SOLCA
NÚCLEO DE QUITO

7 DÉCADAS
desde 1954
de atención especializada en cáncer

Radiogenómica en cáncer de mama identificación de predictores genéticos y genómicos de la toxicidad asociada a la radioterapia

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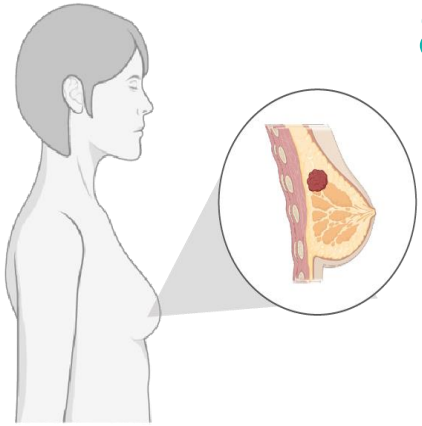
20 de julio de 2024

VHIO VALL D'HEBRON Institut d'Oncologia | Vall d'Hebron Barcelona Hospital Campus

Radiotherapy (RT) plays a crucial role in breast cancer treatment

50%
of patients diagnosed with cancer will receive some form of radiation therapy.

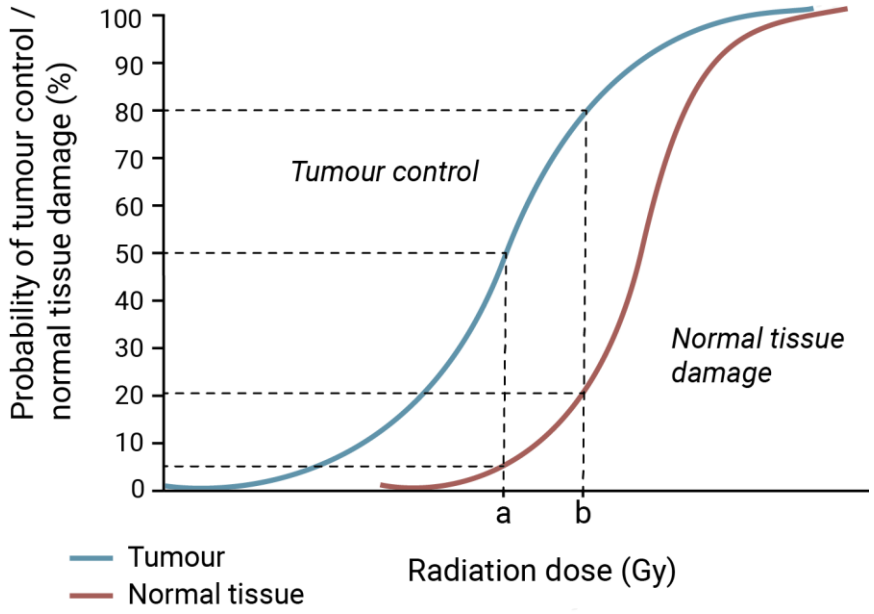


80%

Radiotherapy has been shown to decrease local recurrence and to have a beneficial effect on survival.

Delaney G et al., Cancer (2005)
McGale et al., The Lancet (2014)

The therapeutic ratio: balancing tumour control and normal tissue complications



The therapeutic ratio describes the balance between tumour control probability and normal tissue complications probablitities.

The probability of tumour control increases with increasing radiation dose, but so does the probability of normal tissue damage.

Figure adapted from Barnett et al., Nat Rev Cancer (2009)

Around 10% of patients treated with RT suffer from adverse effects, which can be acute or late

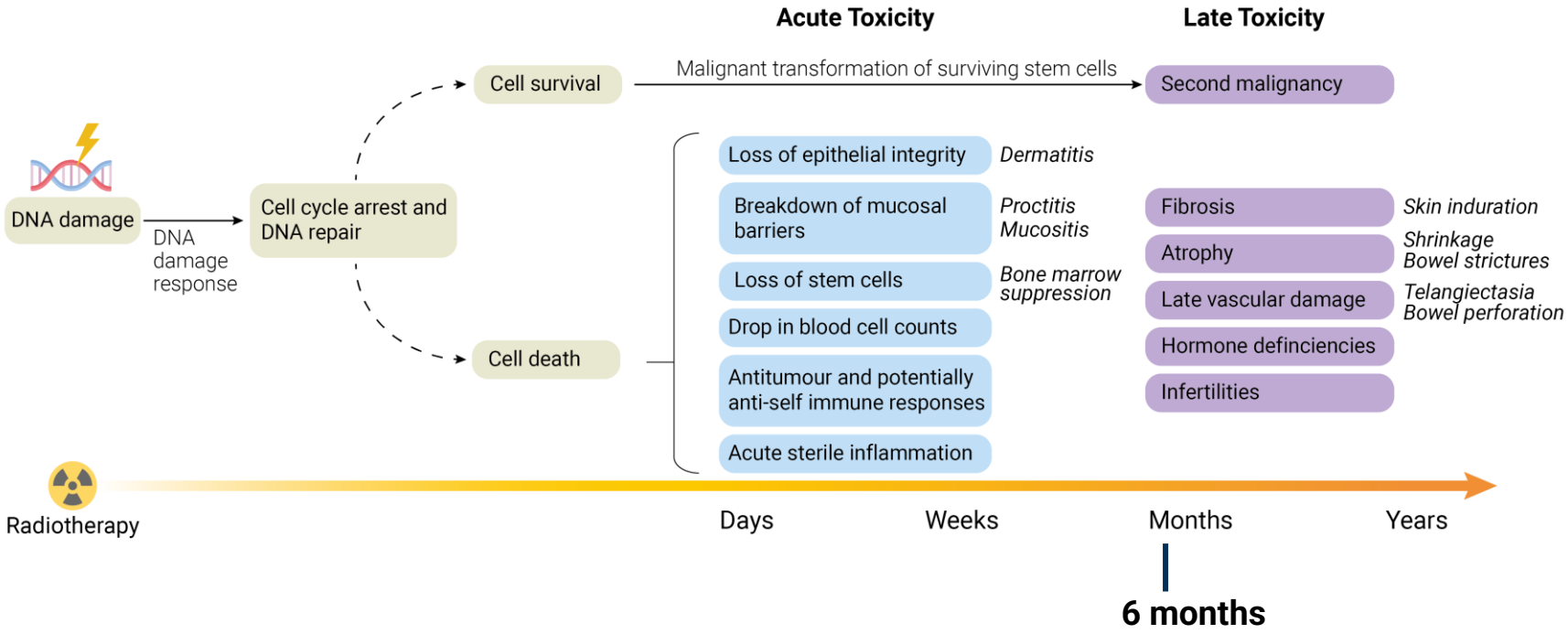
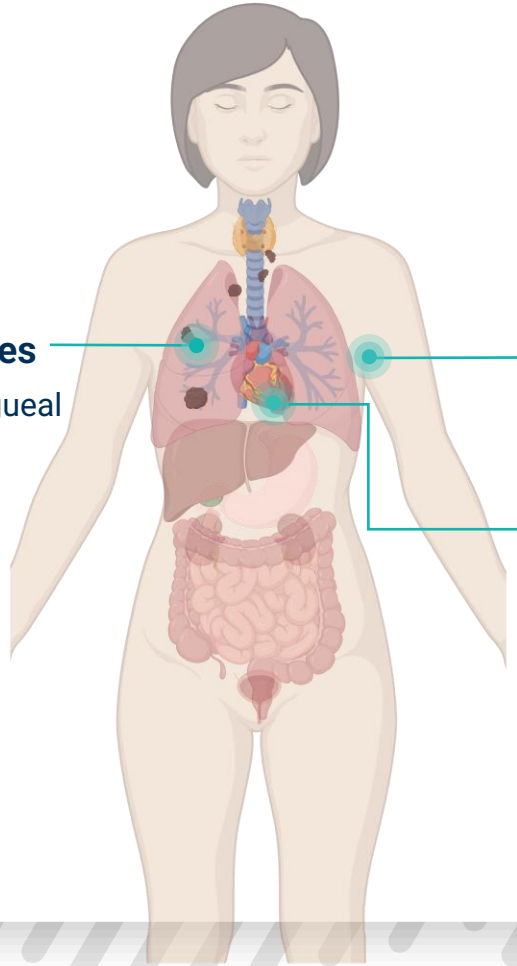


Figure adapted from De Ruysscher et al., Nat Rev Dis Primers (2019) and Barnett et al., Nat Rev Cancer (2009)

Radiation-induced side effects

Breast Cancer



Secondary malignancies

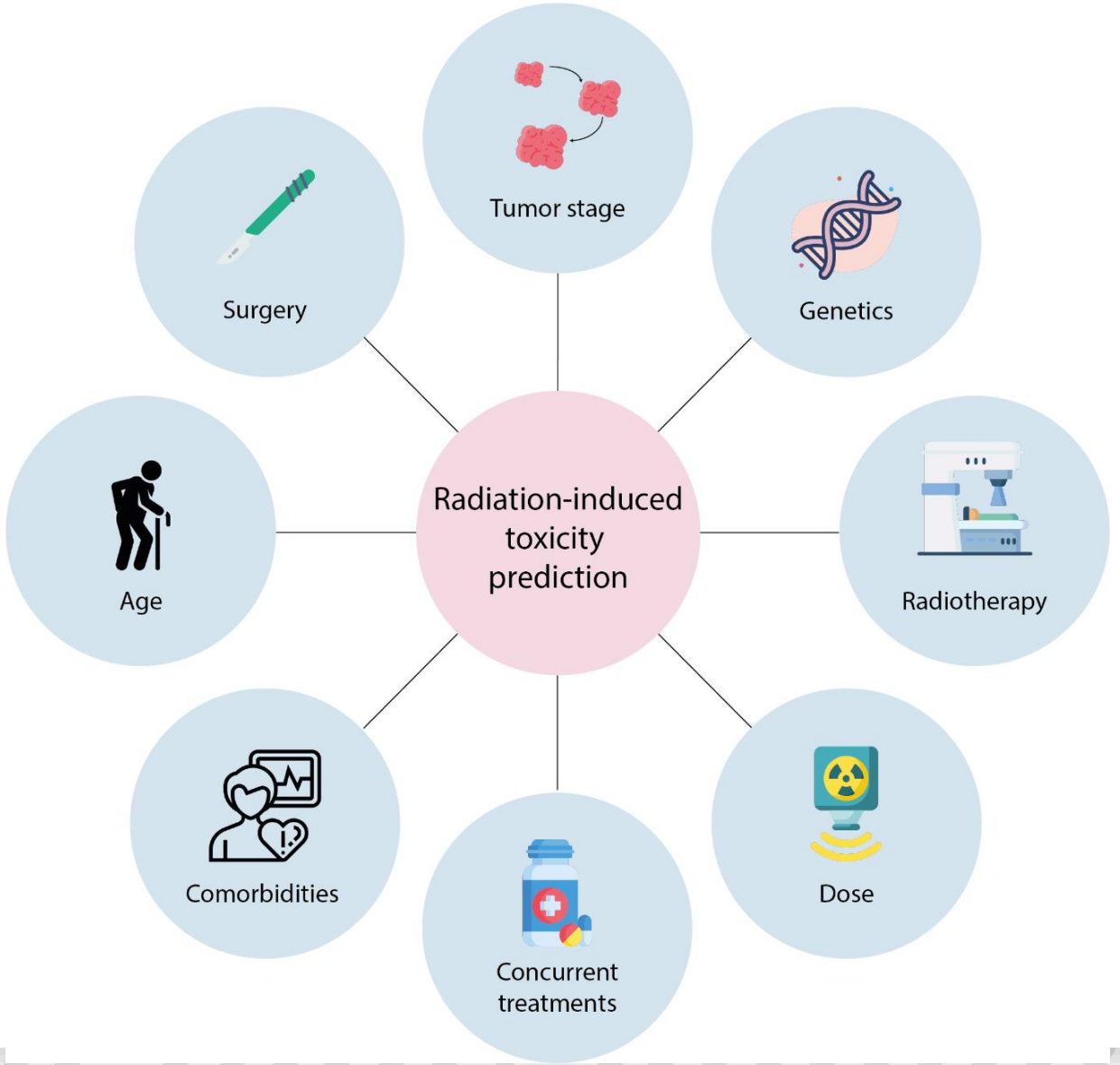
Breast, lung and oesophageal cancers

Skin toxicity

Fibrosis, telangiectasia, skin atrophy and hyperpigmentation

Cardiac toxicity

Germline genetic predictors of radiotherapy-induced toxicity cancer patients



HYPOTHESIS

- Germline genetic variation modulates RT toxicity
- Patient blood cell markers are sensitive and specific indicators of the RT toxicity

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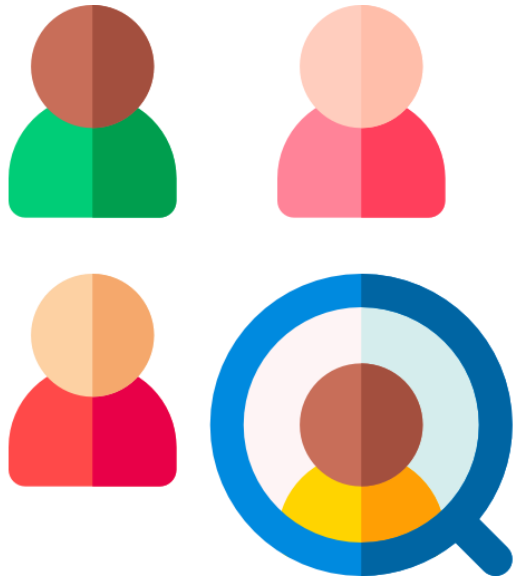
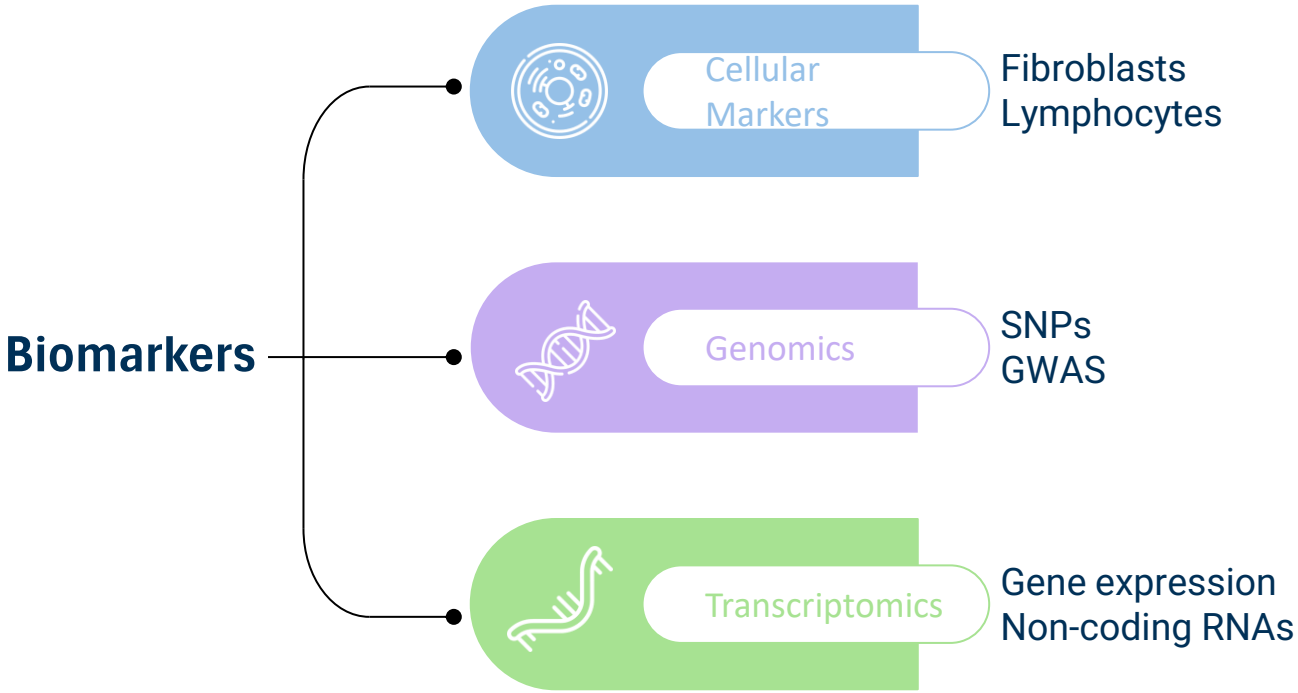
Identifying patients at risk is critical



Ideal: to identify patients with radiation sensitivity before or early in treatment and adapt their treatment plans.

