Role of microenvironment in tumor progression

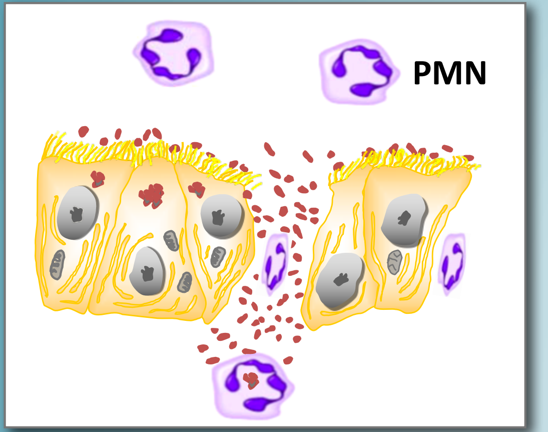
2018-07-11 CIRM, Fr

Patrick Brest
INSERM Researcher
Pr. P. Hofman Team
« Inflammation and Carcinogenesis »

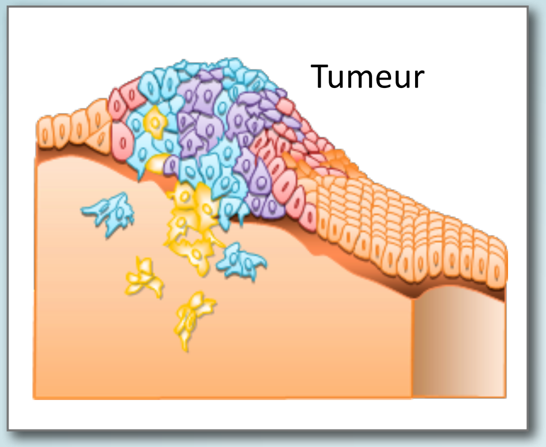
IRCAN
INSTITUT DE RECHERCHE SUR LE CANCER ET LE VIEILLISSEMENT, NICE
INSTITUTE FOR RESEARCH ON CANCER AND AGING, NICE

IRCAN/CHU/CAL

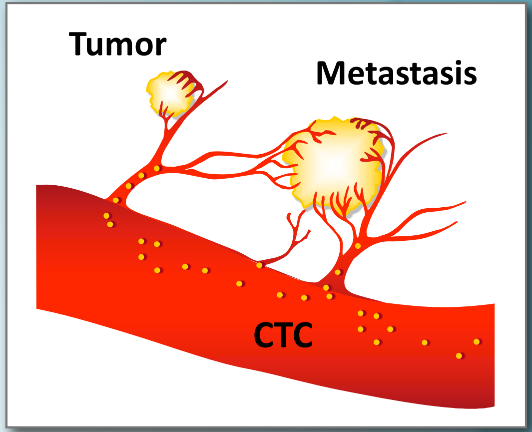
Pollution-Inflammation

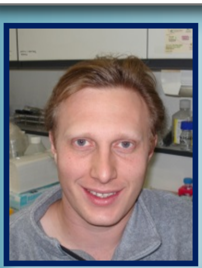


Carcinogenesis



Tumoral Progression





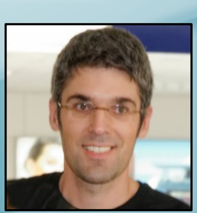
RNA regulation
Genetic predispositions
Tumor µenvironnement

Oncogenes and inflammation

Inflammation and cancer

Circulant Biomarkers
Biobank

Autophagy and cancer

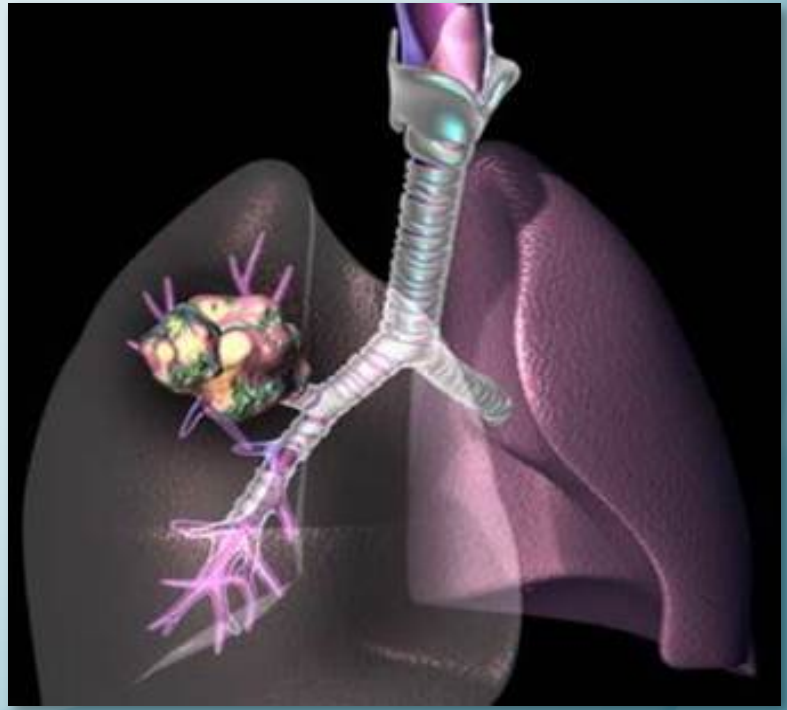


Drug response
System Biology

Non-small-cell lung carcinoma (NSCLC)

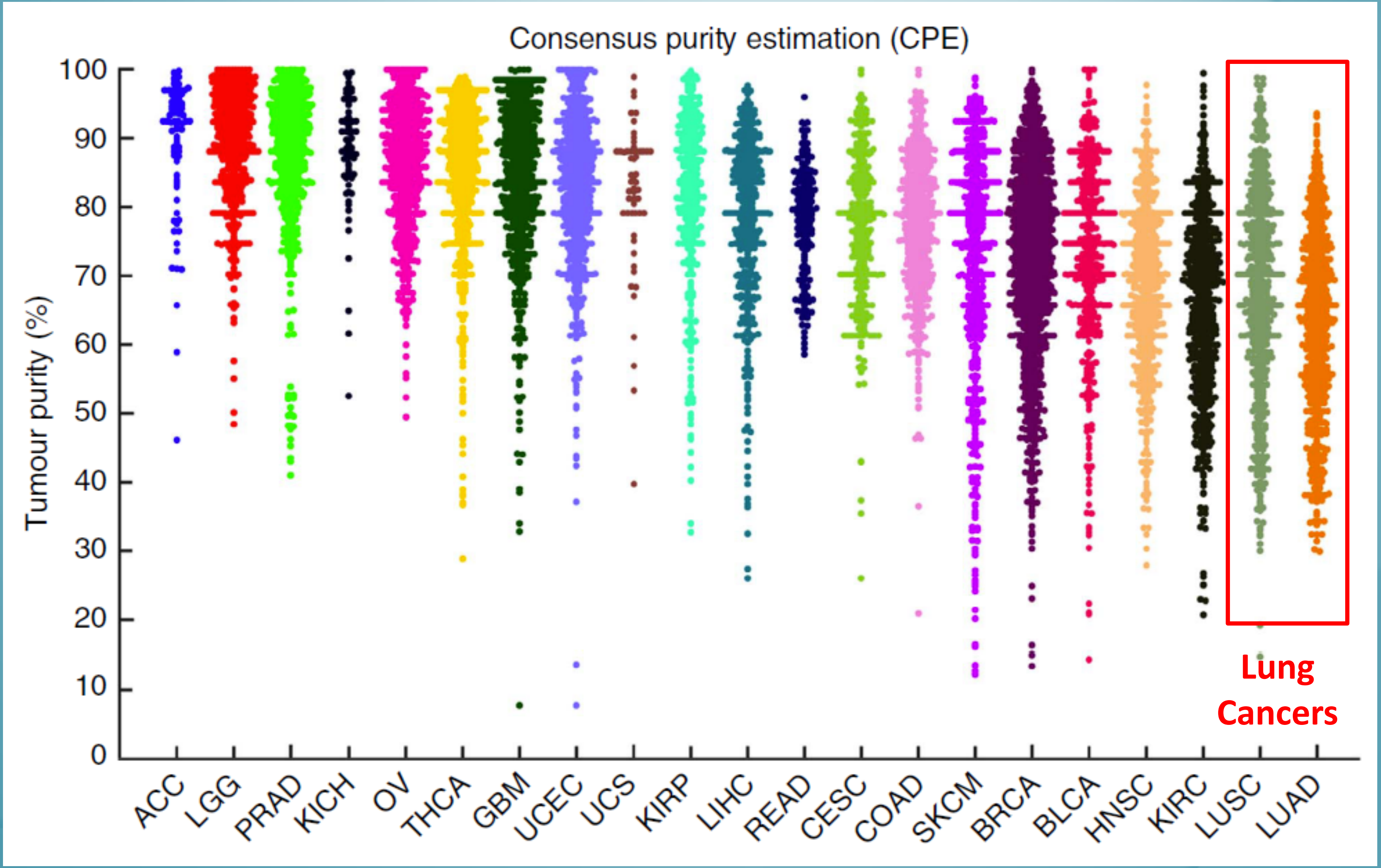
- The leading cause of cancer death in France and Worldwide
- Poor survival
- Late diagnosis
- Ineffective treatments
- Relapse : 40 % early stages I-II

