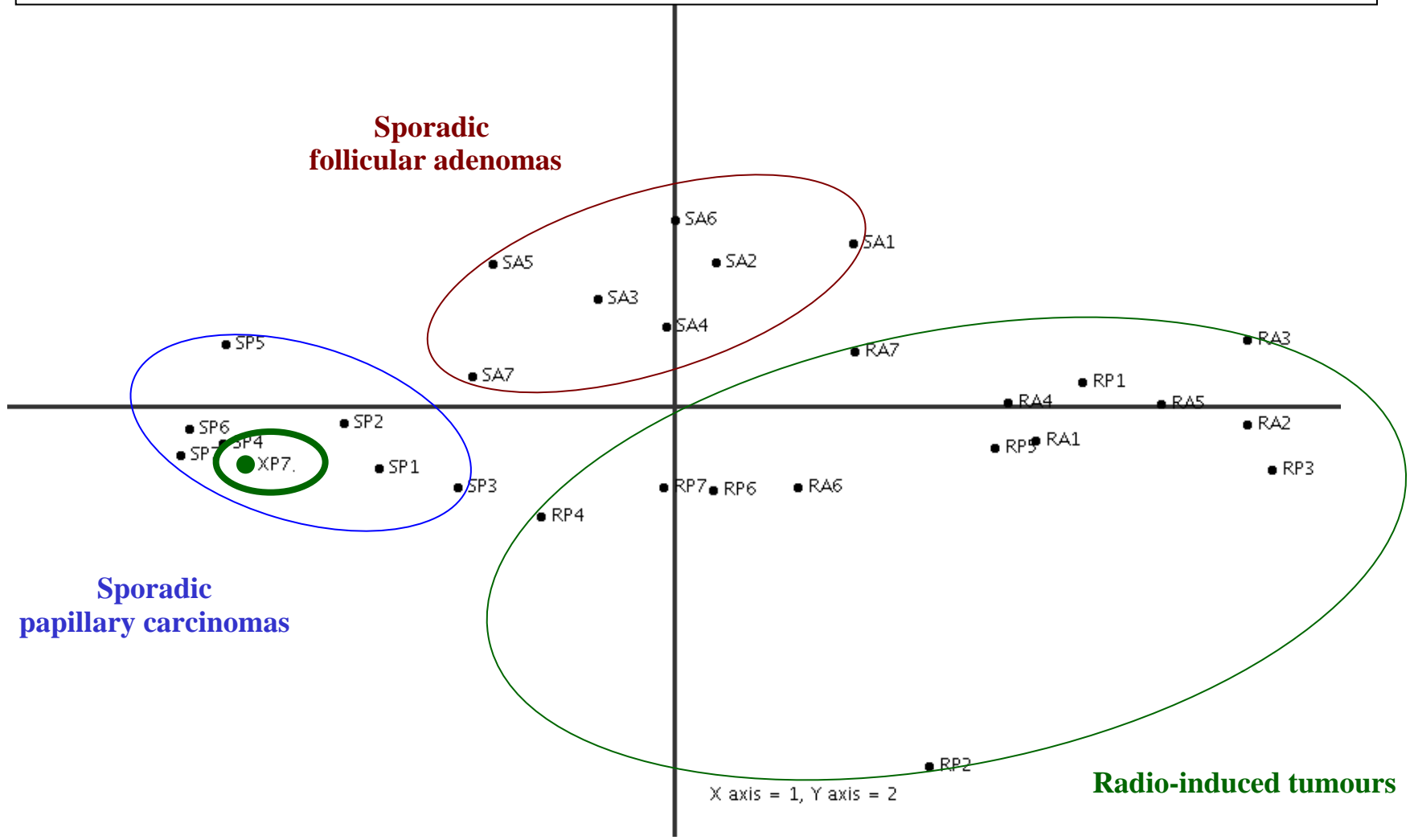
Sporadic follicular adenomas

Sporadic papillary carcinomas

Radio-induced tumours

X axis = 1, Y axis = 2

Patients	Sex	Histology	Signature prediction	Clinical data	Age at IR	Age at tumour diagnosis (yr)	Dosimetry (Gy)	Alteration
XP11	M	PTC	?	R	23	36	20	PTC3