The search for predictive biomarkers of RT toxicity



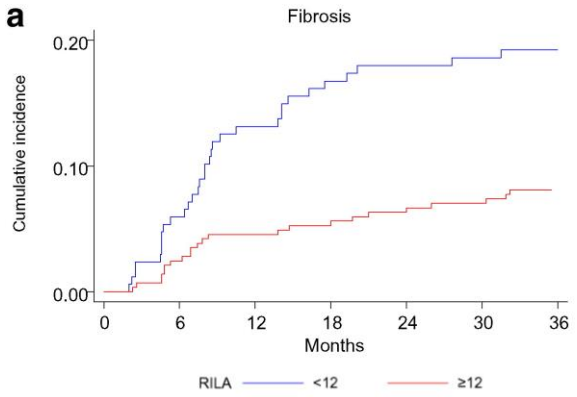
Still, there is not yet an optimisation of RT based on genetic or cell markers in standard practice due to a lack of clinical validation of candidate biomarkers.

Radiation-induced Lymphocyte Apoptosis (RILA): a promising biomarker

Cellular Markers | Genomics | Transcriptomics

Lymphocyte-based assays → good study model due to their higher radiosensitivity in comparison to other cell types and their accessibility.

$$\text{RILA} = \frac{\%\ \text{lymphocyte apoptosis after } \textit{in vitro} \text{ irradiation}}{\%\ \text{spontaneous lymphocyte apoptosis}}$$



↑ RILA ↓ late toxicity

These results have been subsequently replicated in several studies, including a clinical trial (NCT00893035) **demonstrating an inverse relationship between the percentage of apoptosis in irradiated lymphocytes and late toxicities**. However, the mechanism of this inverse association is unclear.

Ozsahin et al., Int J Radiat Oncol Biol Phys (1997)
Ozsahin et al., Clinical Cancer Research (2005)
Azria et al ., EBioMedicine (2015)

Apoptosis (2015) 20:371–382
DOI 10.1007/s10495-014-1056-2

ORIGINAL PAPER

Apoptosis for prediction of radiotherapy late toxicity: lymphocyte subset sensitivity and potential effect of *TP53* Arg72Pro polymorphism

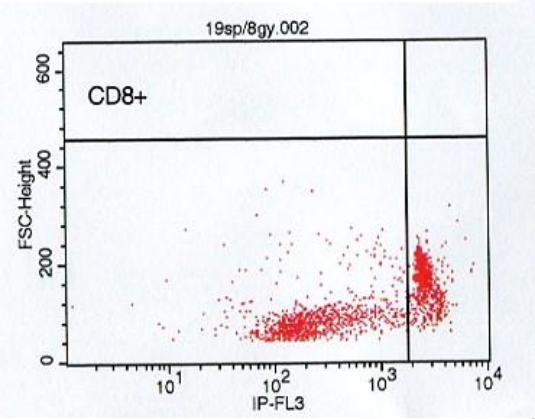
María J. Fuentes-Raspall · Isabel Caragol · Carmen Alonso · Teresa Ramón y Cajal · David Fisas · Alejandro Seoane · Nerea Carvajal · Sandra Bonache · Orland Díez · Sara Gutiérrez-Enríquez

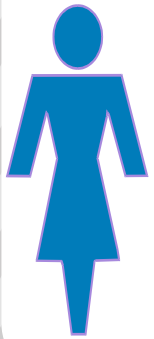
10 breast cancer patients with severe skin long term side effects

37ºC incubation

0 h

48h





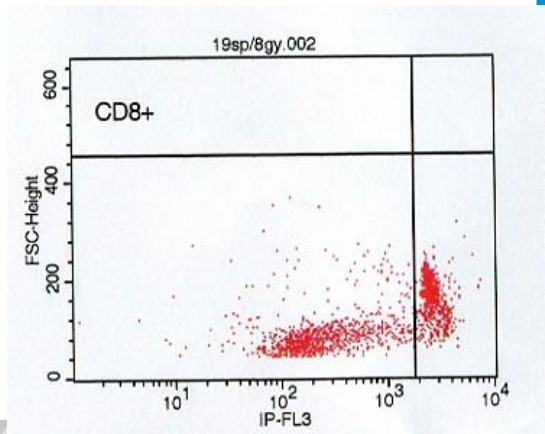
10 matched breast cancer patients without skin toxicity

8 Gy irradiation

37ºC incubation

0 h

48h



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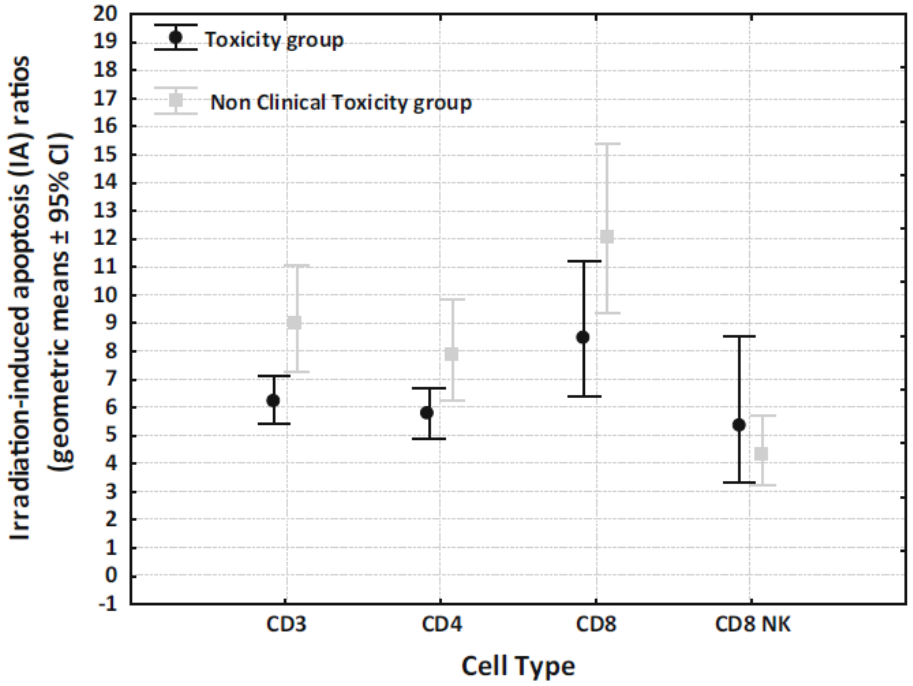
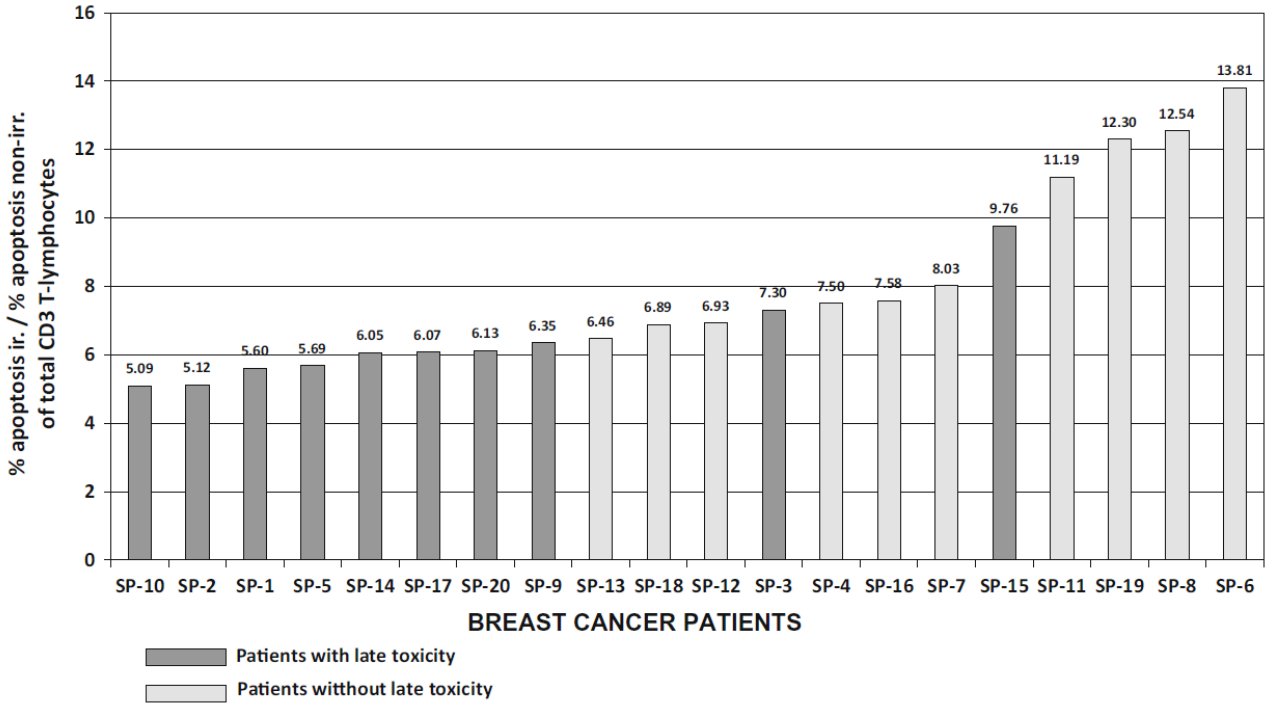
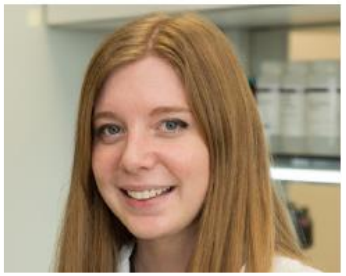




The cohort of patients with late toxicities is characterised by a low level of radiation-induced apoptosis (RILA)

Identify the **molecular basis** underlying radiation-induced CD8 T-lymphocyte apoptosis (**RILA**) by using **blood gene expression** analysis in **BC patients** with and without **late effects** and in whom we had also first identified **differences in RILA**.



Ester Aguado Flor

frontiers | Frontiers in Oncology

ORIGINAL RESEARCH
published: 24 May 2022
doi: 10.3389/fonc.2022.825703

Cell Senescence-Related Pathways Are Enriched in Breast Cancer Patients With Late Toxicity After Radiotherapy and Low Radiation-Induced Lymphocyte Apoptosis

Ester Aguado-Flor[1], María J. Fuentes-Raspall[2], Ricardo Gonzalo[3], Carmen Alonso[4], Teresa Ramón y Cajal[4], David Fisas[4], Alejandro Seoane[5], Álex Sánchez-Pla[3,6], Jordi Giralt[7,8], Orland Díez[1,9] and Sara Gutiérrez-Enríquez[1]

OPEN ACCESS

Patient selection and microarray setup

Apoptosis (2015) 20:371–382
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ORIGINAL PAPER