- Prone to metastasize

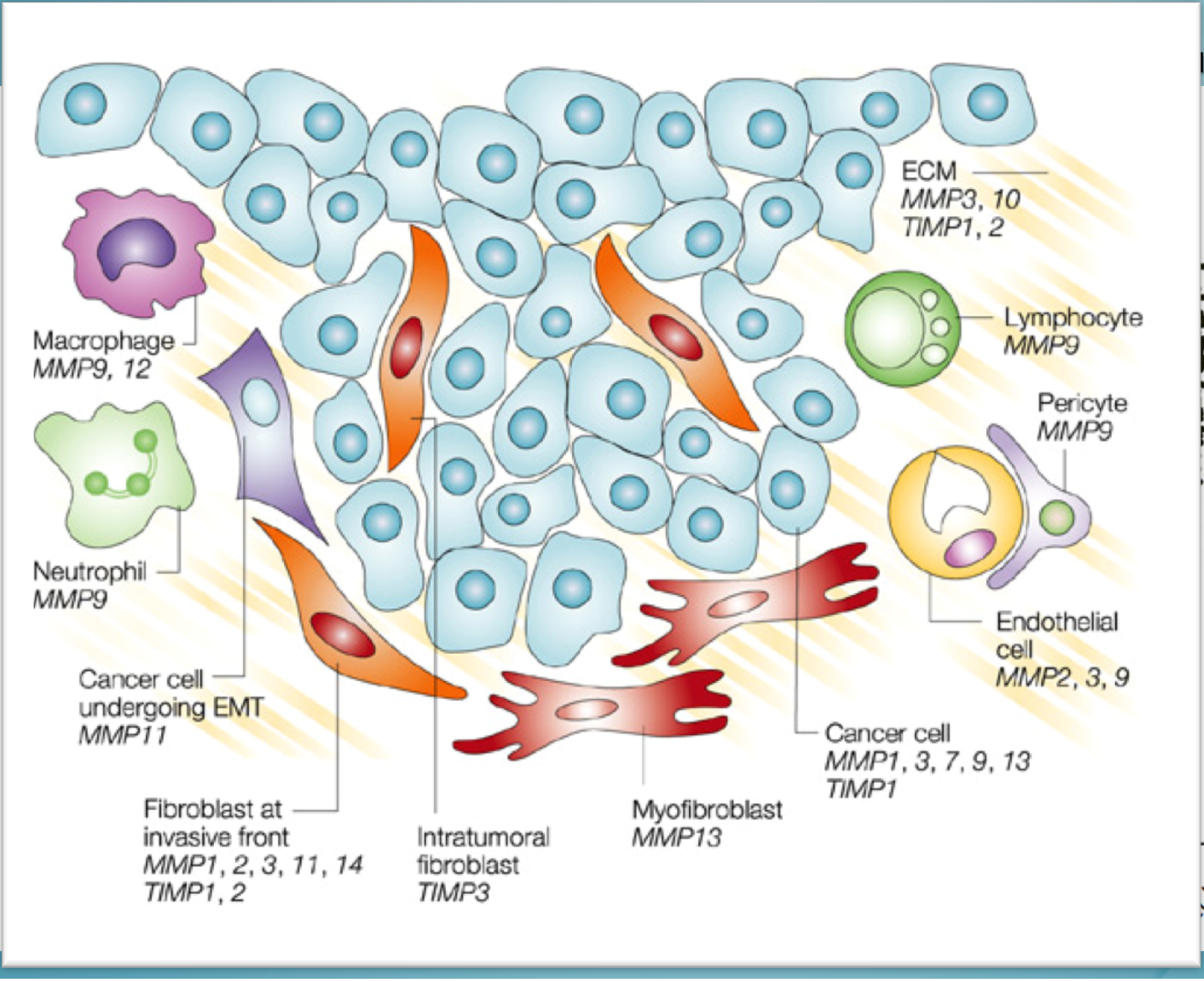


Lung cancer is heterogenous

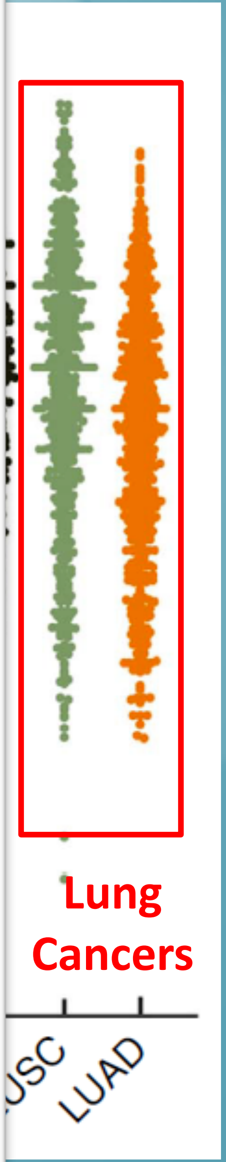
Aran D, et al. Nat Commun. 2015



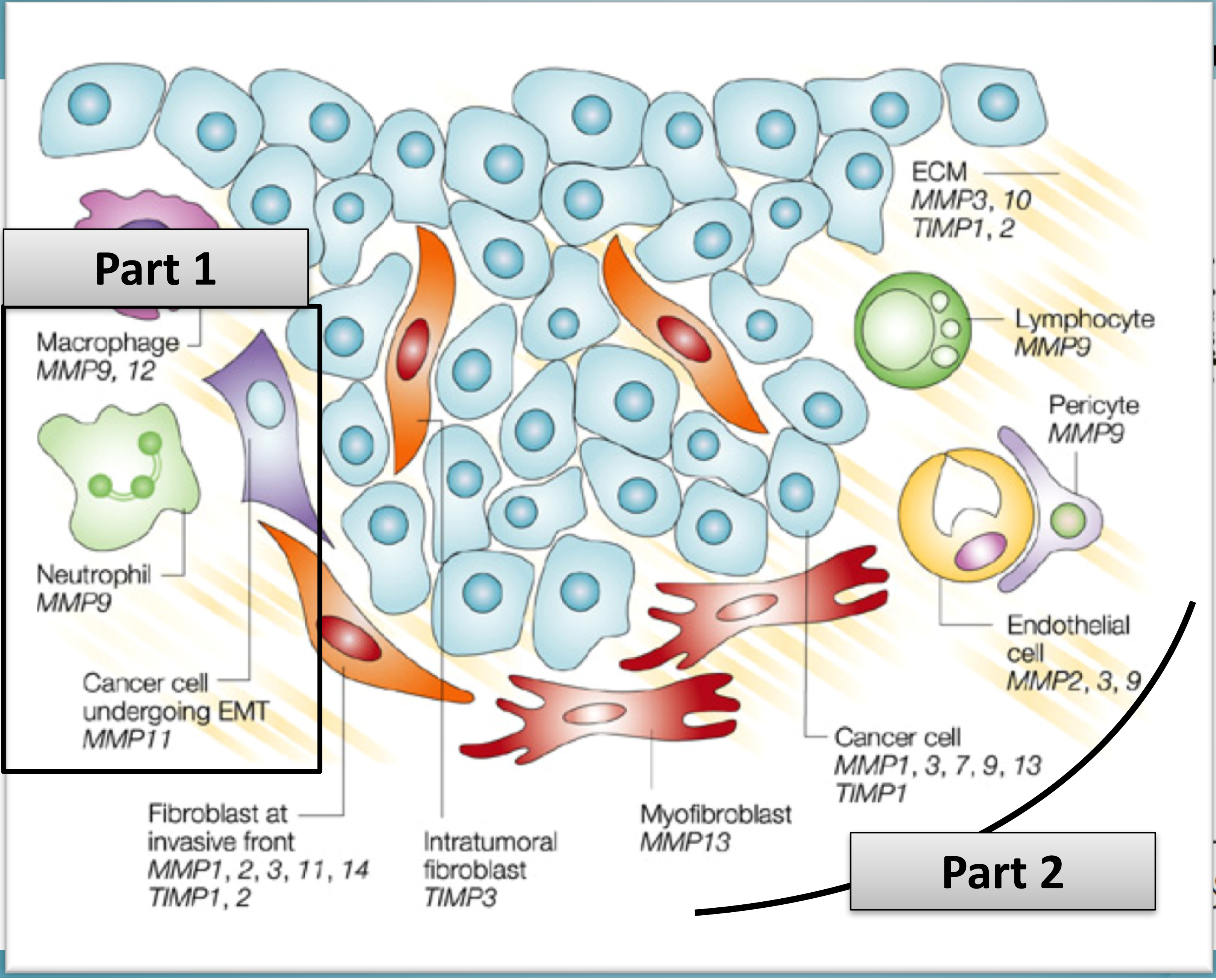
Lung cancer is heterogenous



n. 2015



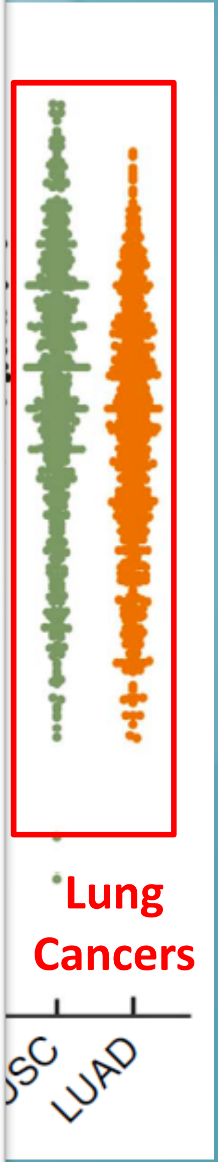
Lung cancer is heterogenous



Part 1

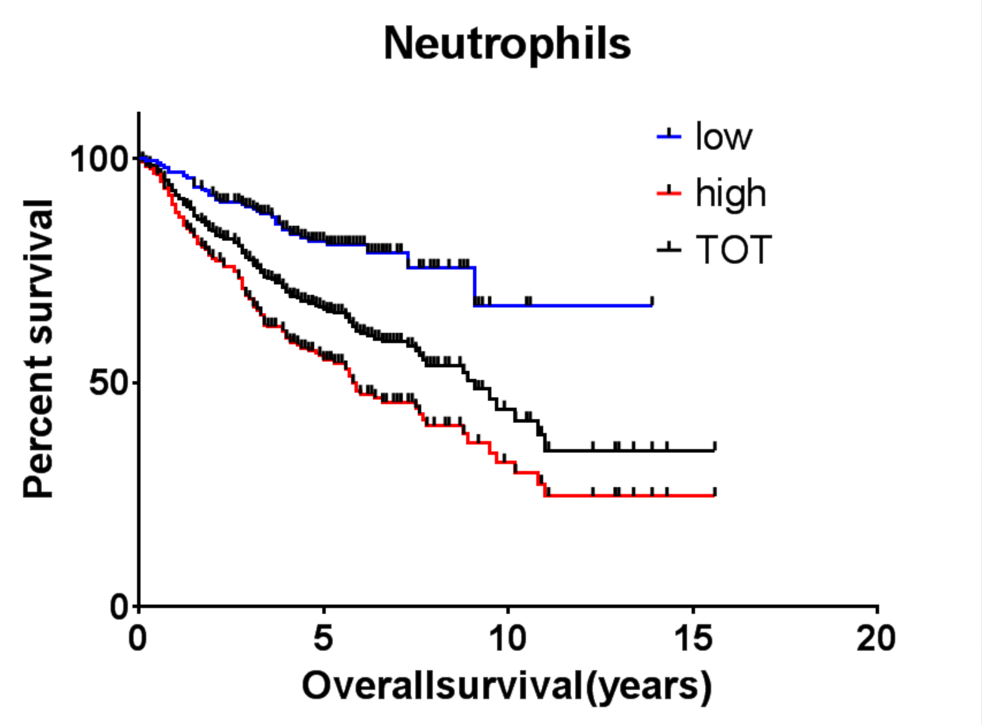
Part 2

n. 2015

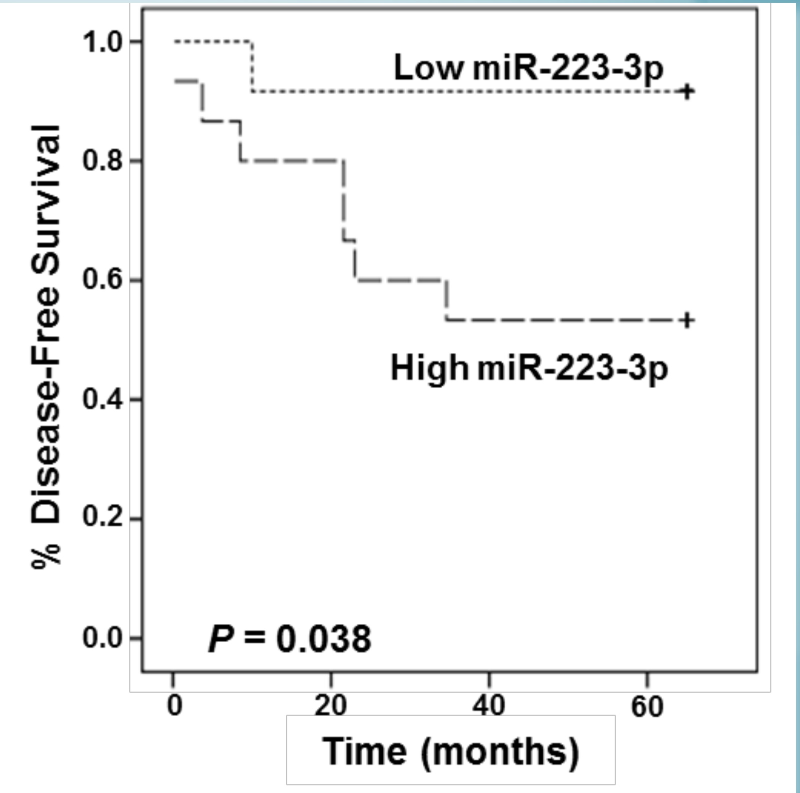


Lung Cancers

Neutrophils and miRNA-223 as biomarkers for lung cancer prognosis?