Sporadic follicular adenomas

Sporadic papillary carcinomas

Radio-induced tumours

X axis = 1, Y axis = 2
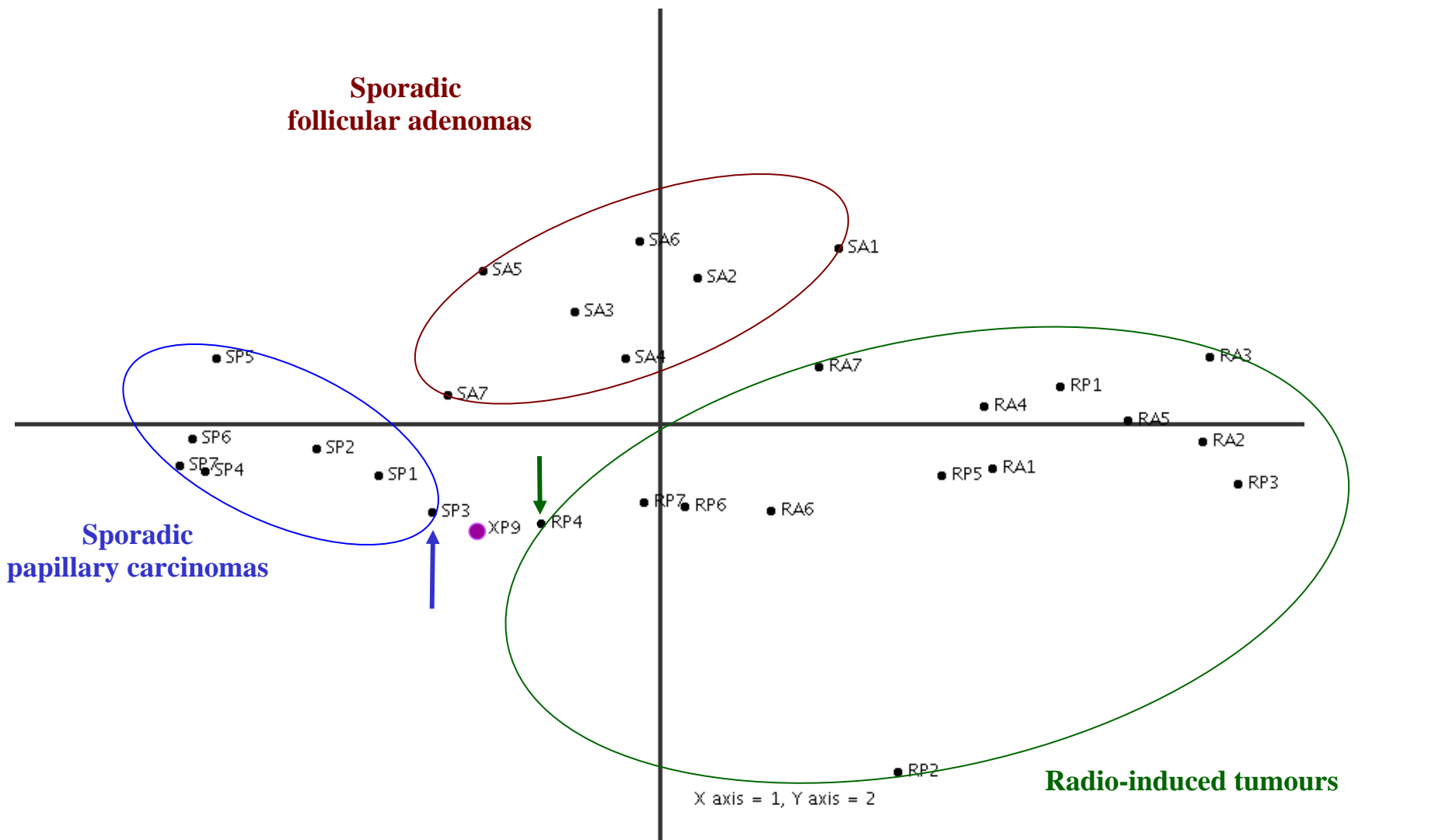
Blind validation of the R/S signature