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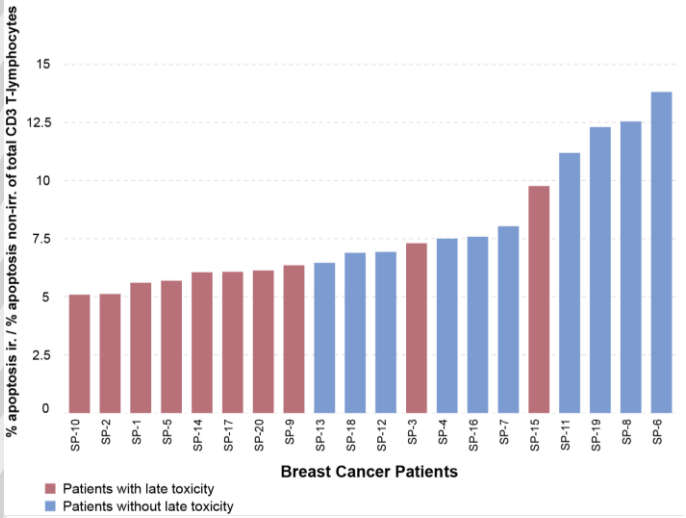
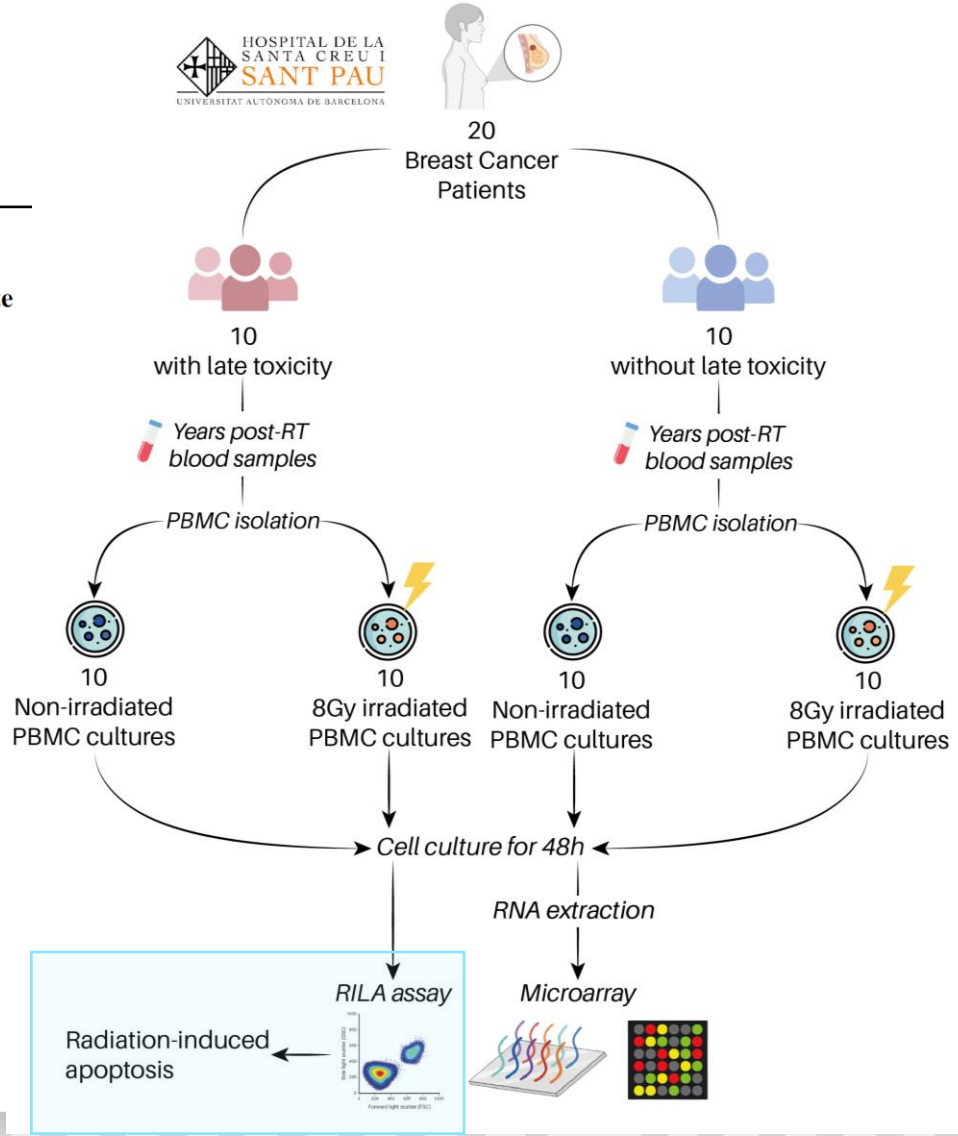




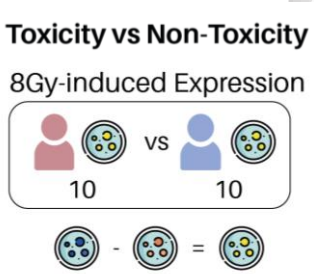
Late clinical toxicity after BC RT and low level of RILA is associated with an altered transcriptional profile of PBMCs?

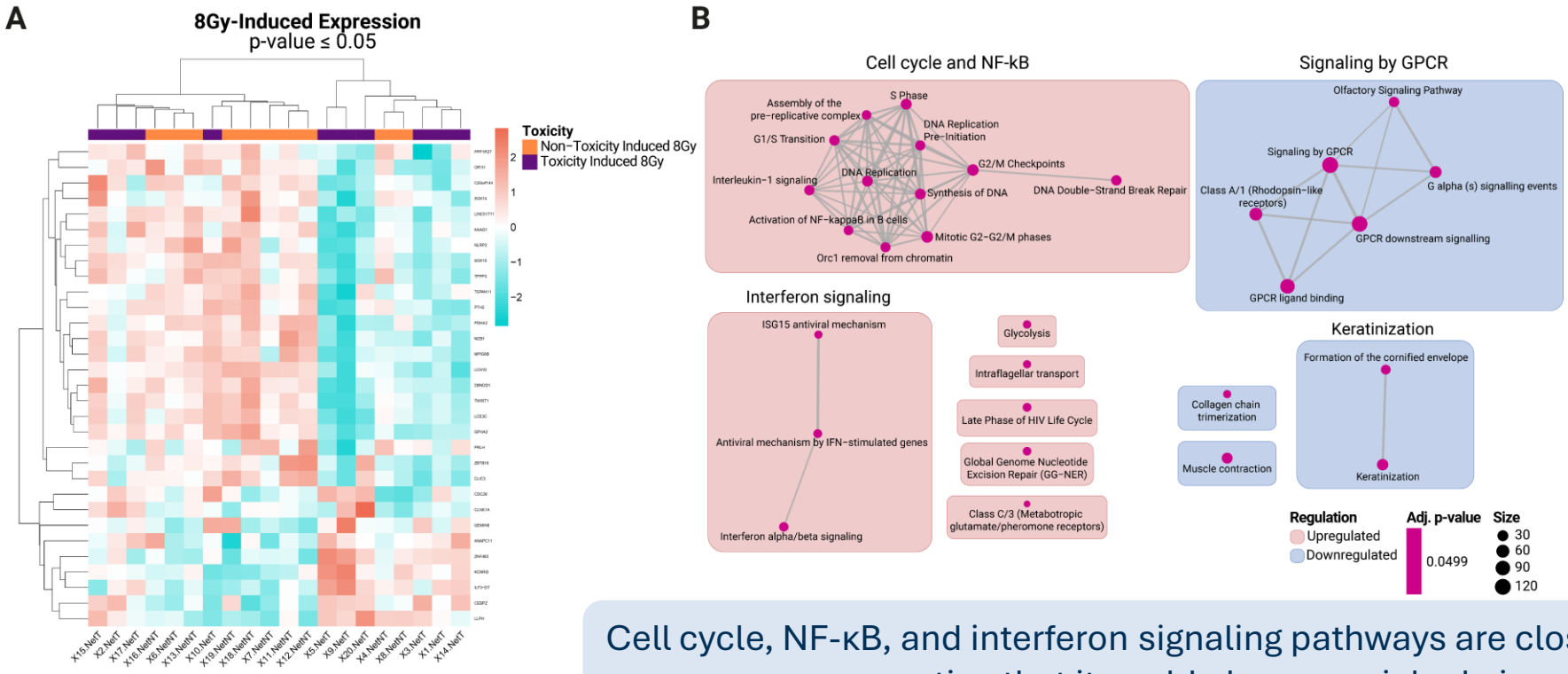
Fuentes-Raspall et al., Apoptosis (2015)

Cell senescence-related pathways enriched after irradiation in patients with toxicity

Toxicity vs non-toxicity patients, 8Gy-induced expression

No genes with an adj. p-value <0.05.
45 pathways with an adj. p-value <0.05.





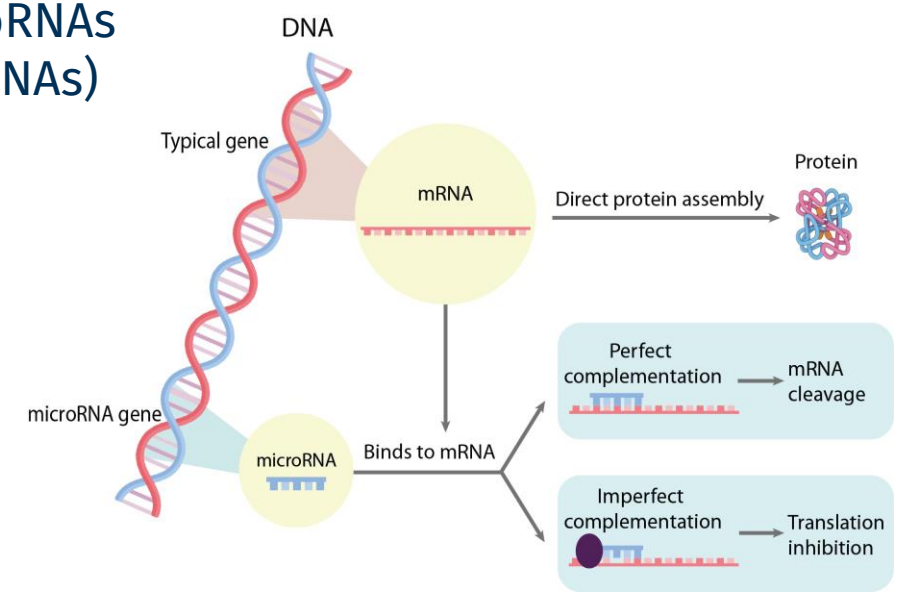
Cell cycle, NF-κB, and interferon signaling pathways are closely linked to cellular senescence, suggesting that it could play a crucial role in radiation-induced toxicity.

Li et al., Front Pharmacol (2018)
Leysen et al., Int J Mol Sci (2018)

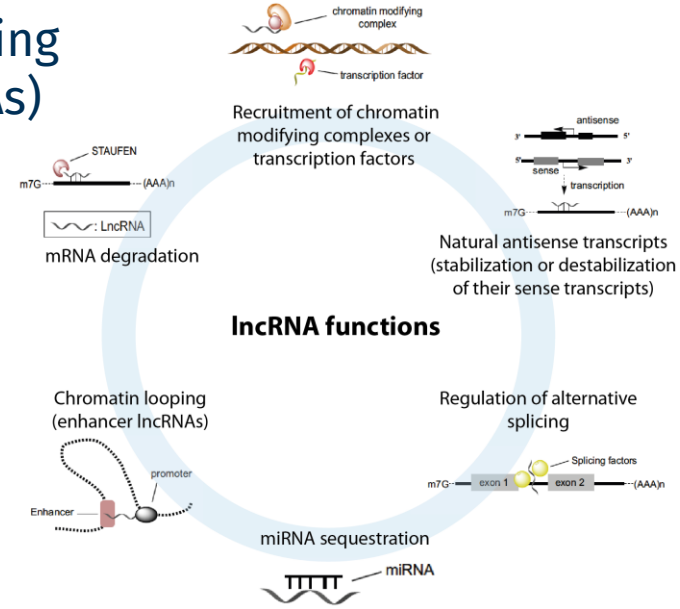
Non-coding RNAs: an unexplored biomarker of RT-induced toxicity

Cellular Markers | Genomics | Transcriptomics

MicroRNAs (miRNAs)



Long non-coding RNAs (lncRNAs)



Both non-coding RNAs modulate key cellular pathways that mediate response to radiation.
However, their role in radiotherapy-induced toxicity in normal tissues remains largely unexplored.

Zhang et al., Nat Commun (2014)
Yu et al., Journal of Gene Medicine (2023)

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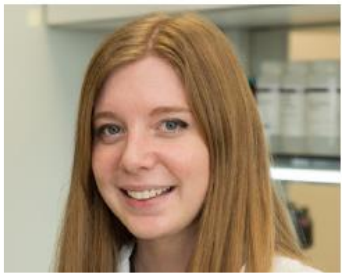
Develop a **genetic association study** of **novel** candidate **miRNA** and **lncRNA SNPs** as well as of **previously** related SNPs to **acute** and **late radiosensitivity** in **BC patients**.



Ester Aguado Flor

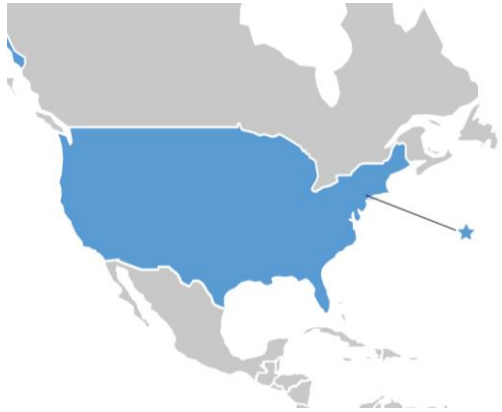
REQUITE

REQUITE is an international project whose aim is to predict which patients are more likely to have side effects from RT.

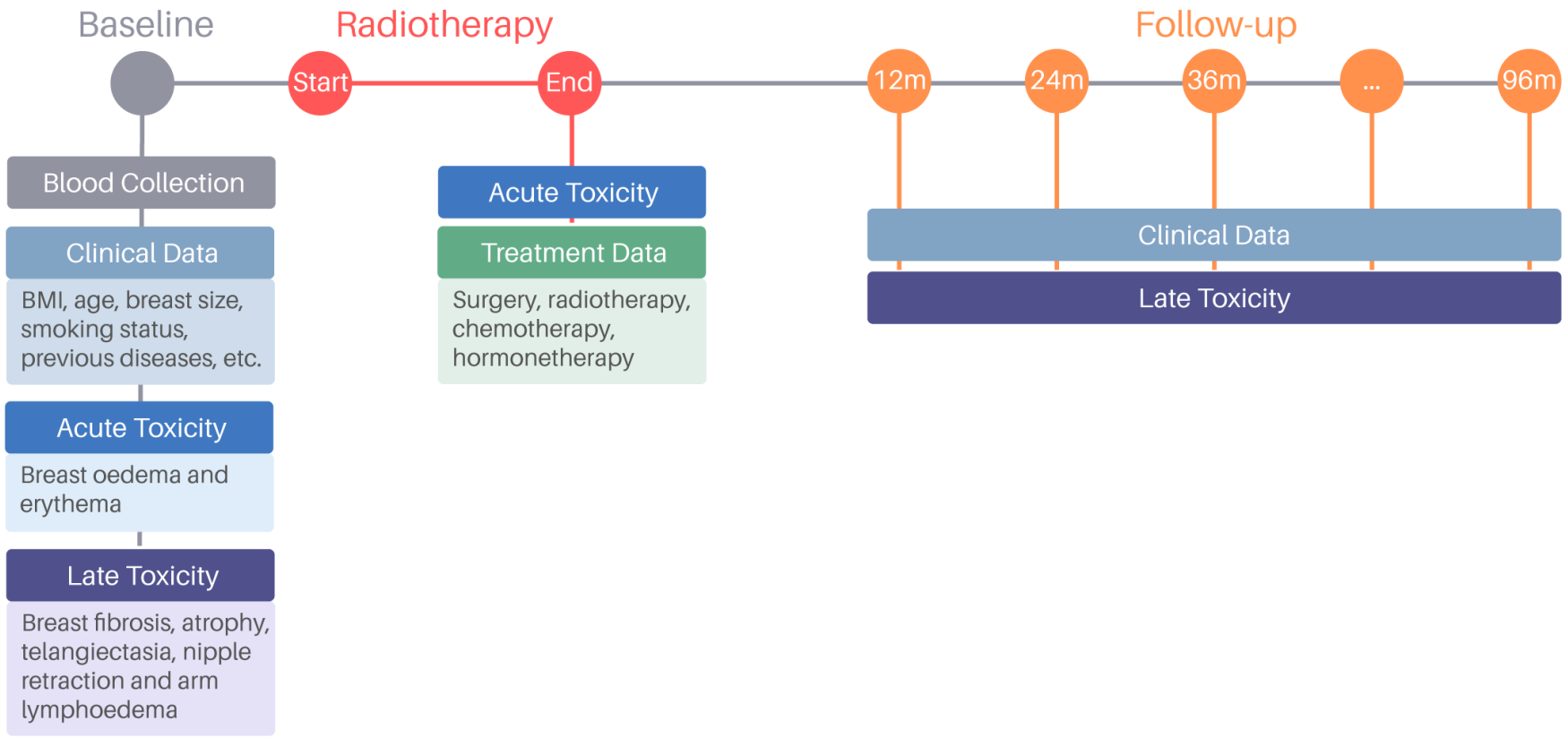
Between 2014 - 2016, eligible patients who received RT for breast, prostate and lung cancer according to local protocols were enrolled into the REQUITE study from several hospitals in multiple countries including Germany, Italy, Spain, UK and US.