Ilie et al. 2012

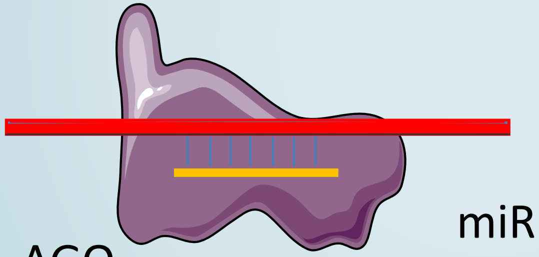


Sanfiorenzo et al. 2013

miRNA

Small non coding RNA(~20nt)

Eukaryotes



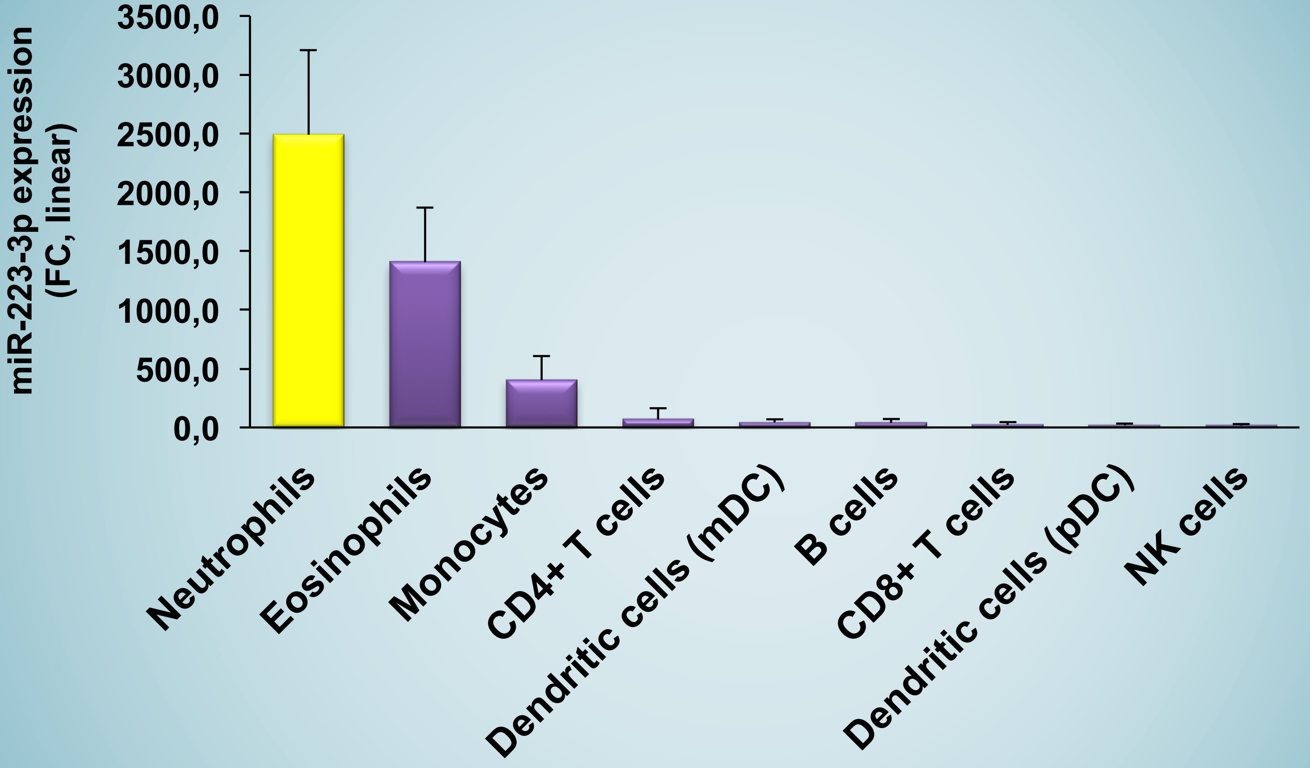
Pathologies

Translation regululators

Differenciation, proliferation
And cell survival

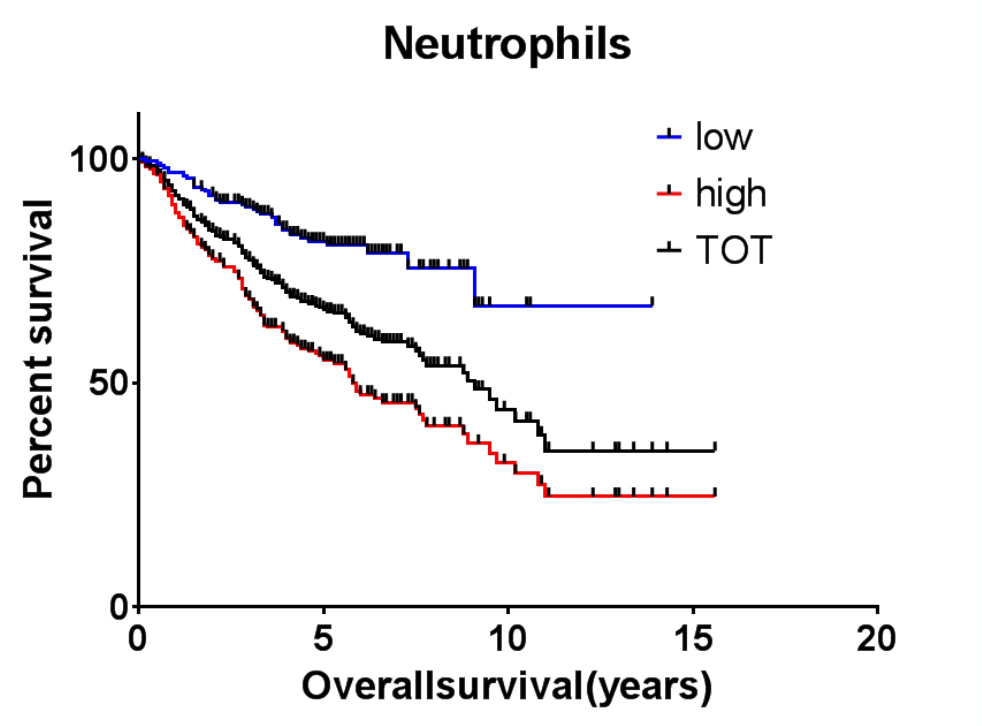
target 1/3 of human mRNA

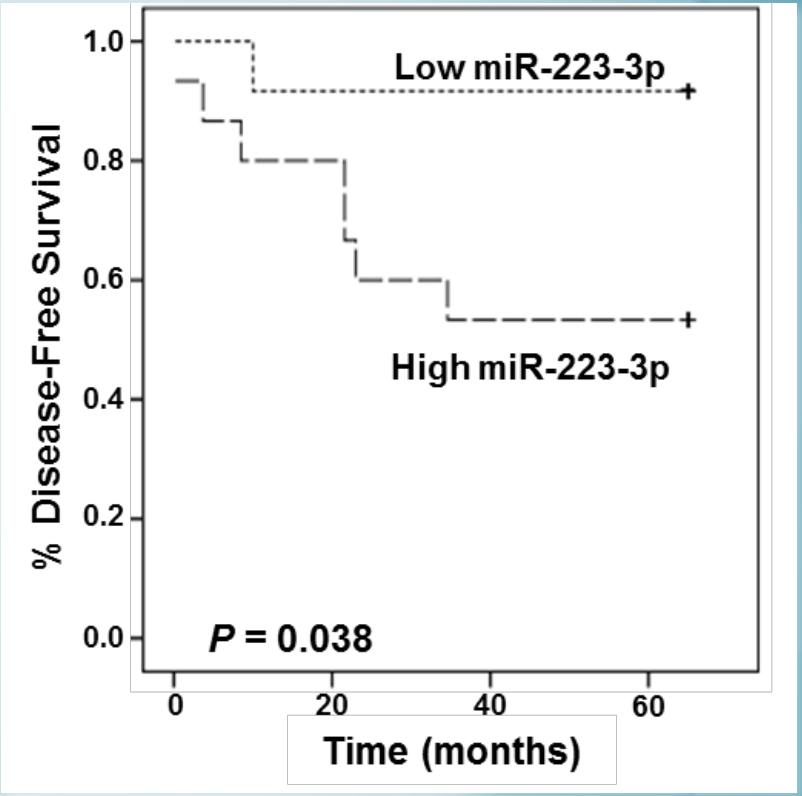
Neutrophils and miRNA-223 as biomarkers for lung cancer prognosis?



Allantaz et al, 2012

Neutrophils and miRNA-223 as biomarkers for lung cancer prognosis?