Validation set   16 follicular adenomas
                      13 papillary carcinomas

Clinical datas

8 SA
8 RA
8 SP
5 RP

Signature prediction

6 SA   **2 RA**
8 RA
8 SP
4 RP   1 ?

26/29 well classified
2/29 miss-classified
1/29 undetermined etiology

	RI	S
+ test (RI)	12	2
- test (S)	0	14

Sensitivity	12/13	0.92
Specificity	14/16	0.87
Positive predictive value	12/14	0.85
Negative predictive value	14/14	1

Proportion of RI tumours well concluded among RI tumours
Proportion of S tumour well concluded among S tumours
Proportion of tumour with + test (R), well concluded
Proportion of tumours with – test (S), well concluded

Signature of radiation-induced tumours after radiotherapy

Molecular signature : 325 genes
when considering all sporadic tumours versus all RI-induced tumours

138 over-expressed and 187 under-expressed genes
RI-induced compared with sporadic tumours

Less stringent analysis
dysregulated pathways 656 genes

313 over-expressed genes and 343 under-expressed genes
in RI tumours as compared with sporadic tumours

Transcriptome analysis

981 genes

170 genes already reported in thyroid microarrays
80 genes associated to normal or pathological thyroid molecular mechanisms

8 genes dysregulated in sporadic PTC vs post-Tchenobyl PTC (Port et Al. 2007)
MST150, RARRES1, GZMH, C13orf3, SFRP1, RIPK4, EPB41L3, RERG

Comparaison difficile à cause des références qui sont différentes

MAPK, EGFR, WNT, TGFβ
Regulation of p53 and p53-dependant regulation
Angiogenesis, Hypoxia

Signatures in each histological group

Signatures were found when considering :
Sporadic PTC versus RI PTC
Sporadic FA versus RI FA

Less robust in term of well classification than all sporadic vs all RI tumours signature

- **Nicolas Ugolin**
- **Kathy Ory**
- **M Schlumberger**
- **P Hofman**
- **Adel El Nagar**