Achievements:

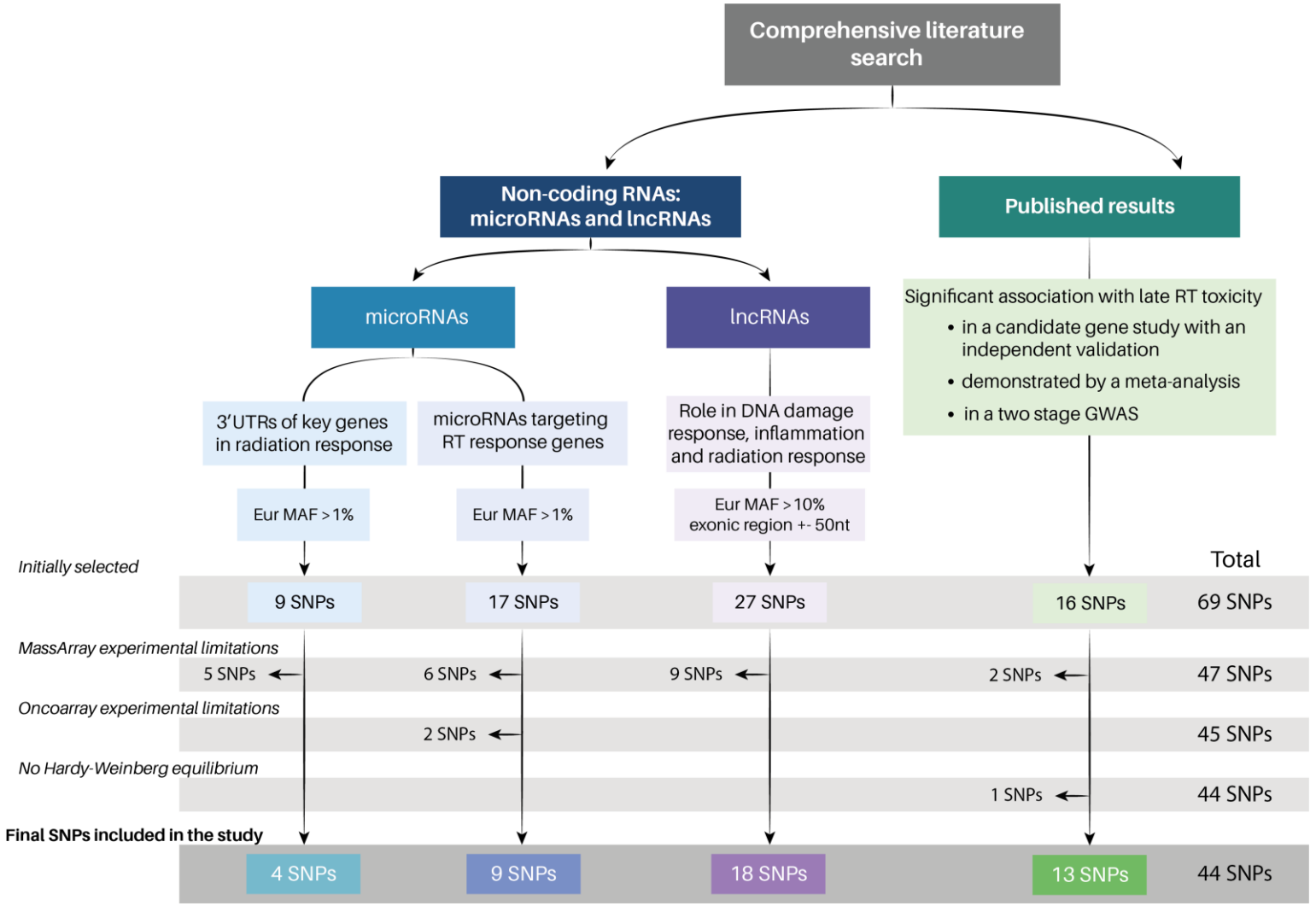
- 4730 breast, prostate and lung cancer patients recruited
- Standardized collection of clinical treatment
- Dosimetric and toxicity data (Common Terminology Criteria for Adverse Events **(CTCAE)**
- DNA and RNA
- A minimum of 2 years' follow-up by end of September 2018



Sample and data were collected following REQUITE protocols

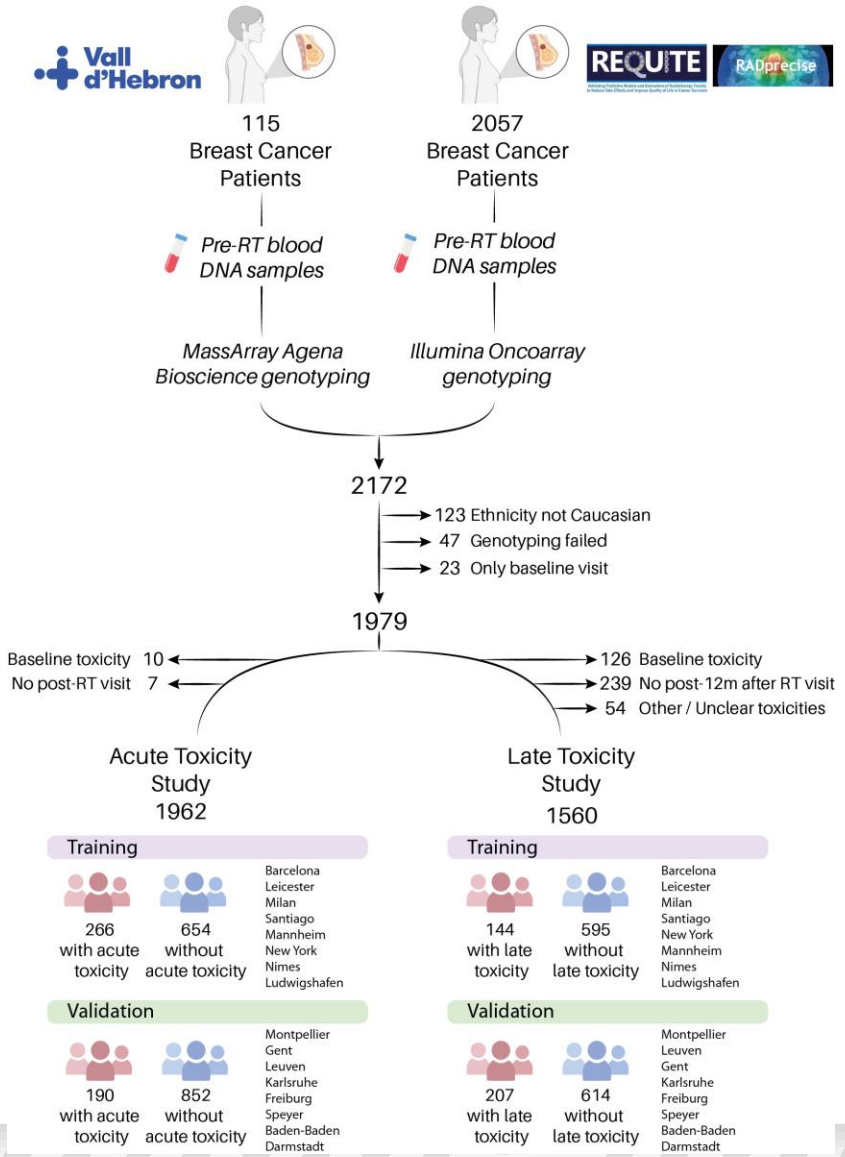


44 SNP were selected for this study



1962 and 1560 BC patients for studying acute and late toxicity respectively

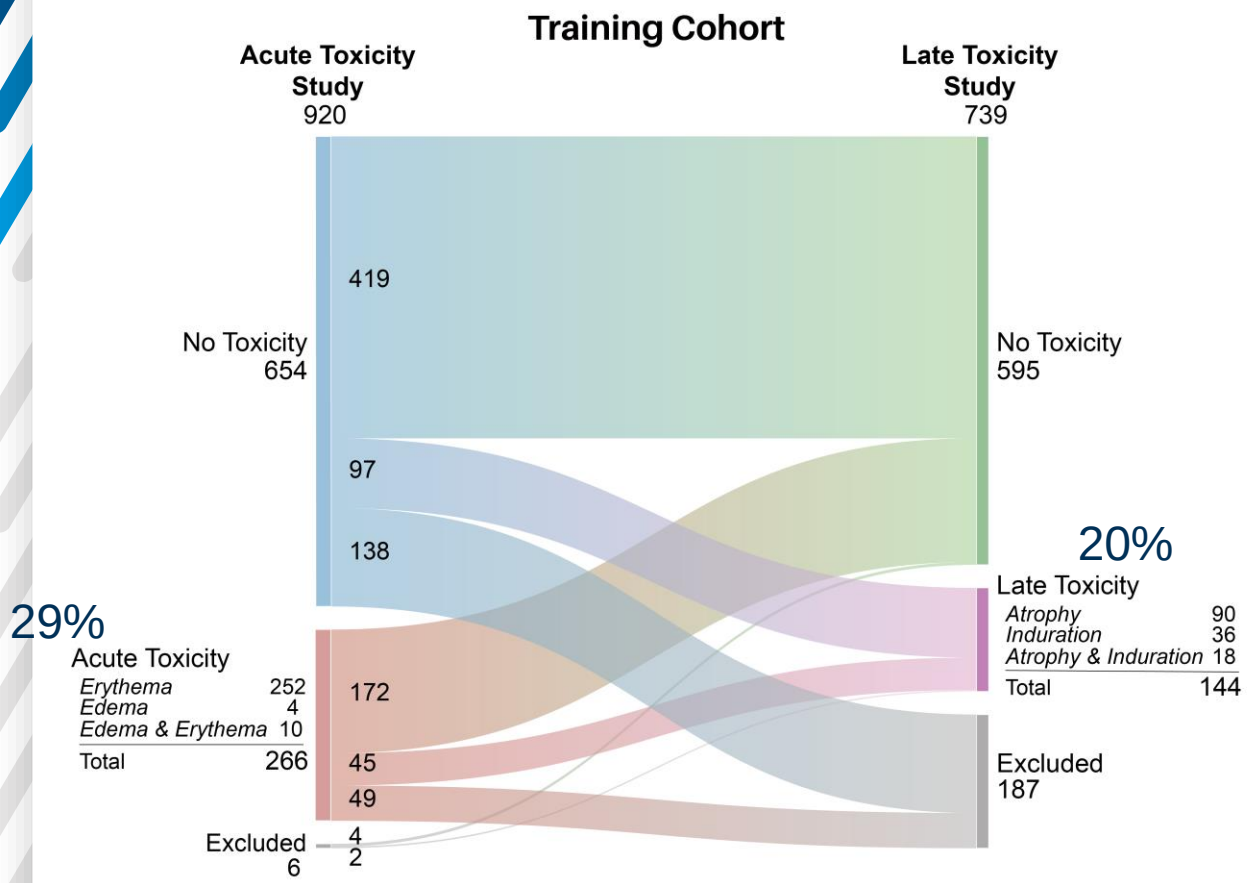
Breast cancer patients suitable for adjuvant RT including those receiving neo-adjuvant chemotherapy (Excluding those with mastectomies and/or bilateral cancers)