Ilie et al. 2012

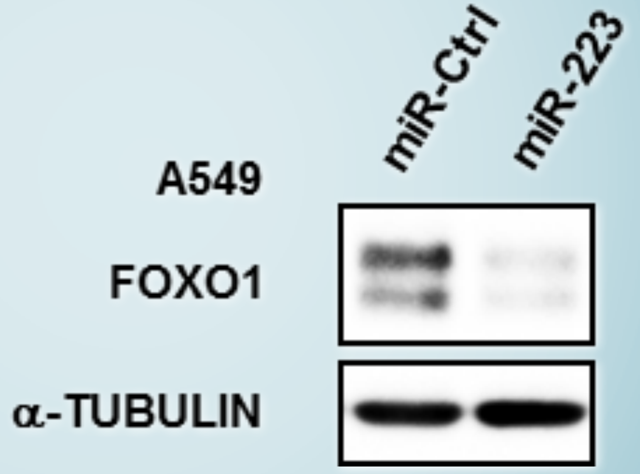
Sanfiorenzo et al. 2013

miRNA-223 is protumoral and associated with poor survial !

miRNA-223 effets in cancer cells

miR-223-3p

FOXO1A invalidation

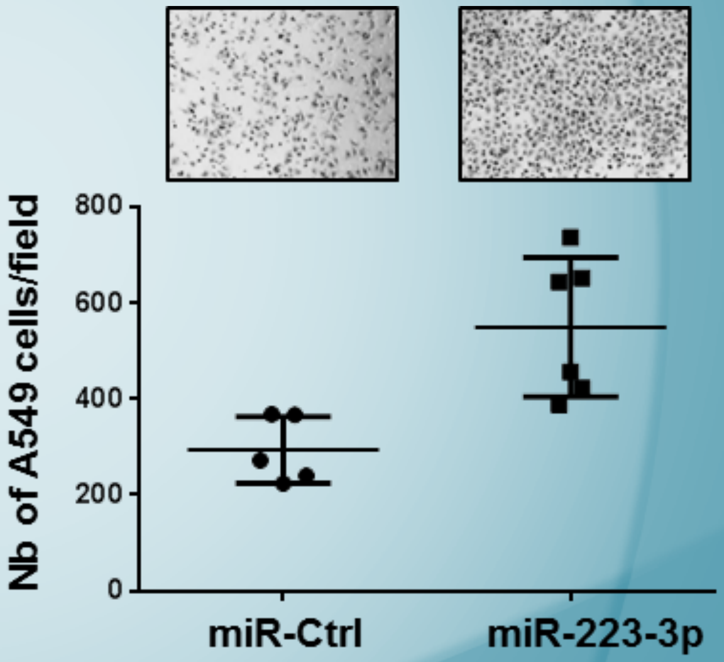


miRNA-223 effets in cancer cells

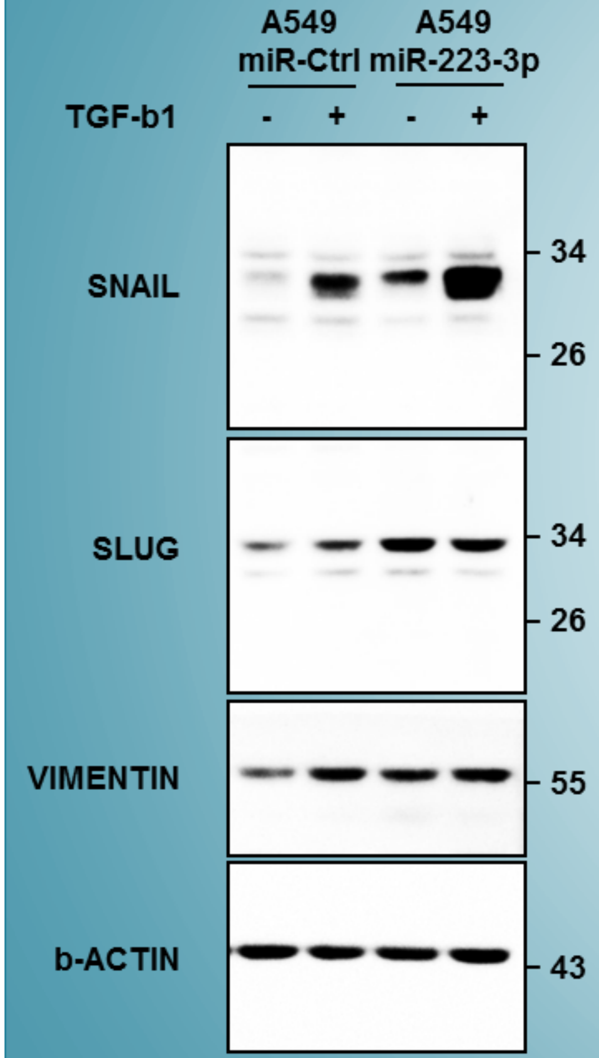
miR-223-3p

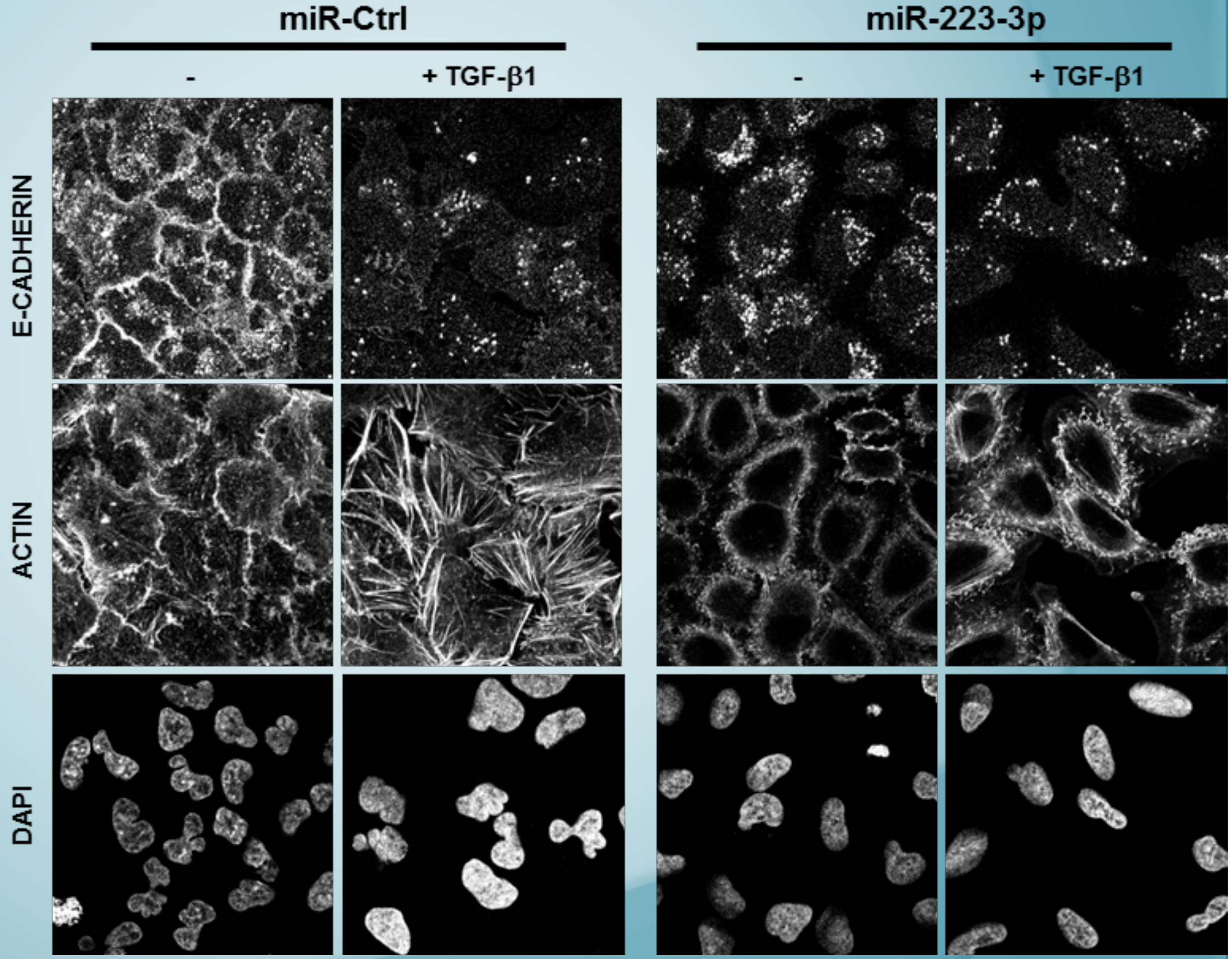
FOXO1A invalidation

EMT/Migration

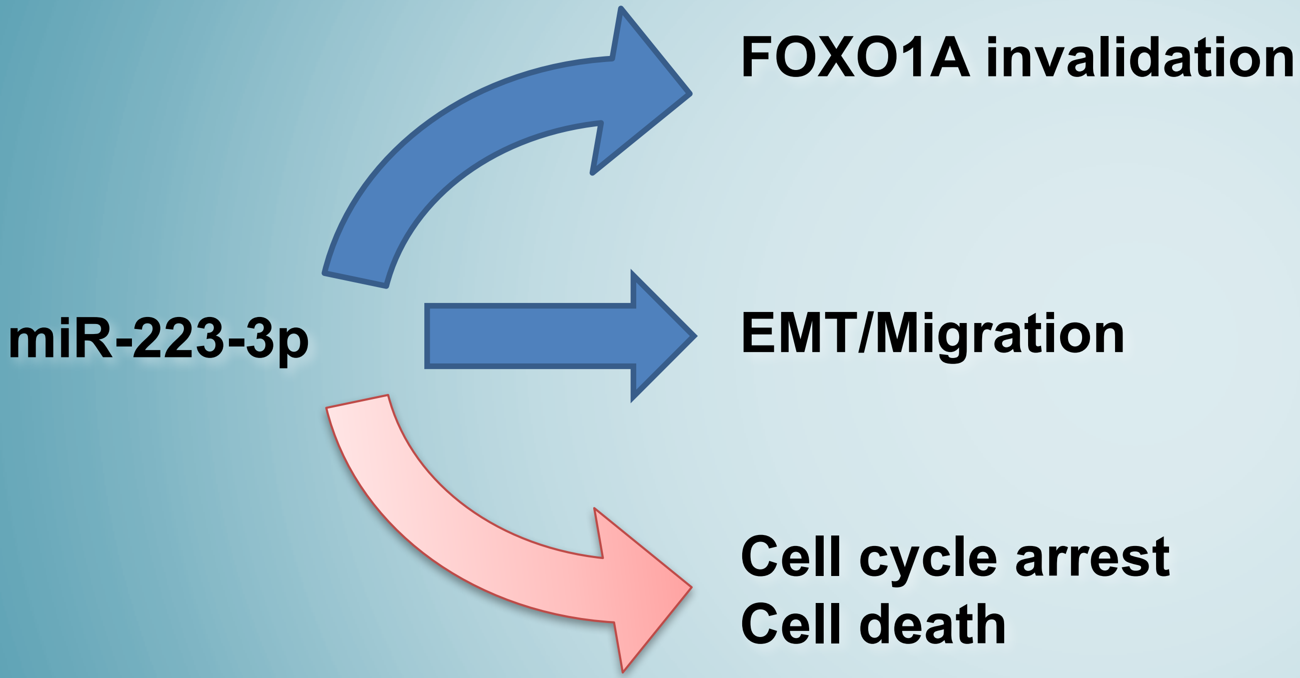


miR-223-3p promotes EMT phenotype





miRNA-223 effets in cancer cells



miR-223-3p

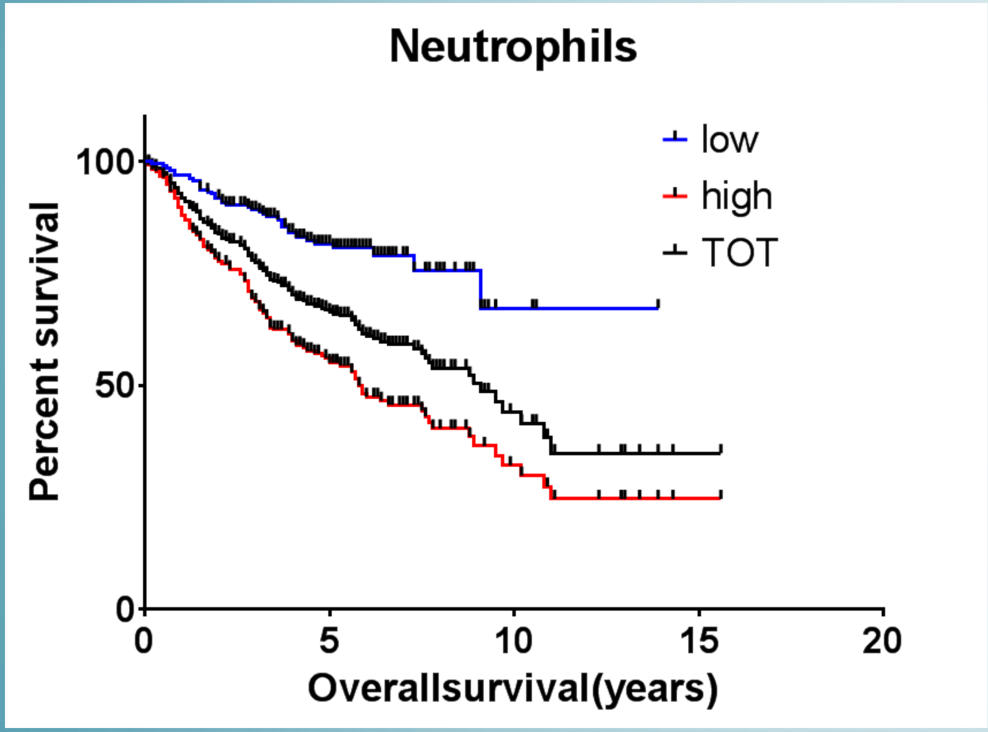