- **CE FP6**
- **EDF**

Sporadic PTC vs Tchernobyl PTC

Tchernobyl signature
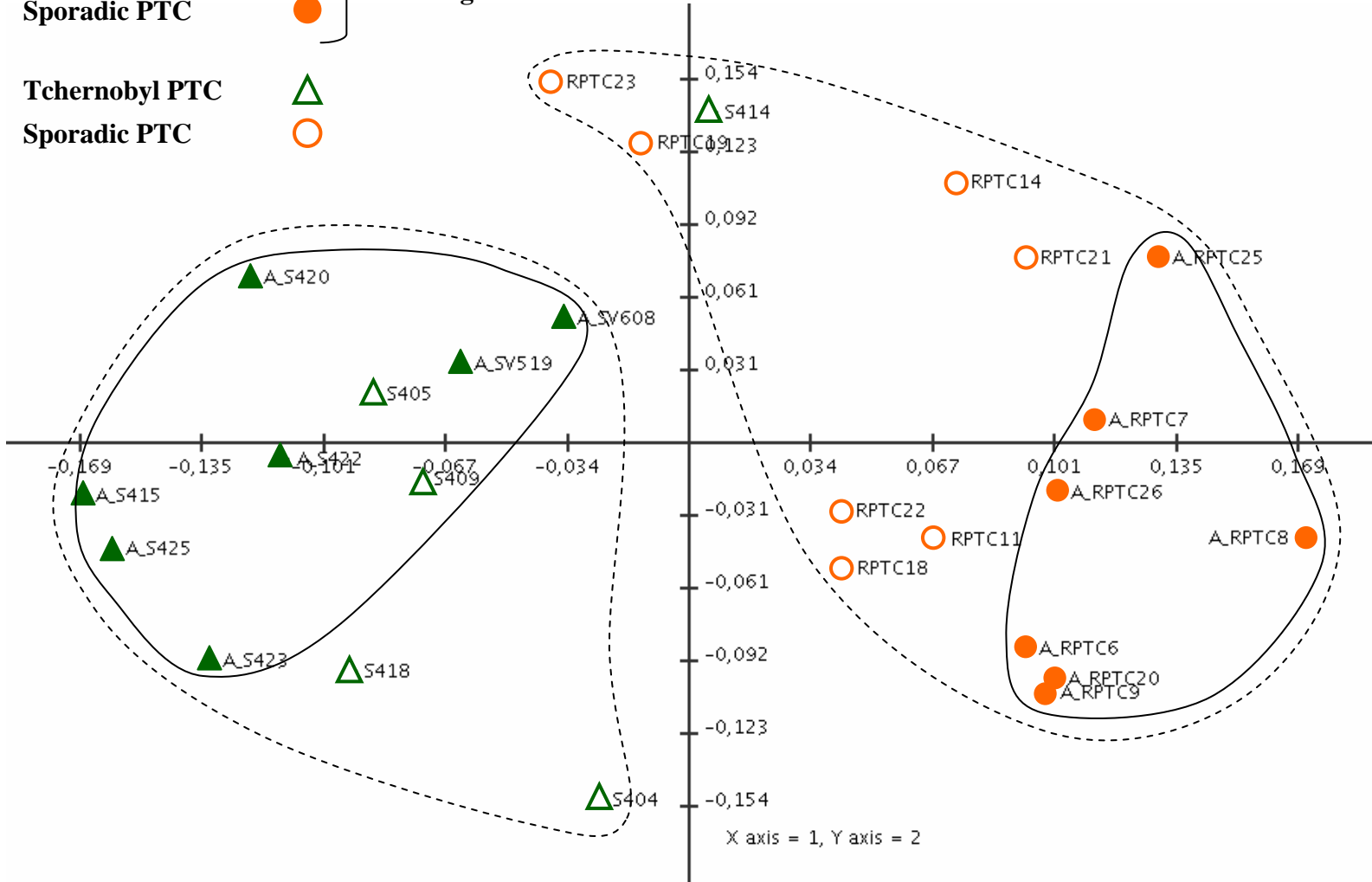
Signature of sporadic tumours versus post-Tchernobyl tumours
495 genes

S   R ⇌ ↗
           ↘
Ref   Ref —

Pool R ⇌ ↗
           ↘
           ↗
Pool S ⇌
           ↘

S   R ⇌ ↗
              ↘
              ↗
Normal Normal ⇌
              ↘
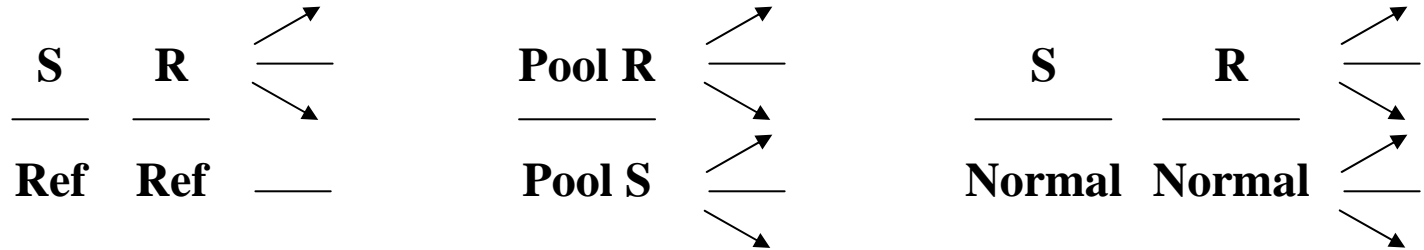
12 genes in common with the 325 genes post-radiotherapy signature
19 genes in common when post-radiotherapy signature is extended (less stringency)

66 genes already reported in thyroid microarrays

4 genes deregulated in sporadic PTC vs post-Tchenobyl PTC (Port et Al. 2007)