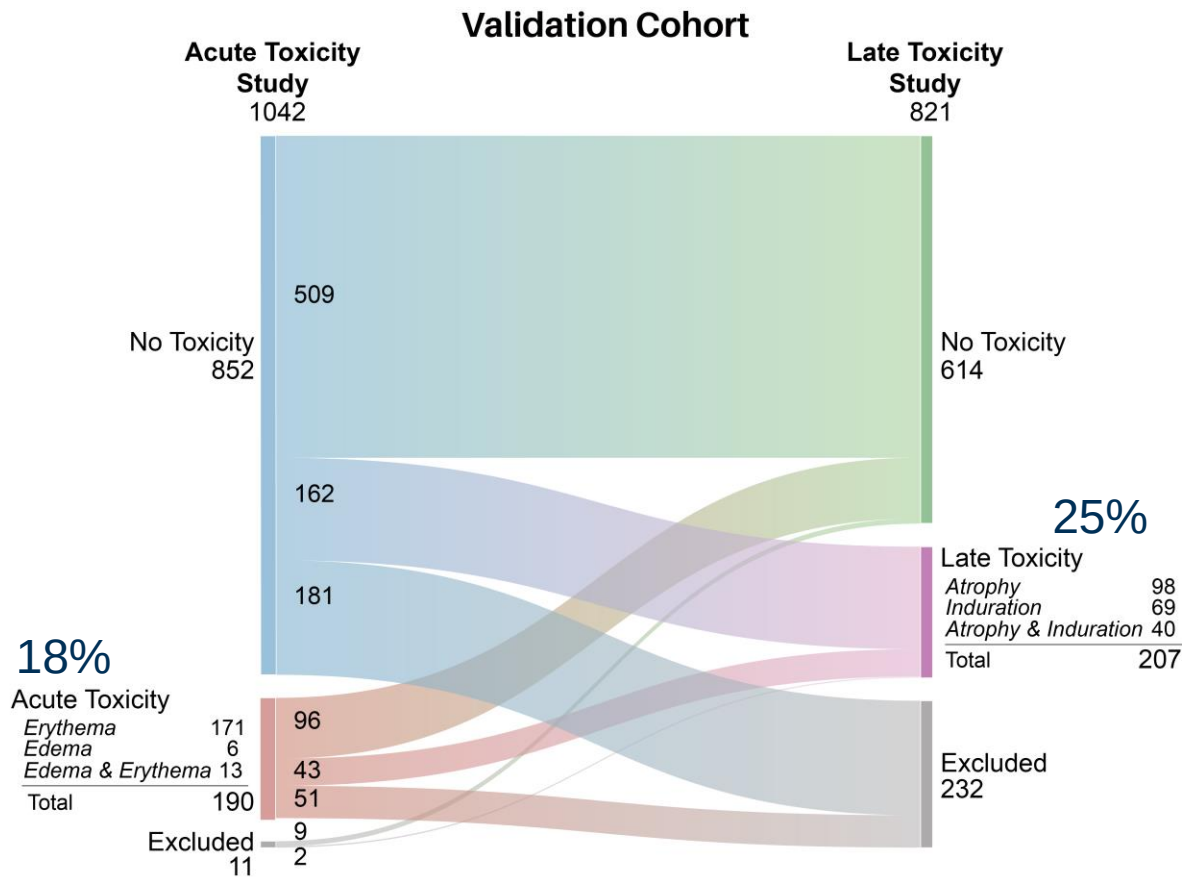


Oedema and Erythema

Atrophy, Fibrosis and Oedema

Around 23% of patients had acute or late RT-induced toxicities





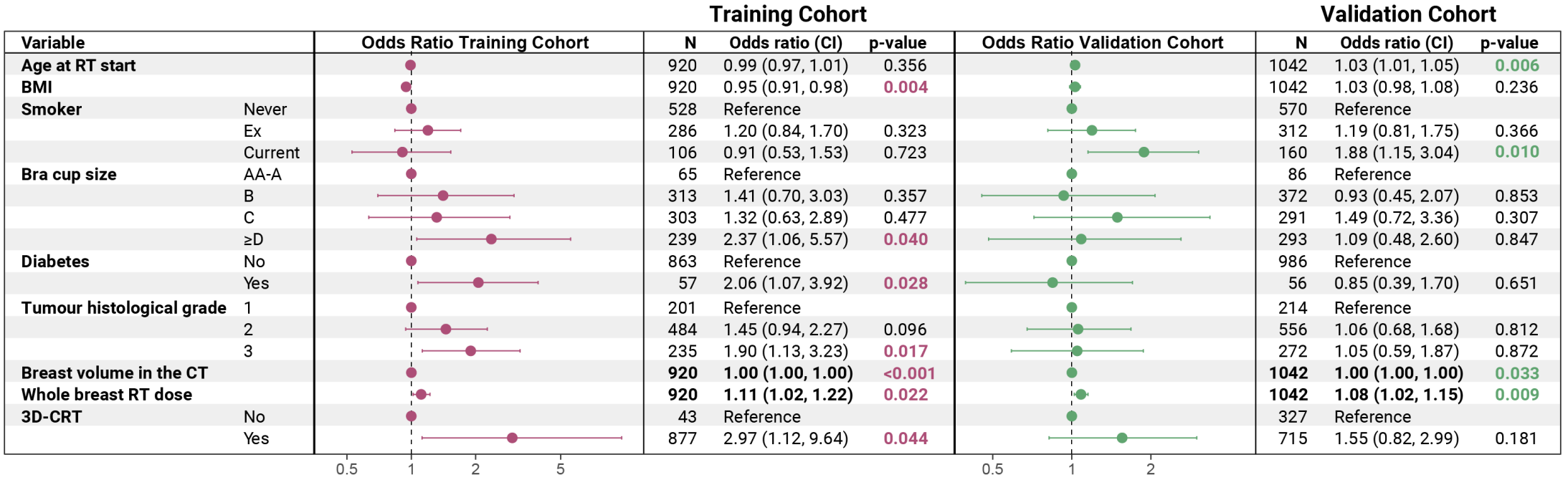
The most common acute toxicity was erythema (around 93%) and atrophy was the most common late toxicity (around 55%).

“Breast volume in the CT” and “Whole breast RT dose” variables associated with acute toxicity

Acute toxicity | Multivariate Logistic Regression Model - Clinical Data

77 clinical variables → **20** selected included in the model → **9** significant associations → **2** validated associations

Significant associations:



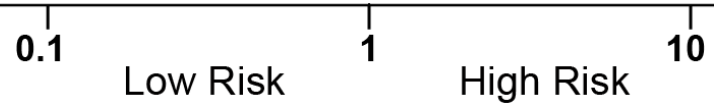
		Training Cohort				Validation Cohort		
Variable		**Odds Ratio Training Cohort**	**N**	**Odds ratio (CI)**	**p-value**	**Odds Ratio Validation Cohort**	**N**	**Odds ratio (CI)**	**p-value**
Age at RT start			920	0.99 (0.97, 1.01)	0.356		1042	1.03 (1.01, 1.05)	**0.006**
BMI			920	0.95 (0.91, 0.98)	**0.004**		1042	1.03 (0.98, 1.08)	0.236
Smoker	Never		528	Reference			570	Reference	
	Ex		286	1.20 (0.84, 1.70)	0.323		312	1.19 (0.81, 1.75)	0.366
	Current		106	0.91 (0.53, 1.53)	0.723		160	1.88 (1.15, 3.04)	**0.010**
Bra cup size	AA-A		65	Reference			86	Reference	
	B		313	1.41 (0.70, 3.03)	0.357		372	0.93 (0.45, 2.07)	0.853
	C		303	1.32 (0.63, 2.89)	0.477		291	1.49 (0.72, 3.36)	0.307
	≥D		239	2.37 (1.06, 5.57)	**0.040**		293	1.09 (0.48, 2.60)	0.847
Diabetes	No		863	Reference			986	Reference	
	Yes		57	2.06 (1.07, 3.92)	**0.028**		56	0.85 (0.39, 1.70)	0.651
Tumour histological grade	1		201	Reference			214	Reference	
	2		484	1.45 (0.94, 2.27)	0.096		556	1.06 (0.68, 1.68)	0.812
	3		235	1.90 (1.13, 3.23)	**0.017**		272	1.05 (0.59, 1.87)	0.872
Breast volume in the CT			**920**	**1.00 (1.00, 1.00)**	**<0.001**		**1042**	**1.00 (1.00, 1.00)**	**0.033**
Whole breast RT dose			**920**	**1.11 (1.02, 1.22)**	**0.022**		**1042**	**1.08 (1.02, 1.15)**	**0.009**
3D-CRT	No		43	Reference			327	Reference	
	Yes		877	2.97 (1.12, 9.64)	**0.044**		715	1.55 (0.82, 2.99)	0.181

Combination of clinical variables & SNPs significantly associated with acute toxicity

Acute toxicity | Logistic Regression Model with risk score categories

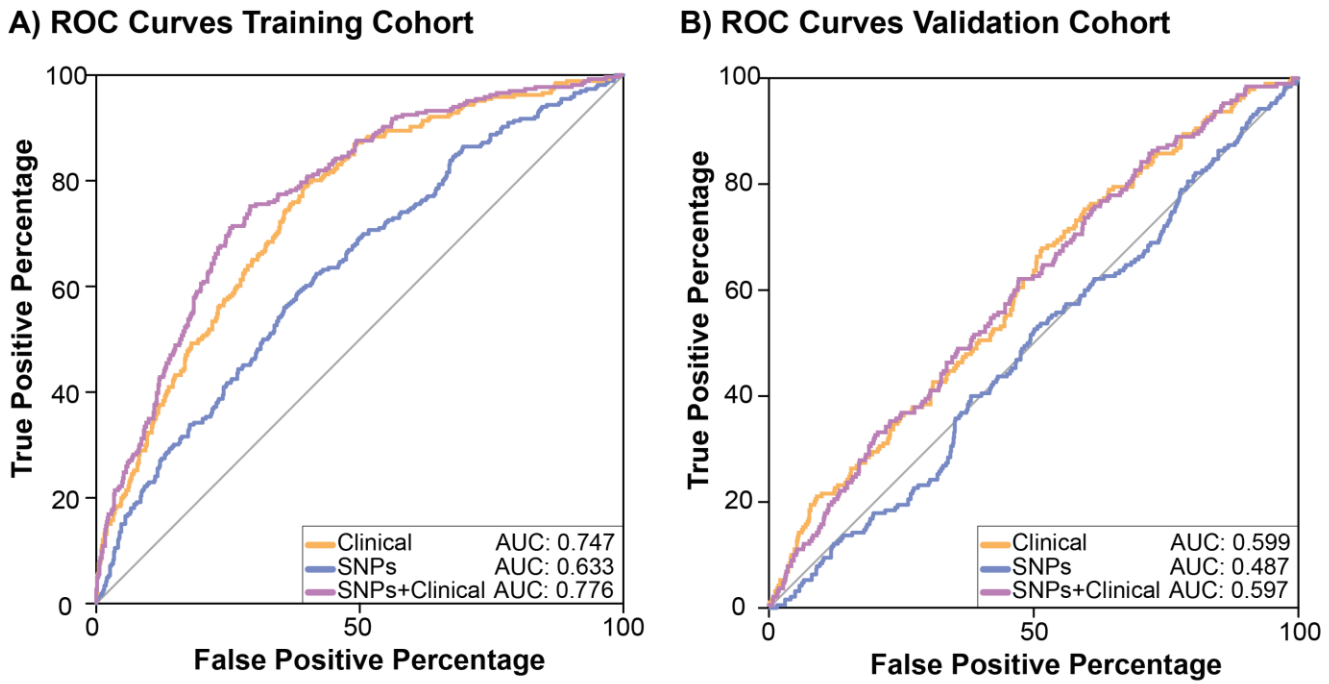
A) Acute Toxicity Study

Data	Cohort	Odds Ratio (log scale)	Odds ratio (CI)	p-value
Clinical	Training		**5.84 (4.19 - 8.24)**	**< 0.001**
	Validation		**1.42 (1.03 - 1.94)**	**0.0305**
SNPs	Training		**2.31 (1.73 - 3.10)**	**< 0.001**
	Validation		0.98 (0.71 - 1.34)	0.8770
SNPs + Clinical	Training		**7.24 (5.26 - 10.07)**	**< 0.001**
	Validation		**1.66 (1.21 - 2.29)**	**0.0017**



Combined/clinical model were the best in the validation cohort with an AUC of 0.60

Acute toxicity | Logistic Regression Model with risk score categories



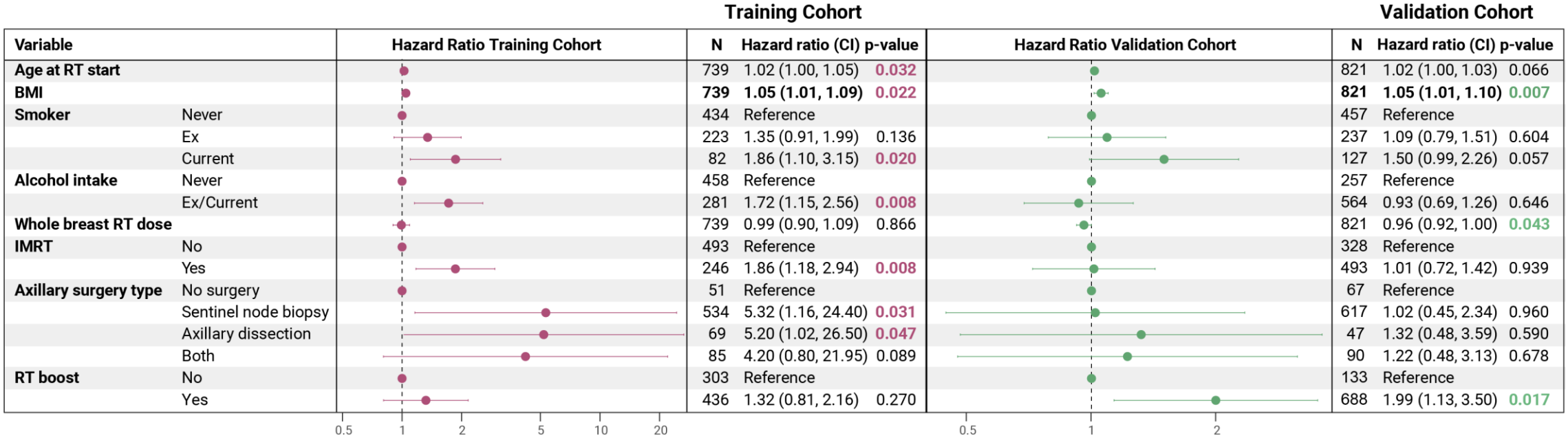
Rattay et al., Frontiers in Oncology (2020)

“Body Mass Index” variable associated with late toxicity

Late toxicity | Multivariate Cox Regression Model - Clinical Data

77 clinical variables → **19** selected included in the model → **8** significant associations → **1** validated associations

Significant associations:

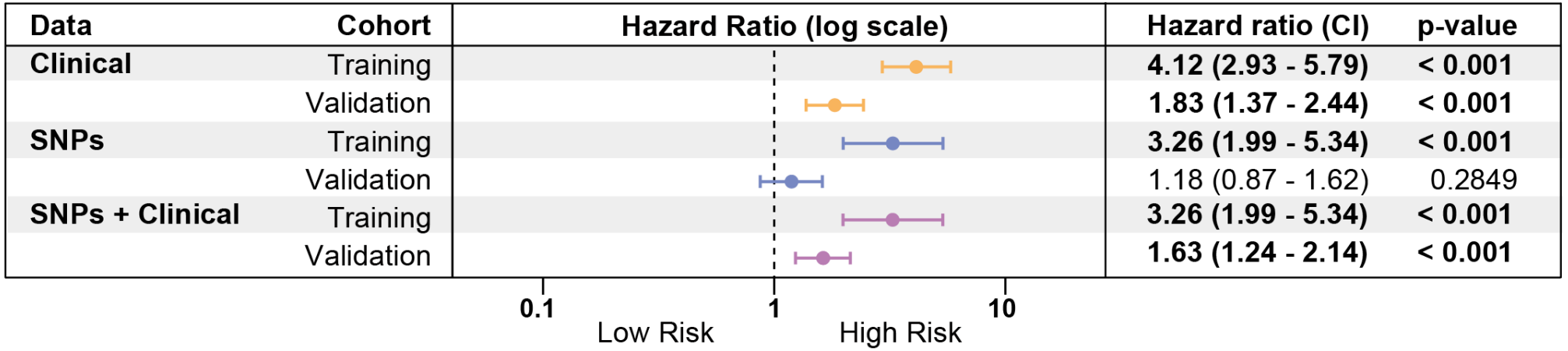


		Training Cohort			Validation Cohort		
Variable		**Hazard Ratio Training Cohort**	**N**	**Hazard ratio (CI) p-value**	**Hazard Ratio Validation Cohort**	**N**	**Hazard ratio (CI) p-value**
Age at RT start			739	1.02 (1.00, 1.05) 0.032		821	1.02 (1.00, 1.03) 0.066
BMI			**739**	**1.05 (1.01, 1.09)** 0.022		**821**	**1.05 (1.01, 1.10)** 0.007
Smoker	Never		434	Reference		457	Reference
	Ex		223	1.35 (0.91, 1.99) 0.136		237	1.09 (0.79, 1.51) 0.604
	Current		82	1.86 (1.10, 3.15) 0.020		127	1.50 (0.99, 2.26) 0.057
Alcohol intake	Never		458	Reference		257	Reference
	Ex/Current		281	1.72 (1.15, 2.56) 0.008		564	0.93 (0.69, 1.26) 0.646
Whole breast RT dose			739	0.99 (0.90, 1.09) 0.866		821	0.96 (0.92, 1.00) 0.043
IMRT	No		493	Reference		328	Reference
	Yes		246	1.86 (1.18, 2.94) 0.008		493	1.01 (0.72, 1.42) 0.939
Axillary surgery type	No surgery		51	Reference		67	Reference
	Sentinel node biopsy		534	5.32 (1.16, 24.40) 0.031		617	1.02 (0.45, 2.34) 0.960
	Axillary dissection		69	5.20 (1.02, 26.50) 0.047		47	1.32 (0.48, 3.59) 0.590
	Both		85	4.20 (0.80, 21.95) 0.089		90	1.22 (0.48, 3.13) 0.678
RT boost	No		303	Reference		133	Reference
	Yes		436	1.32 (0.81, 2.16) 0.270		688	1.99 (1.13, 3.50) 0.017

Combination of clinical variables & SNPs significantly associated with late toxicity

Late toxicity | Cox Regression Model with risk score categories

B) Late Toxicity Study



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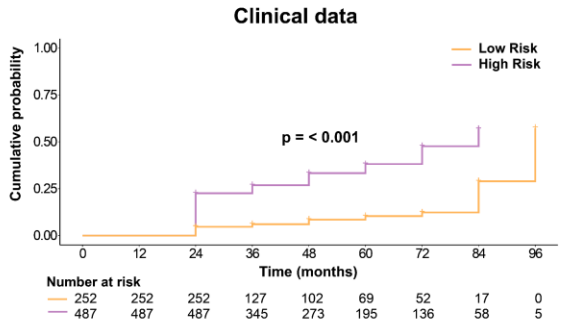
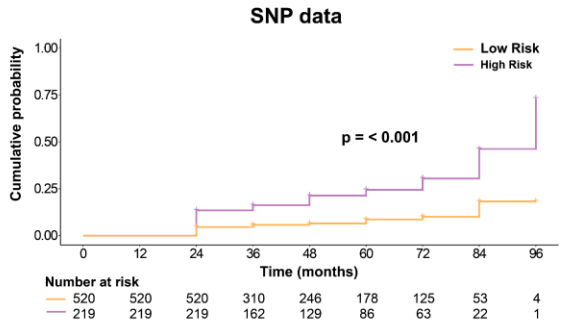
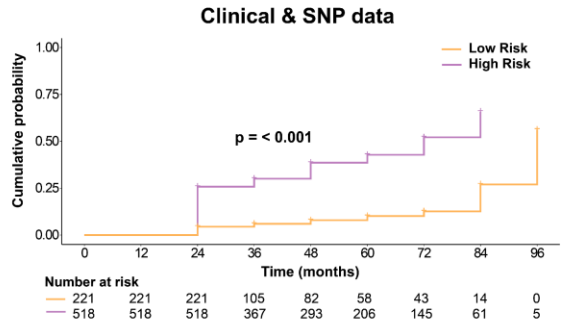
Combination of clinical variables & SNPs significantly associated with late toxicity

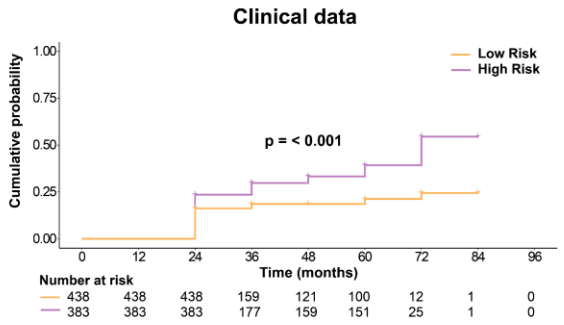
Training Cohort

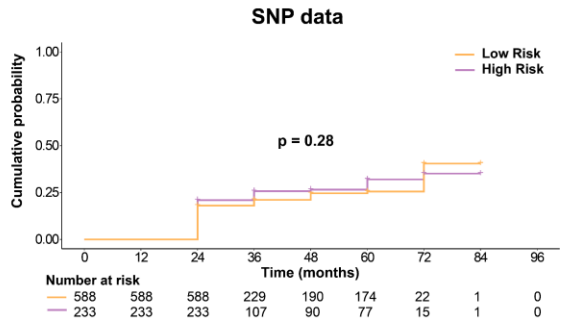


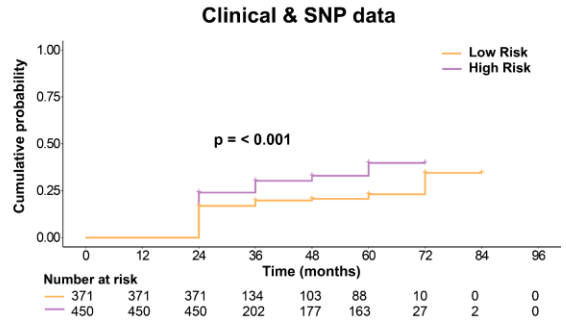




Validation Cohort

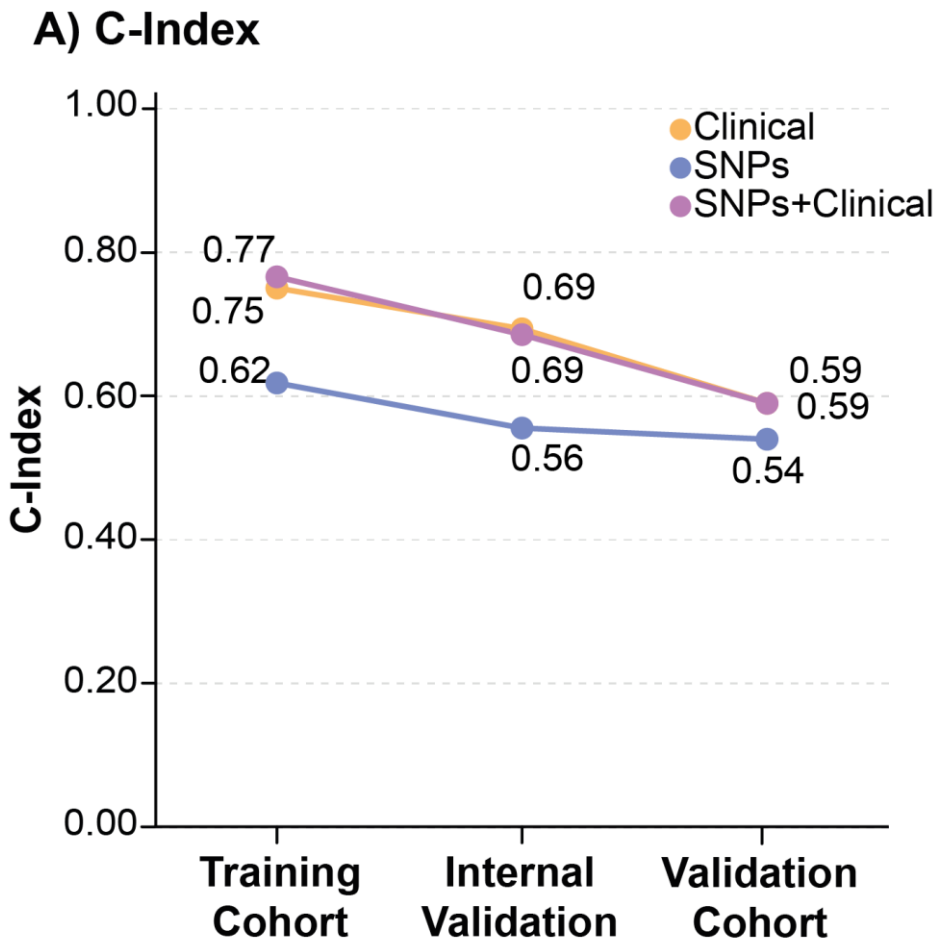






Combined/clinical model were the best in the validation cohort with c-index of 0.59

Late toxicity | Cox Regression Model with risk score categories



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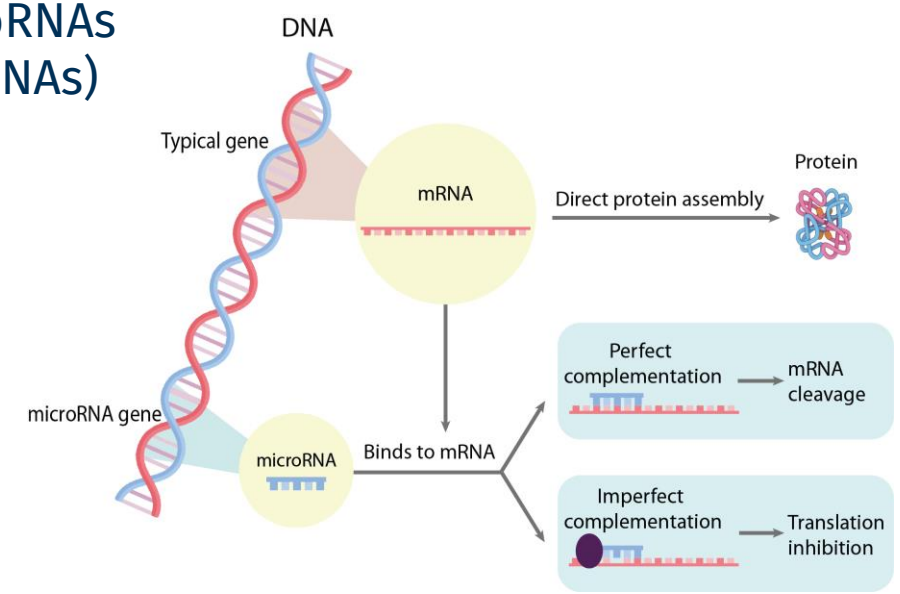
1. The **predictive value** for clinical **toxicity** after radiotherapy in breast cancer patients **of the studied set of candidate SNPs**, composed of previous reported in the literature and those located in non-coding RNAs, was **limited** or **insufficient compared to** the influence of **clinical variables**.

2. Patient-related factors such as **breast volume**, **whole breast radiotherapy dose**, and **BMI** had a significant **role** in determining the likelihood of developing both **acute** and **late** toxicities post-radiotherapy in **breast** cancer patients.

Non-coding RNAs: an unexplored biomarker of RT-induced toxicity

Cellular Markers | Genomics | Transcriptomics

MicroRNAs (miRNAs)



microRNAs modulate key cellular pathways that mediate response to radiation.
However, their role in radiotherapy-induced toxicity in normal tissues remains largely unexplored.

Zhang et al., Nat Commun (2014)
Yu et al., Journal of Gene Medicine (2023)

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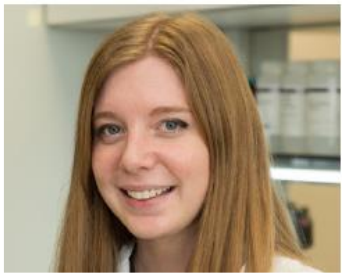
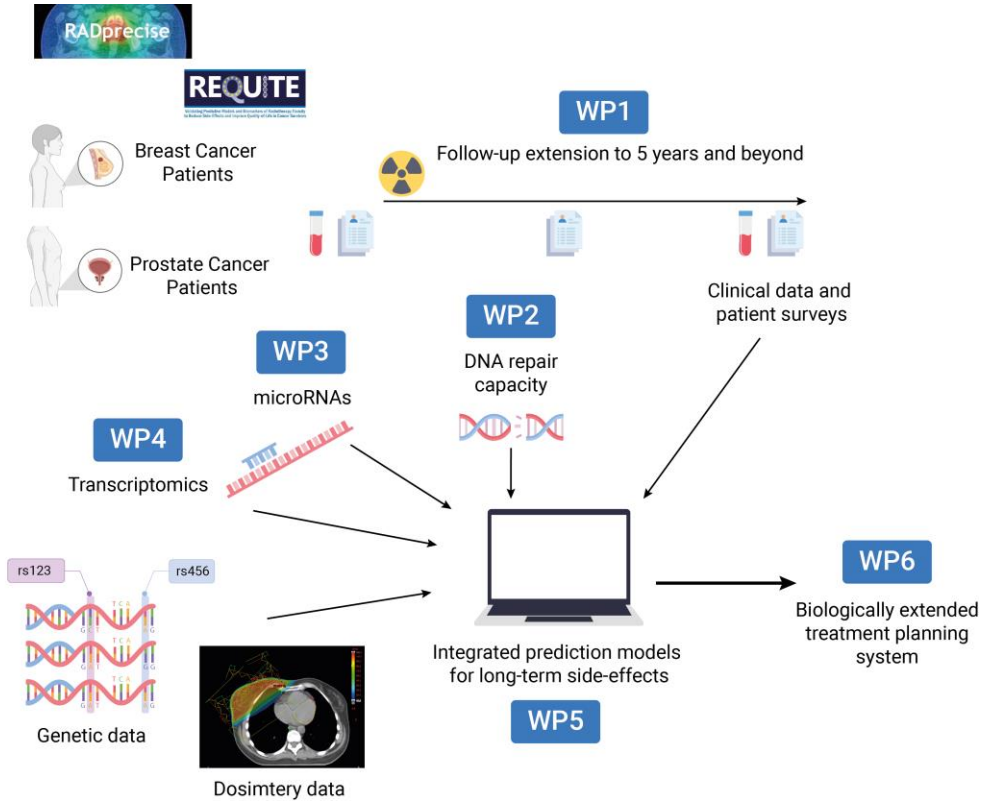
Identify **differentially expressed miRNAs** as **novel biomarkers** for **radiosensitivity** in blood cells of **PC** and **BC patients**.