FOXO1A invalidation

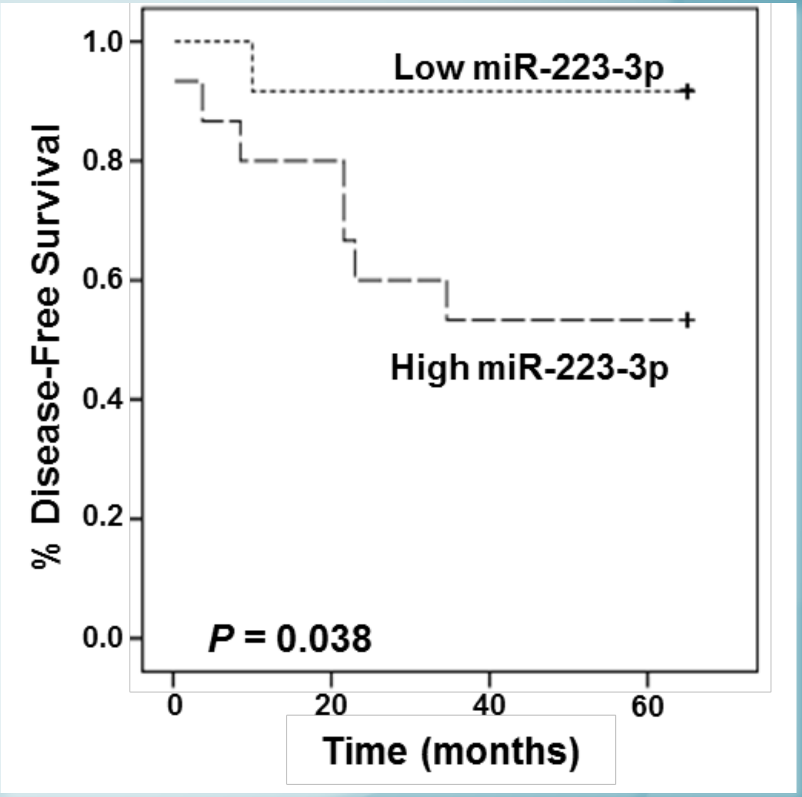
EMT/Migration

Cell cycle arrest
Cell death

miRNA-223 is a antitumoral microRNA !

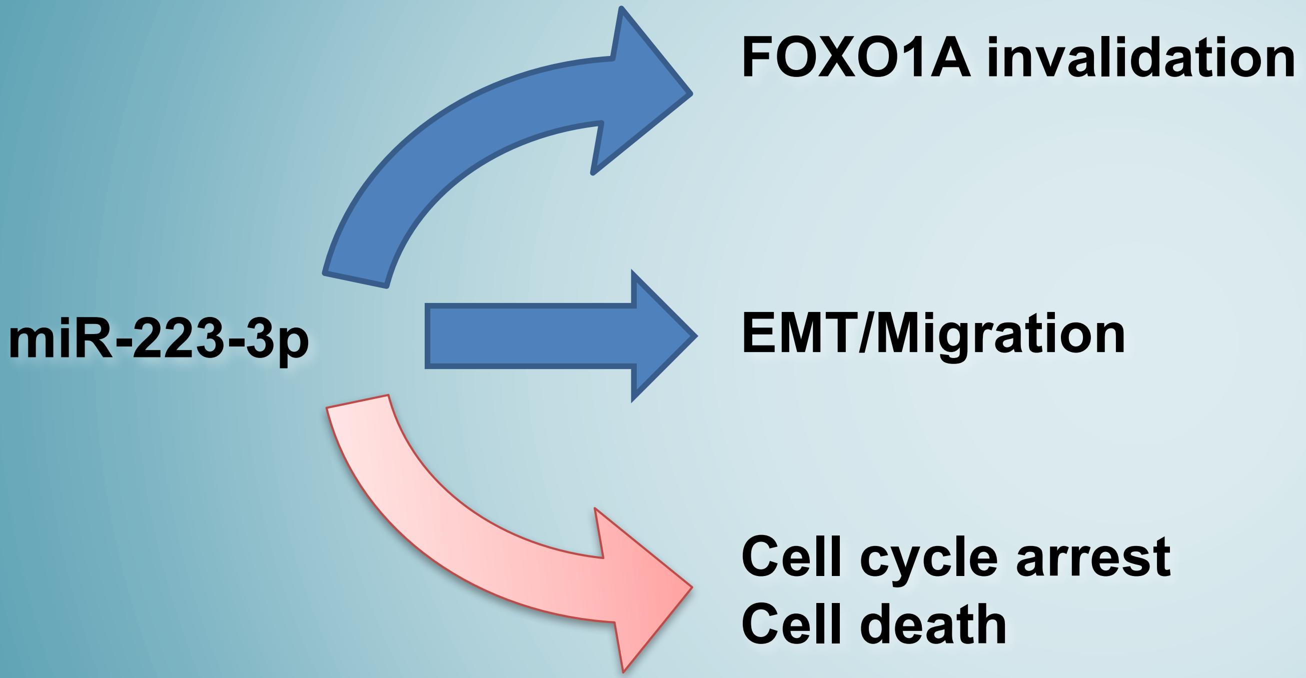
Neutrophils and miRNA-223 as biomarkers for lung cancer prognosis?





miRNA-223 is protumoral and associated with poor survial !

miRNA-223 effets in cancer cells



miR-223-3p

FOXO1A invalidation

EMT/Migration

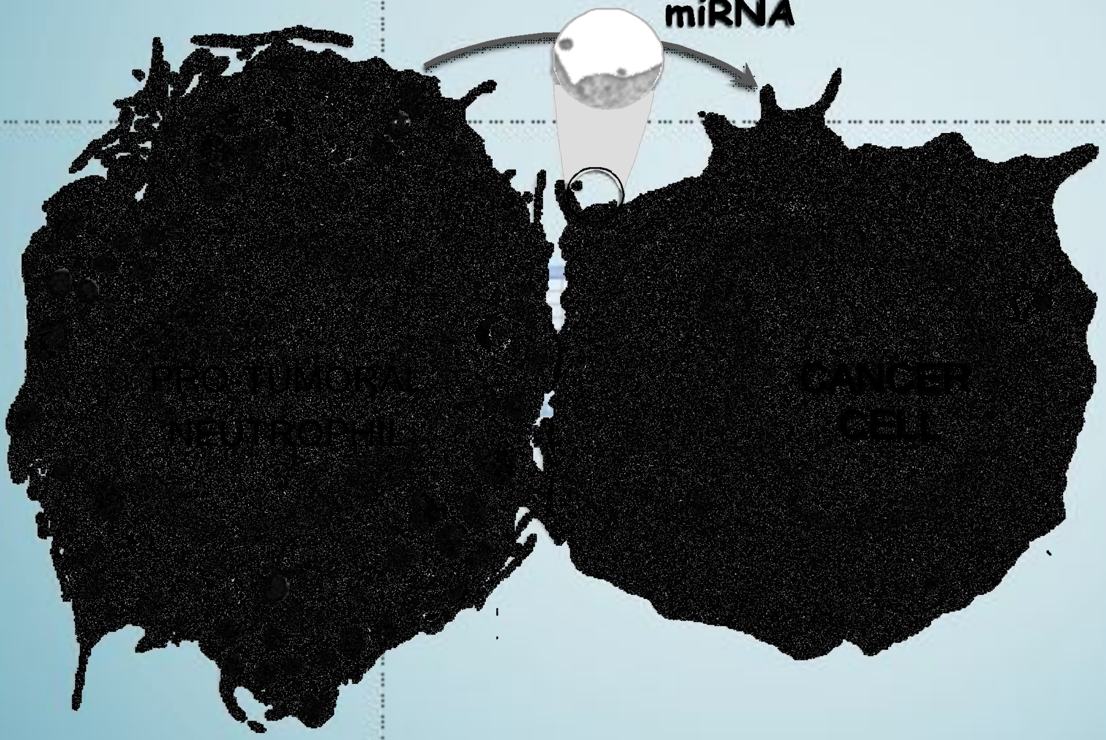
Cell cycle arrest
Cell death

~~miRNA-223 is a ant~~ ~~oRNA !~~

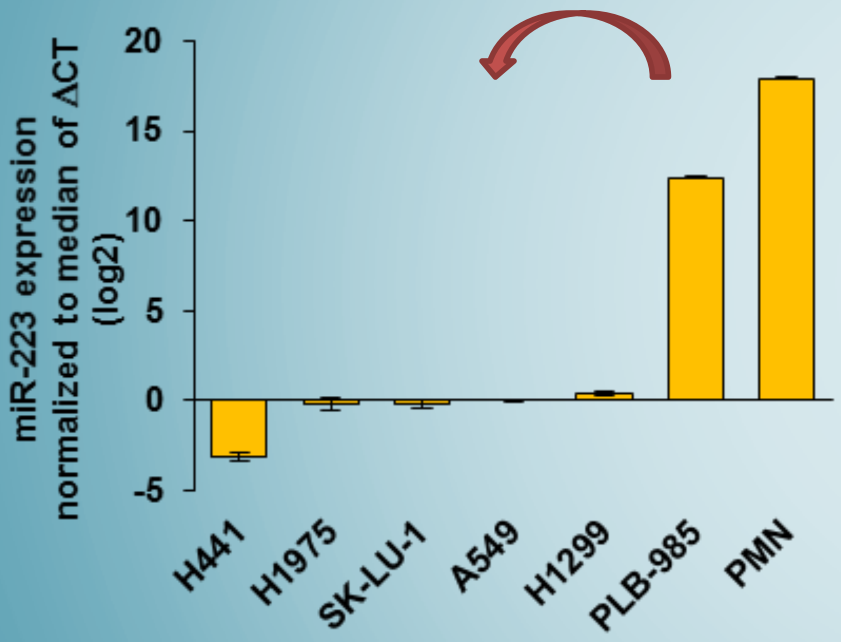
miRNA Dynamics ?