Alterations in RAS, BRAF and RET genes

Papillary carcinomas

		Patients	RET/PTC	BRAF	Ras
RI	Learning	RP1			
		RP2			
		RP3			
		RP4			
		RP5			
		RP6		V600E	
		RP7			
	Validation	XP9	RET/PTC3		
		XP10	RET/PTC1		
		XP11			
		XP12	RET/PTC3		
		XP13			Kras Q61R
S	Learning	SP1		V600E	
		SP2		V600E	
		SP3			
		SP4	RET/PTC3		
		SP5		V600E	
		SP6	RET/PTC1		
		SP7		V600E	
	Validation	XP1		V600E	
		XP2			
		XP3			
		XP4	RET/PTC1		
		XP5		V600E	
		XP6		3bp deletion	
		XP7			
		XP8		V600E	

Follicular adenomas

		Patients	Ras
RI	Learning	RA1	
		RA2	Hras Q61R
		RA3	
		RA4	
		RA5	
		RA6	
		RA7	
	Validation	XA9	
		XA10	
		XA11	
		XA12	
		XA13	Kras Q61K
		XA14	
		XA15	
		XA16	
S	Learning	SA1	
		SA2	
		SA3	
		SA4	
		SA5	Hras Q61R
		SA6	
		SA7	Nras Q61R
	Validation	XA1	
		XA2	
		XA3	
		XA4	
		XA5	
		XA6	
		XA7	Nras Q61K
		XA8	

Clinical data from radiation-induced tumours of the validation set

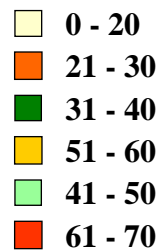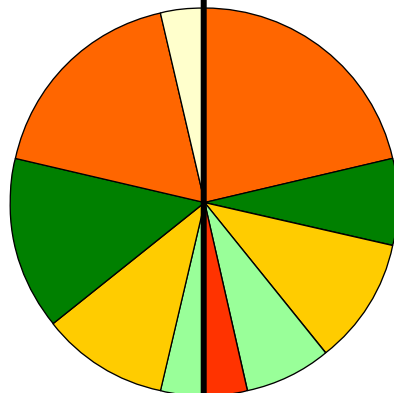| Patient	Histology	Sex	Age at IR	Cause	Age at tumour diagnosis (yr)	Dosimetry (Gy)
XA9	FA	M	19	?	40	?
XA10	FA	F	?	?	35	?
XA11	FA	M	13	Hodgkin's disease	53	Unavailable
XA12	FA	F	23	Hodgkin's disease	40	42.9
XA13	FA	F	29	Hodgkin's disease	37	41.17
XA14	FA	F	16	Hodgkin's disease	60	42.66
XA15	FA	F	19	Non-Hodgkin lymphoma	43	40.67
XA16	FA	F	28	Uterus	60	47.9
XP9	PTC	M	23	Hodgkin's disease	36	20
XP10	PTC	F	13	Ovarian teratoma	30	0.08
XP11	PTC	F	24	Lymphoma	59	44.45
XP12	PTC	F	11	Hodgkin's disease	61	40.23
XP13	PTC	F	19	Graves disease	39	Unavailable

Clinical data from sporadic tumors of the validation set

Patient	Histology	Sex	Age at tumour diagnosis (yr)
XA1	FA	M	58
XA2	FA	F	31
XA3	FA	F	29
XA4	FA	F	29
XA5	FA	F	27
XA6	FA	F	59
XA7	FA	F	22
XA8	FA	F	48
XP1	PTC	F	17
XP2	PTC	F	25
XP3	PTC	F	39
XP4	PTC	F	17
XP5	PTC	M	74
XP6	PTC	F	73
XP7	PTC	M	41
XP8	PTC	F	40

Radiation-induced tumors of the learning set ← → **Sporadic tumors of the learning set**

- 0 - 20
- 21 - 30
- 31 - 40
- 51 - 60
- 41 - 50
- 61 - 70

Age at tumour diagnosis

- BRAF
- RET/PTC
- No alteration

RET/PTC, BRAF

- Women
- Men

Sex