Ester Aguado Flor

RADprecise

This project aims to improve the prediction of the risk for long-term side effects after RT using multiple biomarkers from blood in addition to clinical and personal factors. Information from the prediction models will be incorporated into RT treatment planning systems.



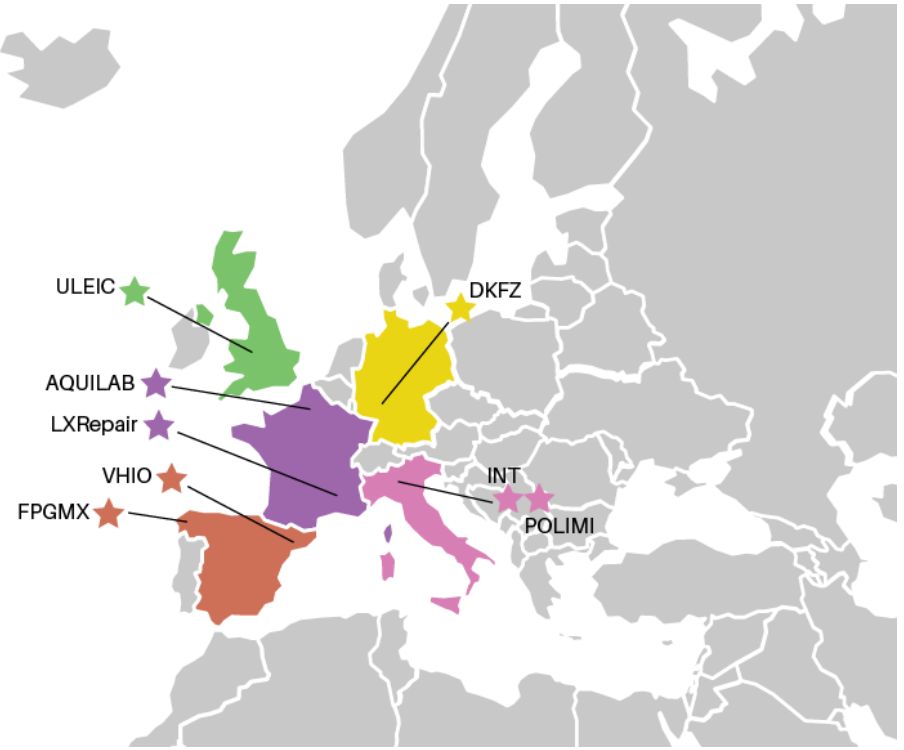
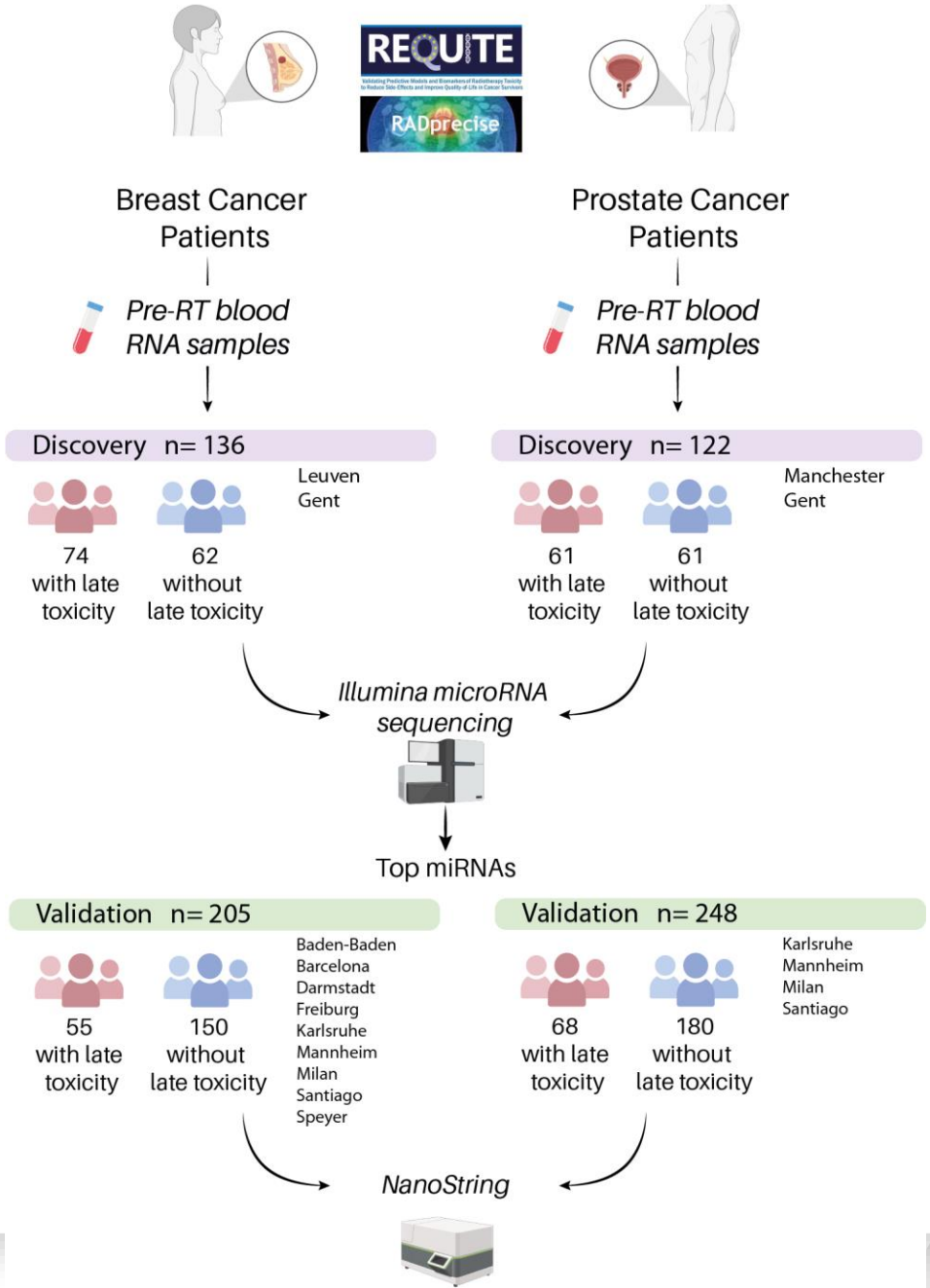


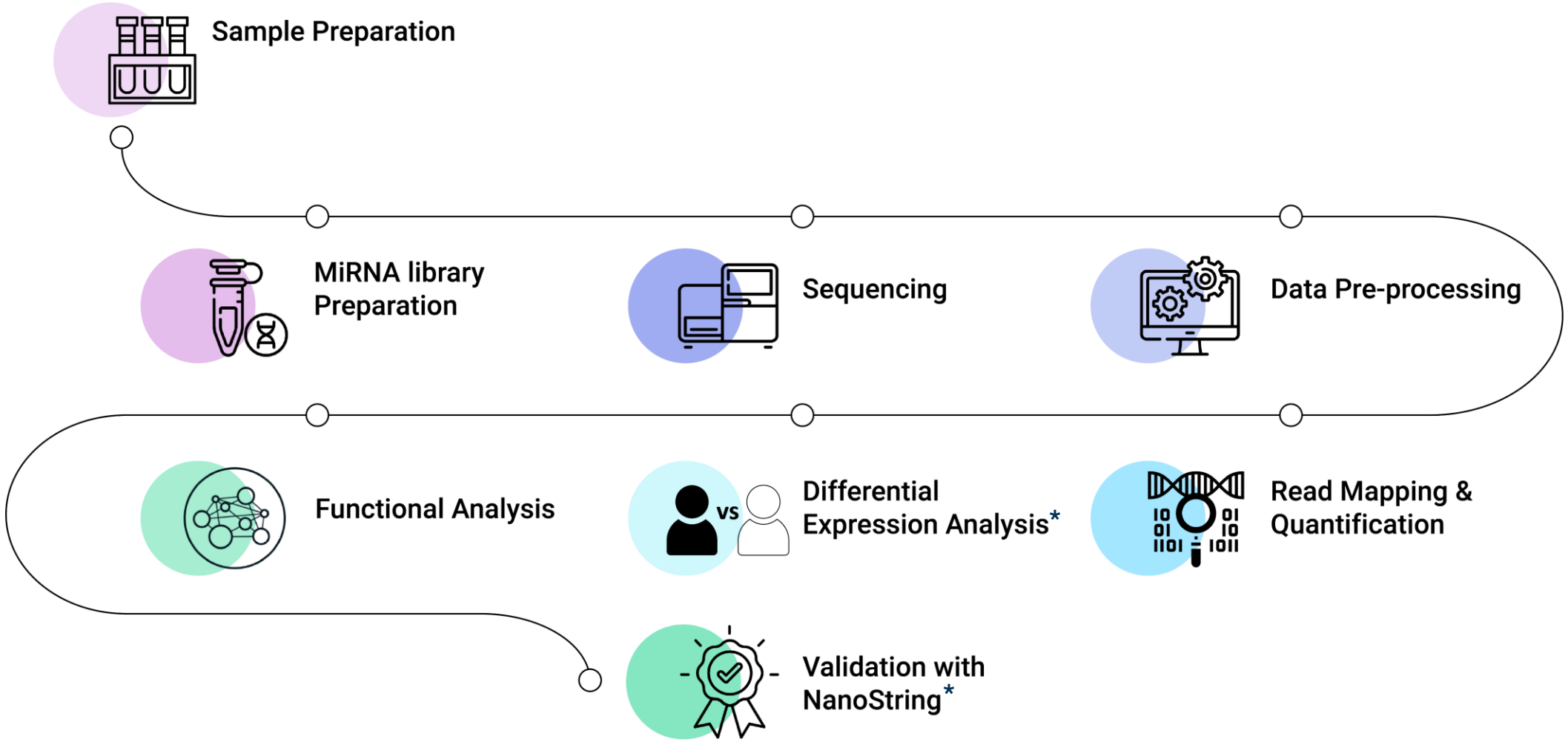
Figure adapted from RADprecise project proposal.

Patient Selection

Atrophy and Fibrosis



Experimental Workflow



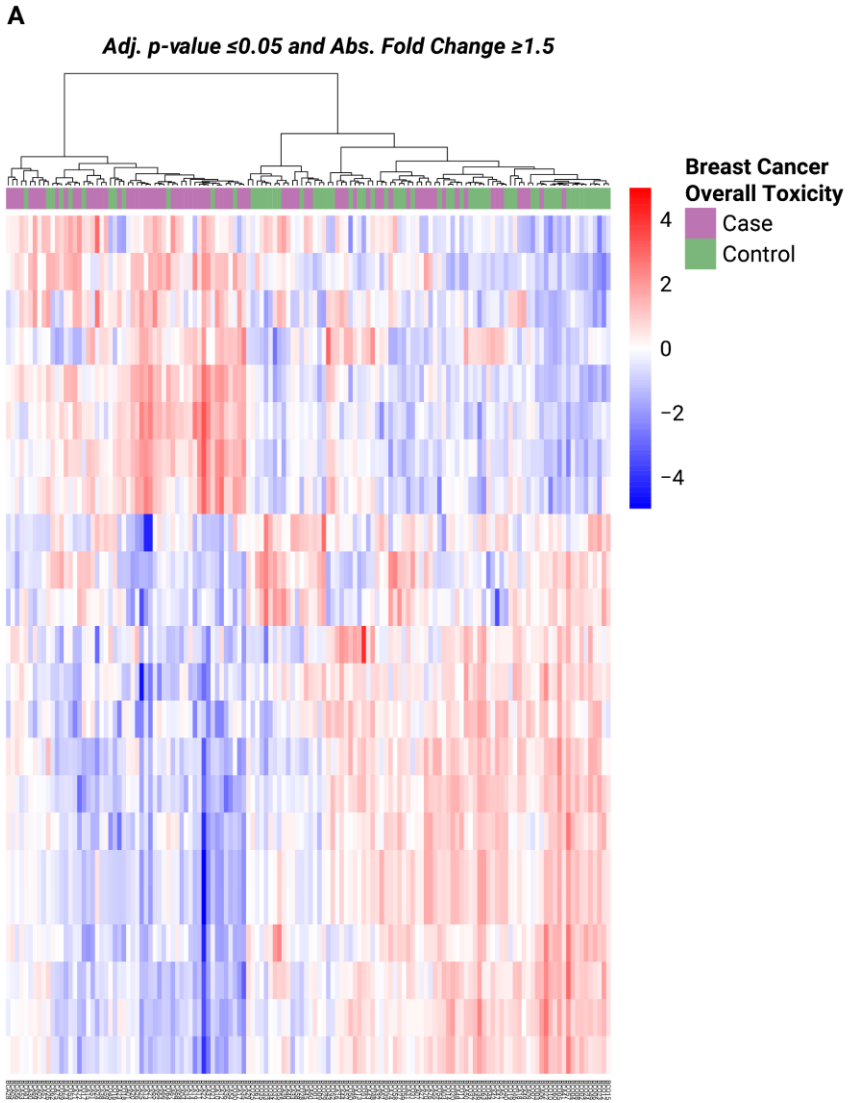
*In collaboration with Bioinformatics Group (VHIO)

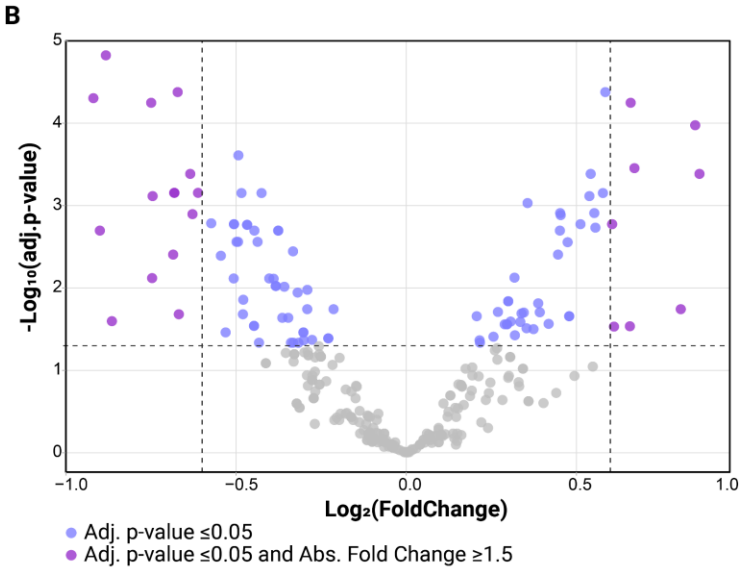
There is a differential expression pattern of microRNAs between BC patients with toxicity and those without

DISCOVERY

Breast Cancer | Overall Toxicity

90 miRNAs with an adj. p-value <0.05.