Dynamics => back to co-culture

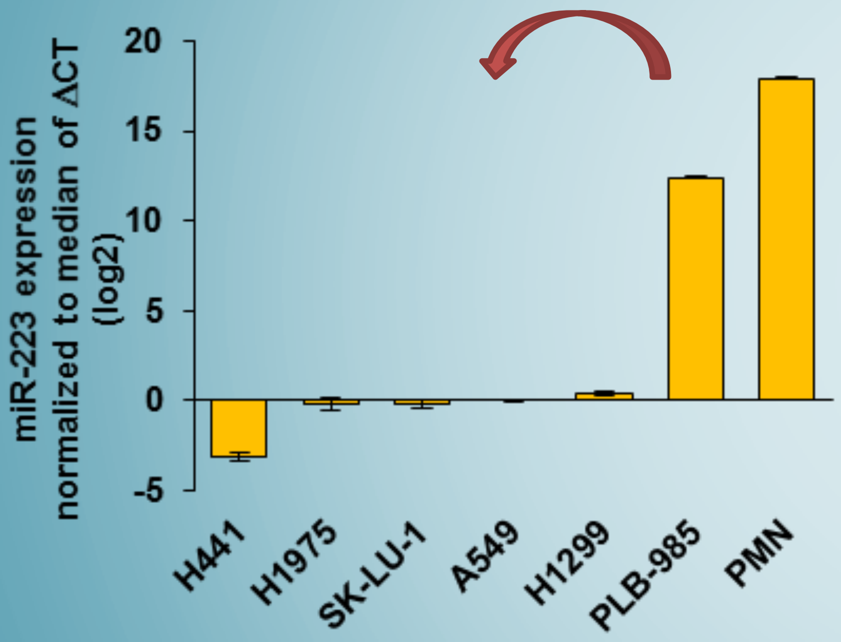
- Cliquez pour ajouter du texte

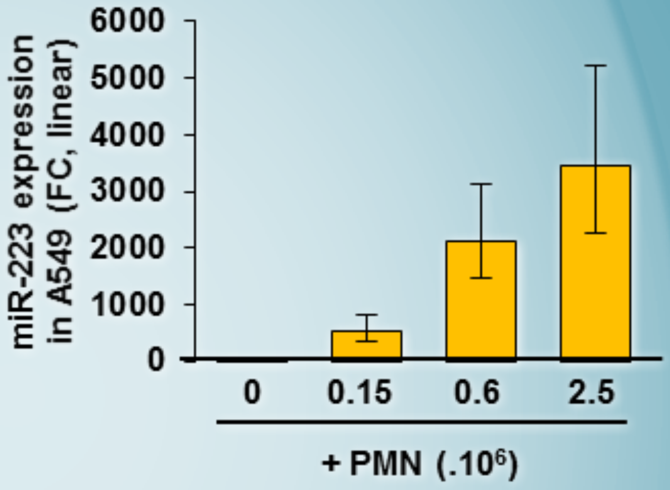


miR-223 is efficiently transferred in epithelial cells

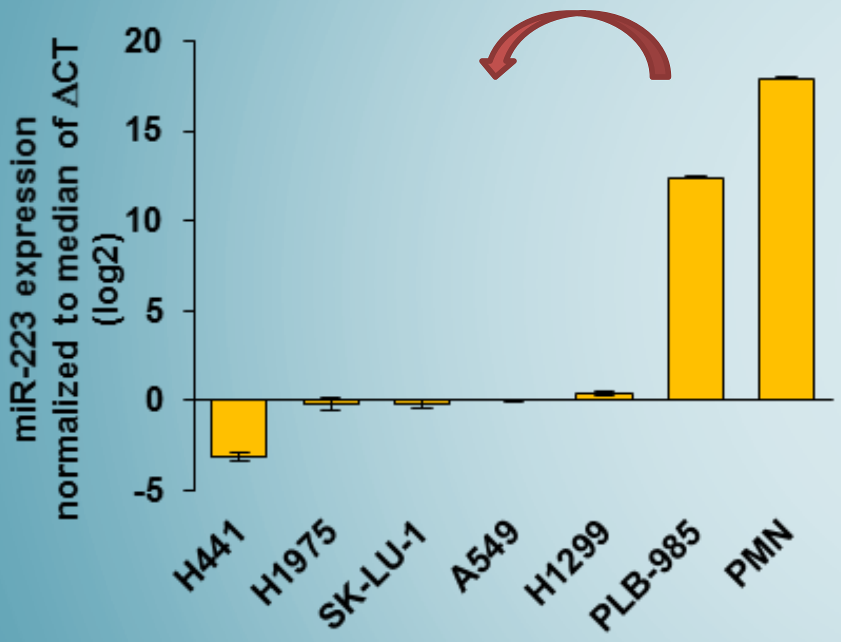


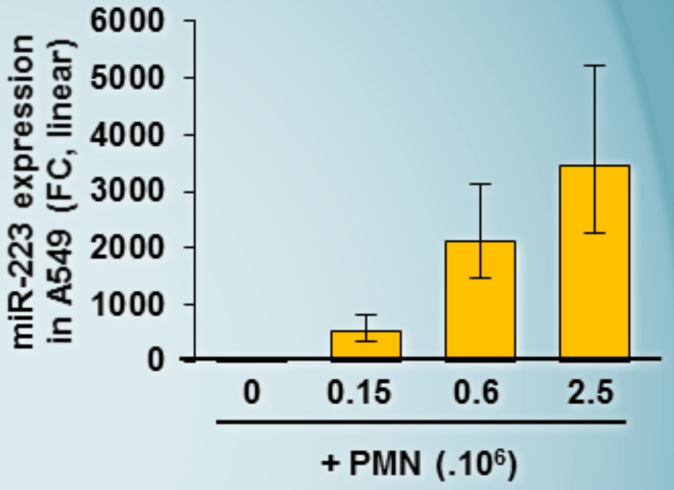
miR-223 is efficiently transferred in epithelial cells

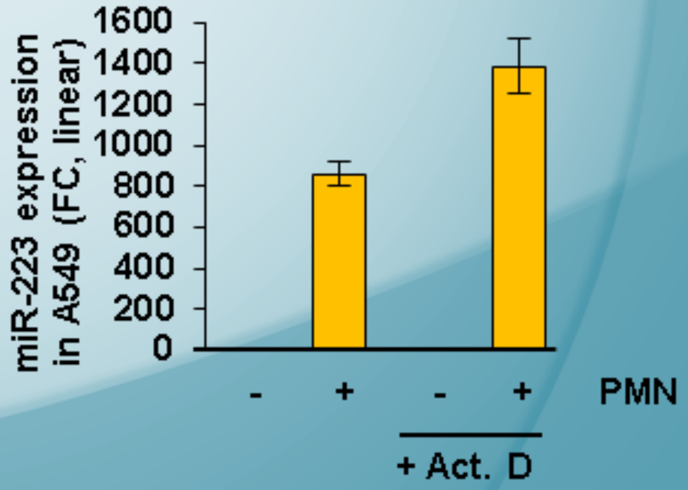




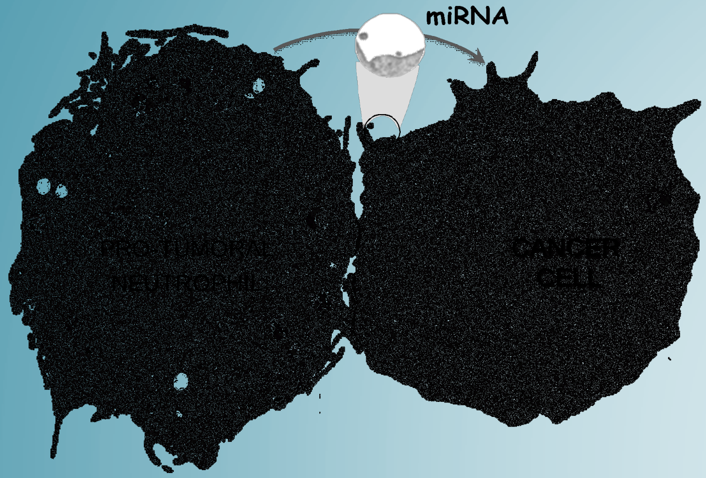
miR-223 is efficiently transferred in epithelial cells

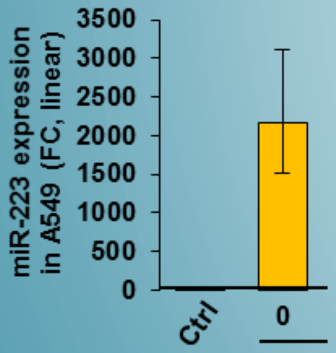




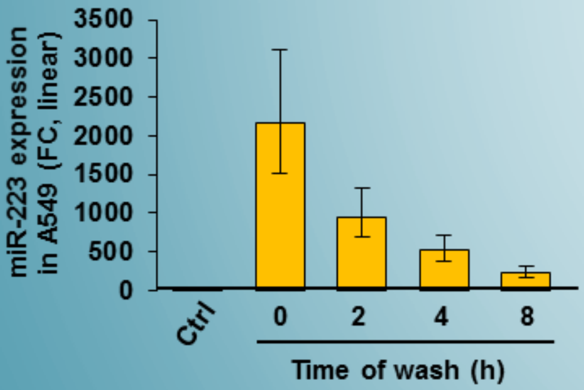


How stable is an ex-miRNA?

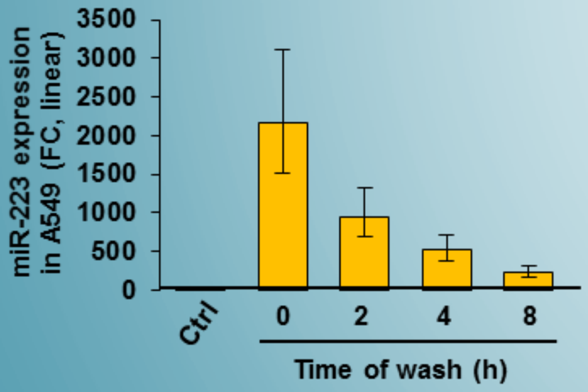


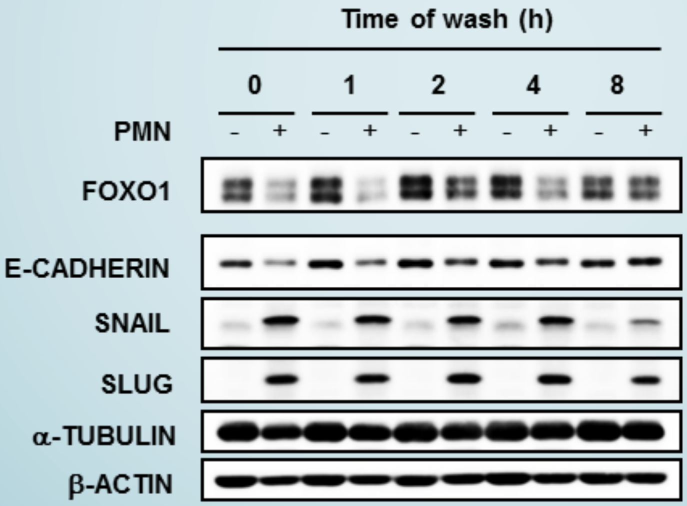


Ex-miRNA are unstable !