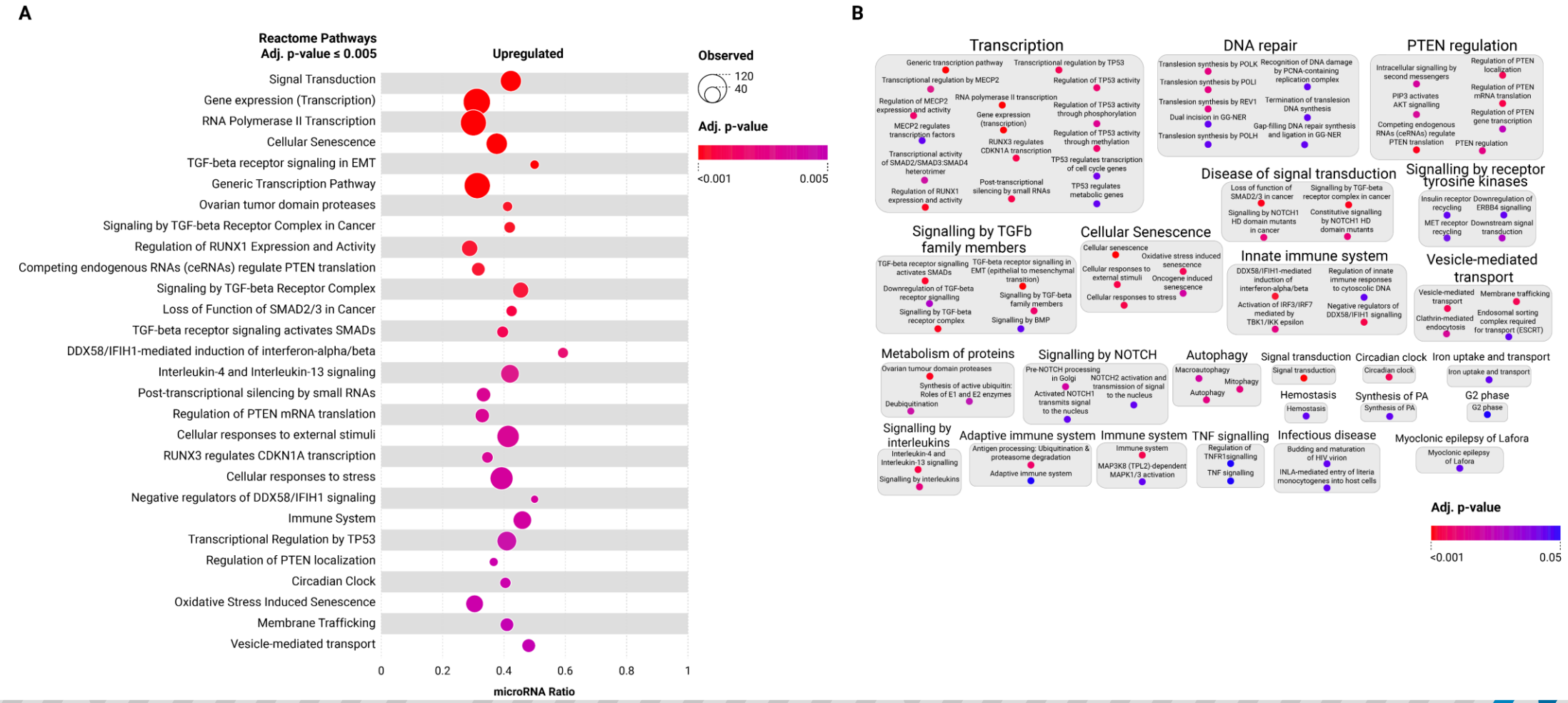




BC patients with toxicity show an enrichment of transcription, DNA repair and cellular senescence

Breast Cancer | Overall Toxicity

84 pathways with an adj. p-value <0.05.

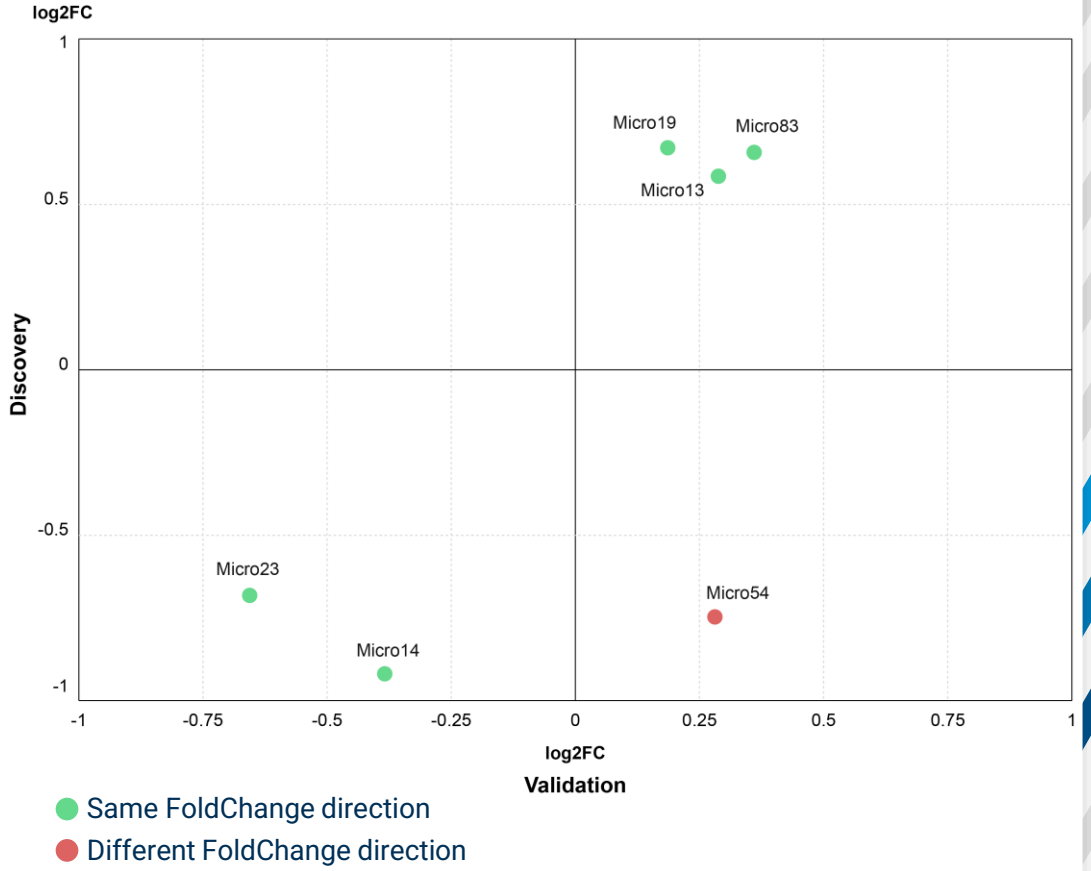


5 microRNAs were validated as biomarkers of overall toxicity in BC patients

Out of the 22 selected for validation, a 5-miRNA signature that was validated externally, indicating the potential use of miRNA profiles as prognostic indicators for severe late radiotherapy-induced toxicity in breast cancer patients.

Encrypted	Discovery Cohort			Validation Cohort		
miRNA	log2FC	p-value	padj	log2FC	p-value	padj
Micro23	**-0.682**	**0.000**	**0.001**	**-0.656**	**0.000**	**0.000**
Micro83	**0.657**	**0.009**	**0.029**	**0.360**	**0.000**	**0.000**
Micro14	**-0.919**	**0.000**	**0.000**	**-0.384**	**0.000**	**0.001**
Micro13	**0.585**	**0.000**	**0.000**	**0.288**	**0.000**	**0.002**
Micro54	-0.747	0.001	0.008	0.281	0.001	0.005
Micro19	**0.671**	**0.000**	**0.000**	**0.186**	**0.002**	**0.006**

Roles in processes like proliferation, fibrosis, inflammation, and apoptosis, which reinforce their utility as biomarkers of radiation toxicity.



What we have learned:

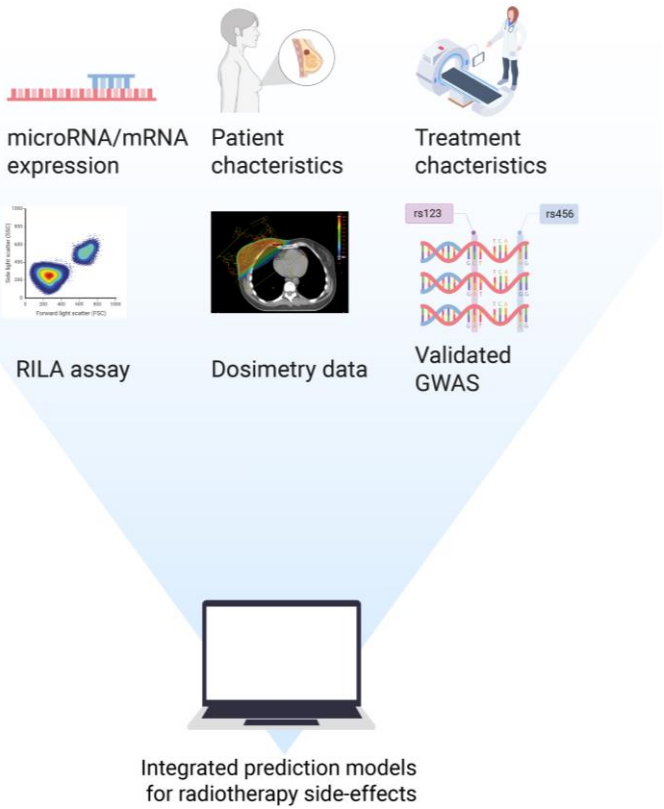
Patients at risk of developing radiotherapy-induced side effects might be identified according to their level of blood markers such as apoptosis and microRNA gene expression

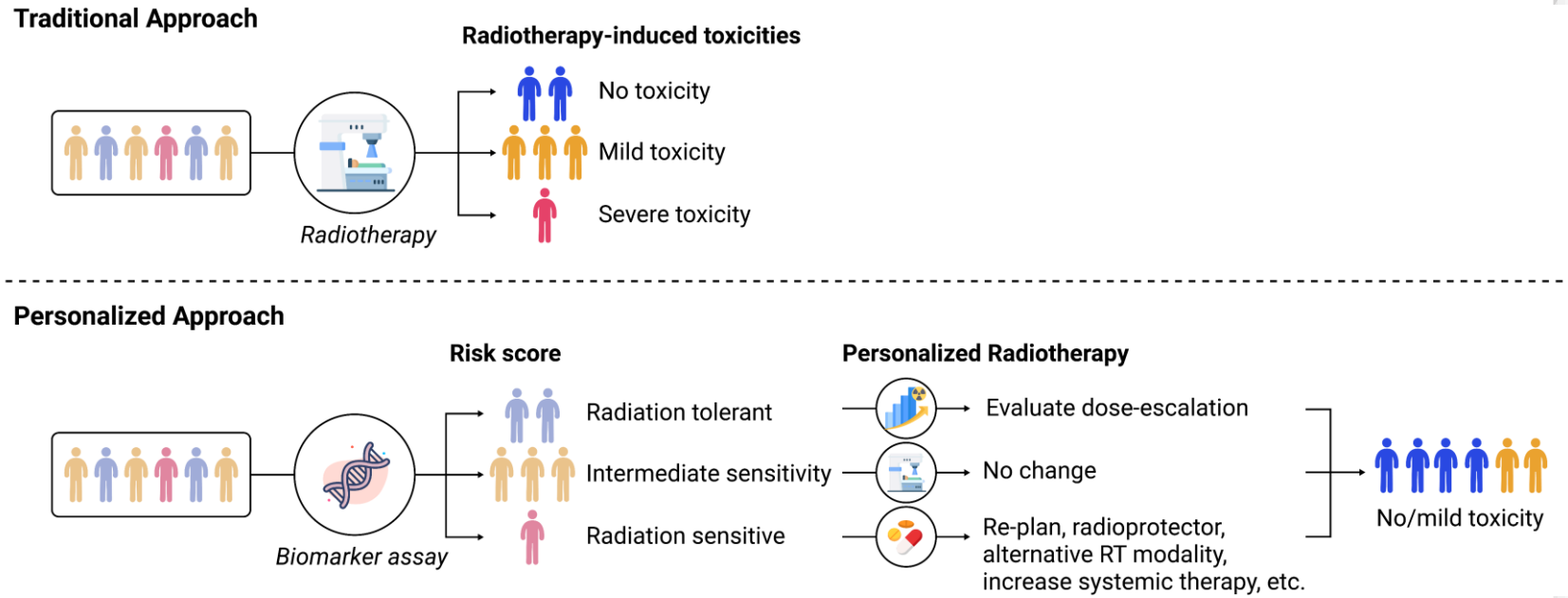
Future Challenges:

Validate the models of prediction toxicity integrating clinical, dosimetry and biological factors

Conduct clinical trials in which patients would be randomized to conventional or biological-modulated treatment groups

A personalized approach, incorporating predictive models, could improve patients' quality of life





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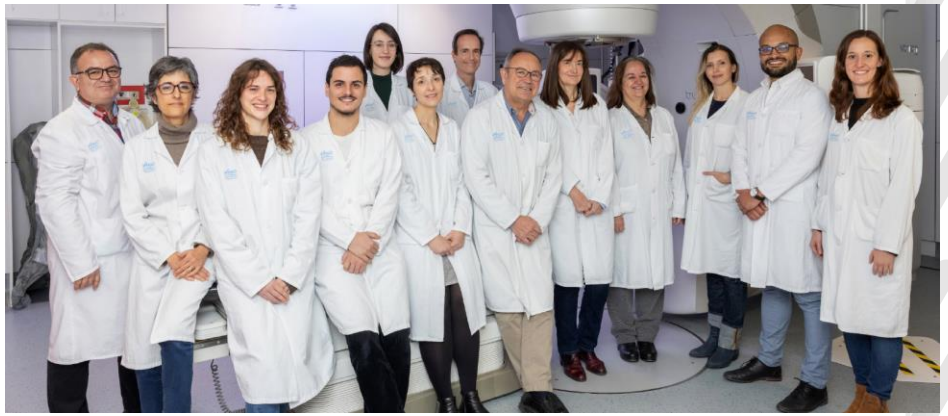

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