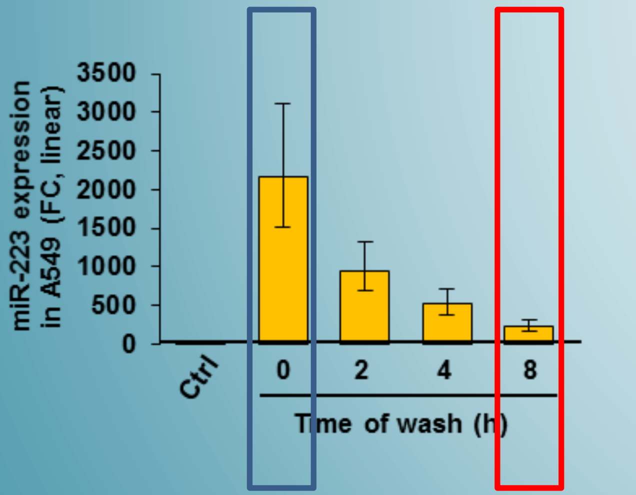


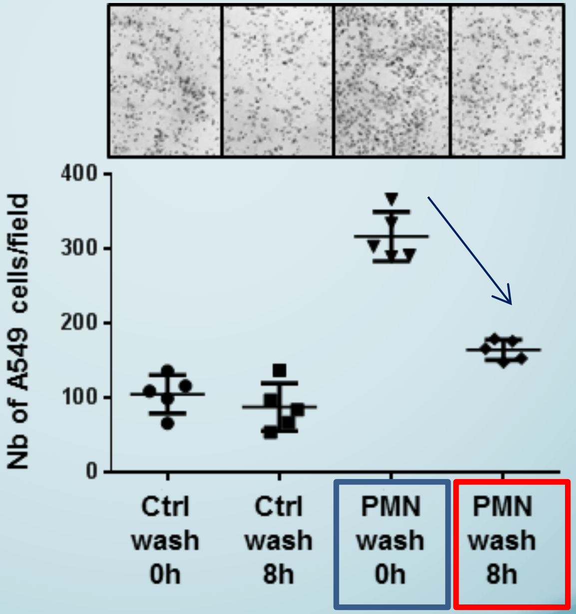
ex-miRNA targets are quickly restored



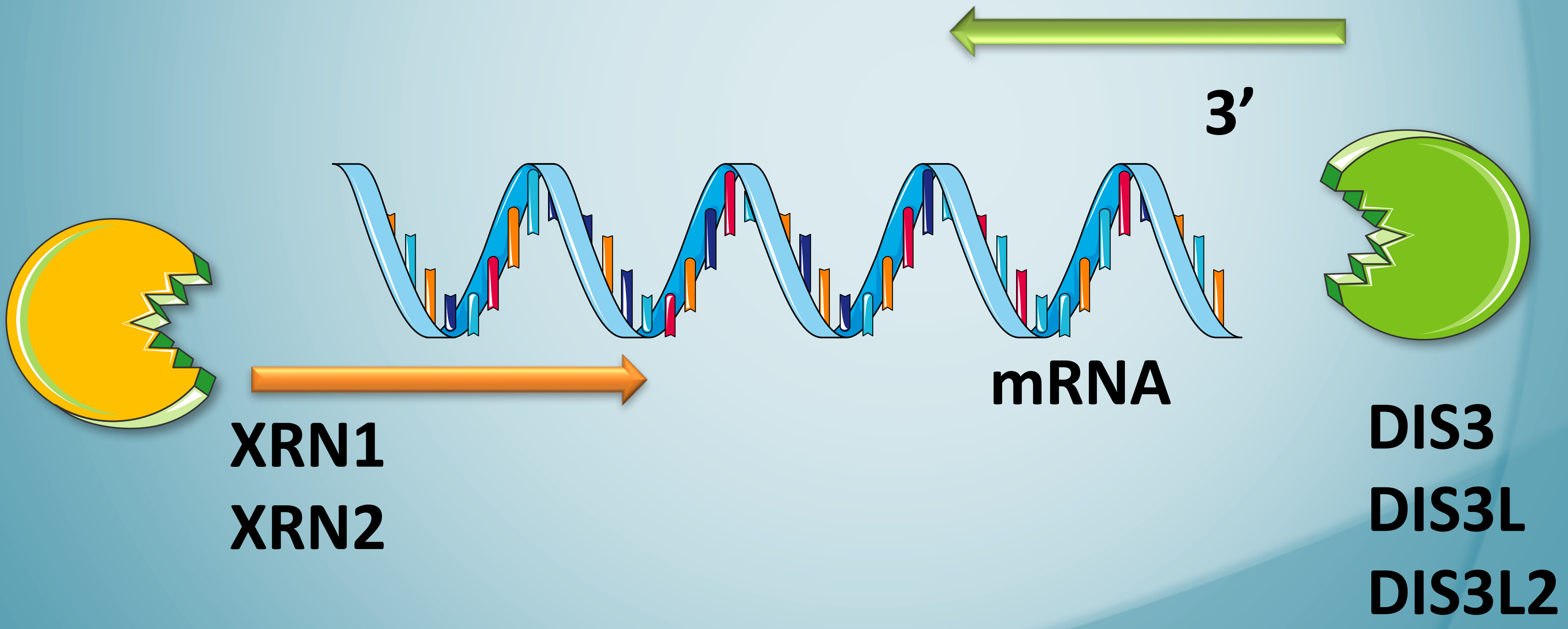


ex-miRNA induced phenotypes are transient

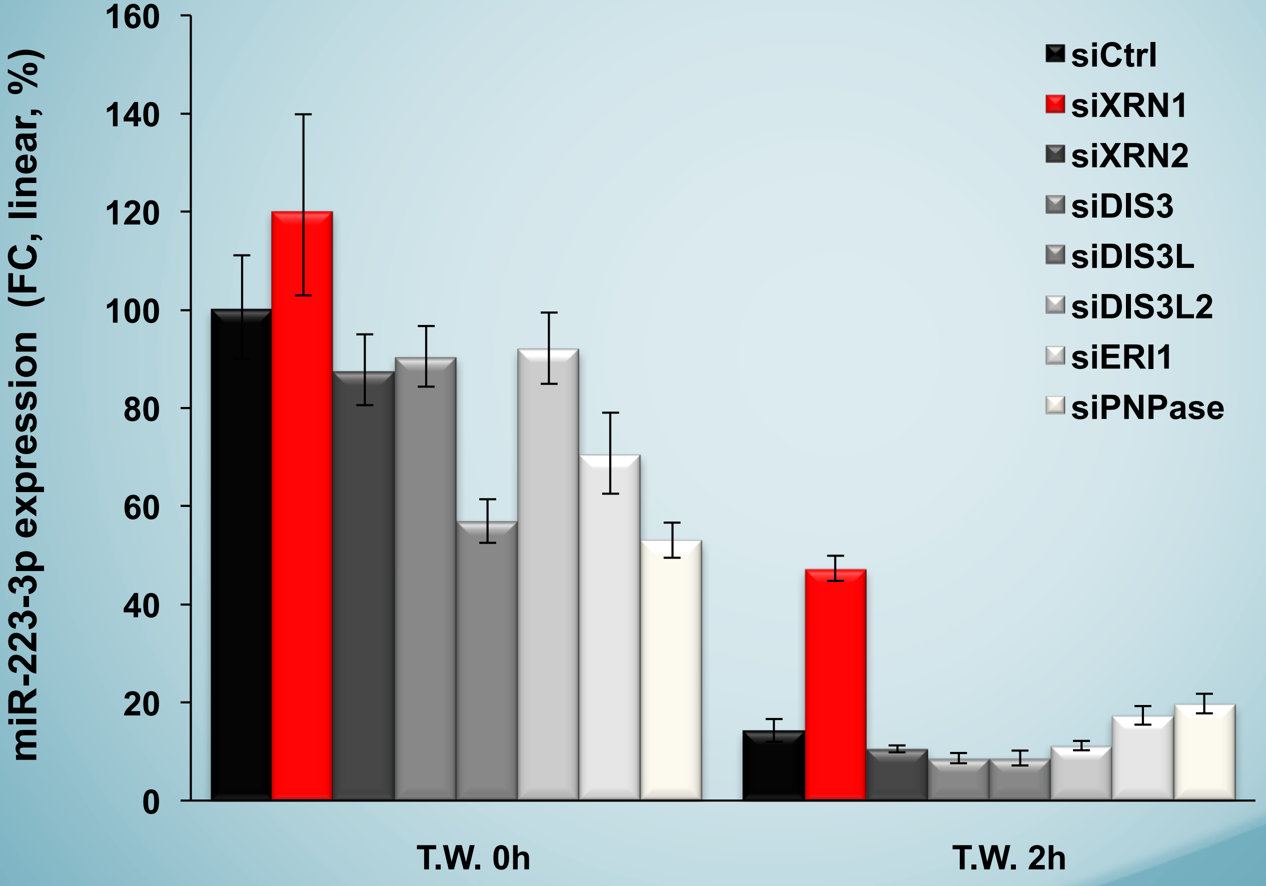




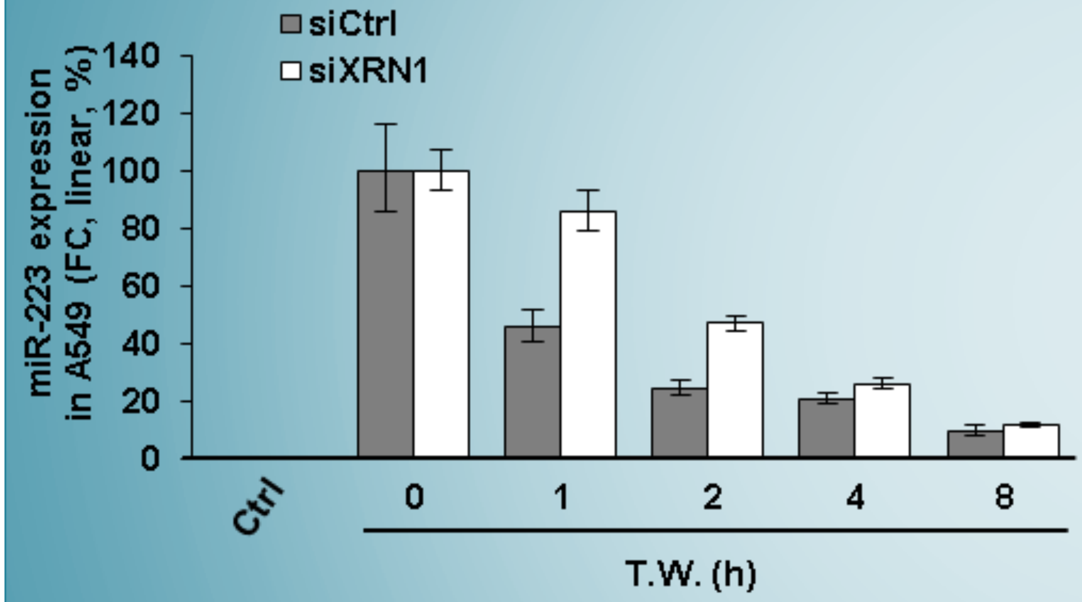
ex-miRNA degradation

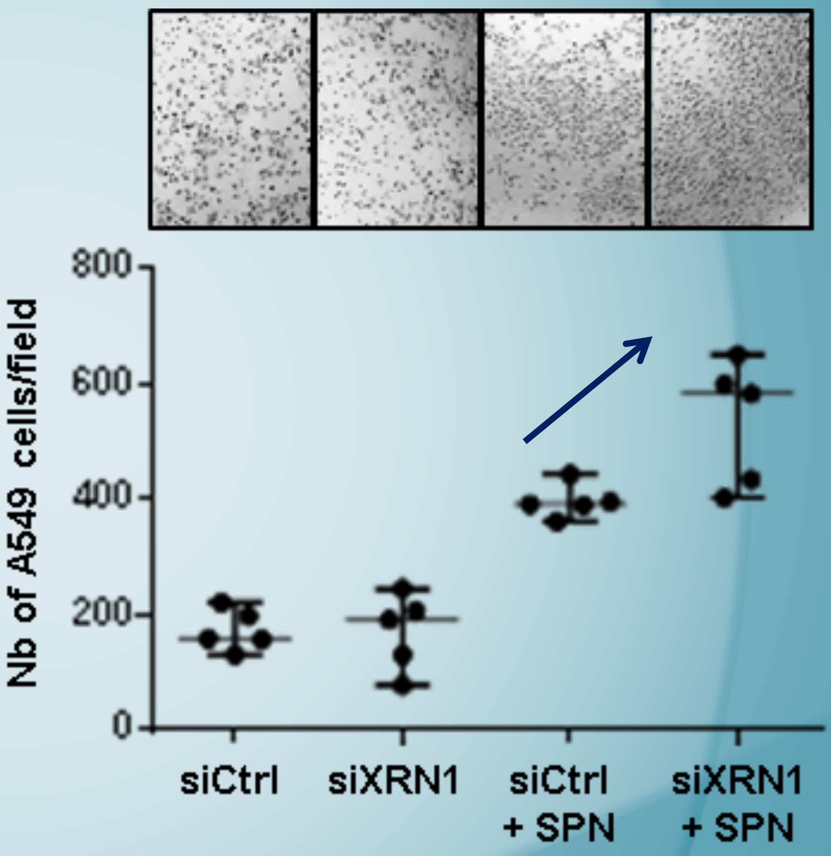


ex-miRNA is degradated by XRN1

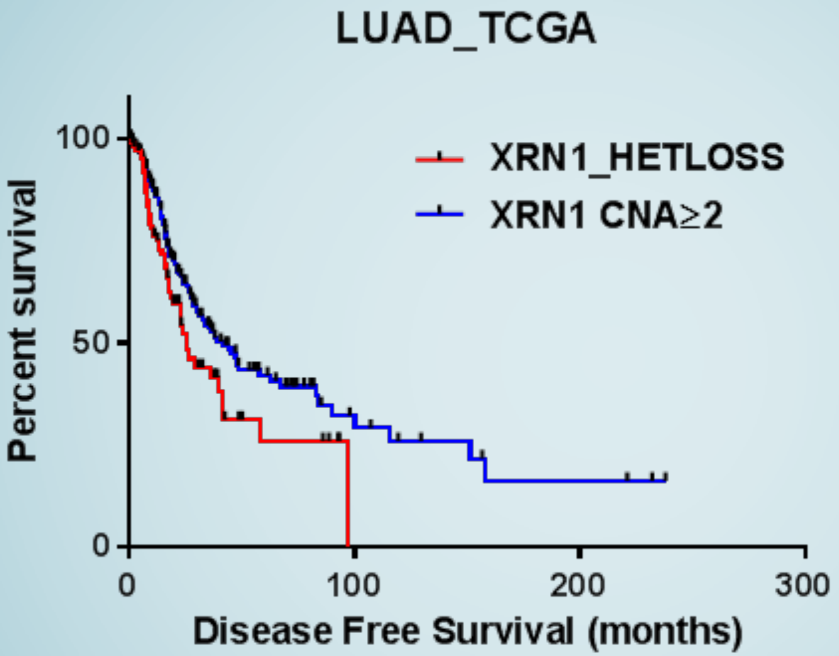


XRN1 invalidation delays ex-miR-223 phenotype





XRN1 deletion is associated with poorer prognosis



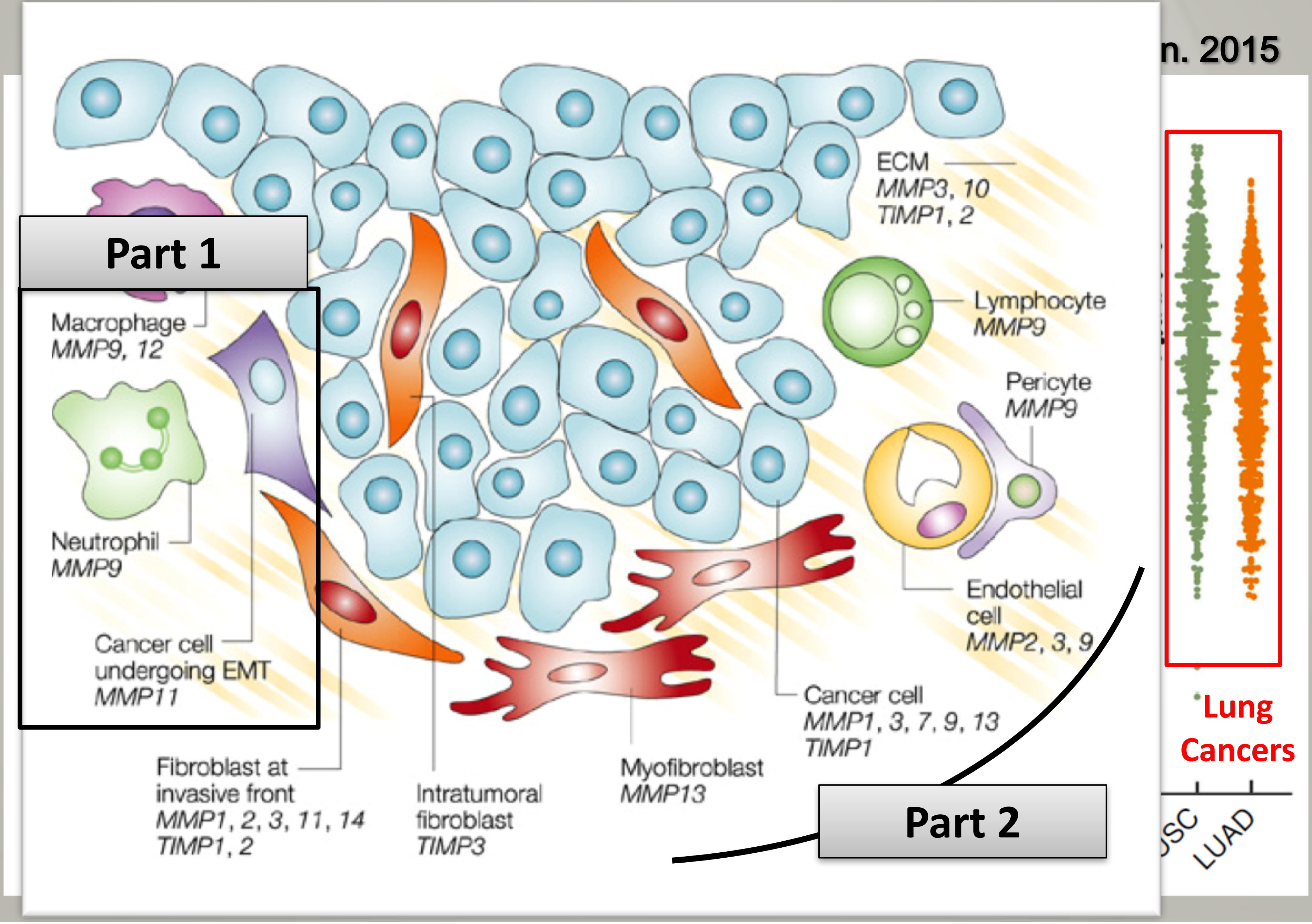
Disease Free Survival	XRN1_HETLOSS	XRN1 CNA≥2
Median survival (months)	25.36	41

miRNA dynamics

- First evidence of rapid decay of ex-miRNA
- miRNA-dependent rapid shift of phenotype
- Involvement of XRN1 in miRNA decay
- Increased diffusion of cell depending of neutrophil proximity
- Role of neutrophils in cancer and metastasis

Zangari et al. 2017

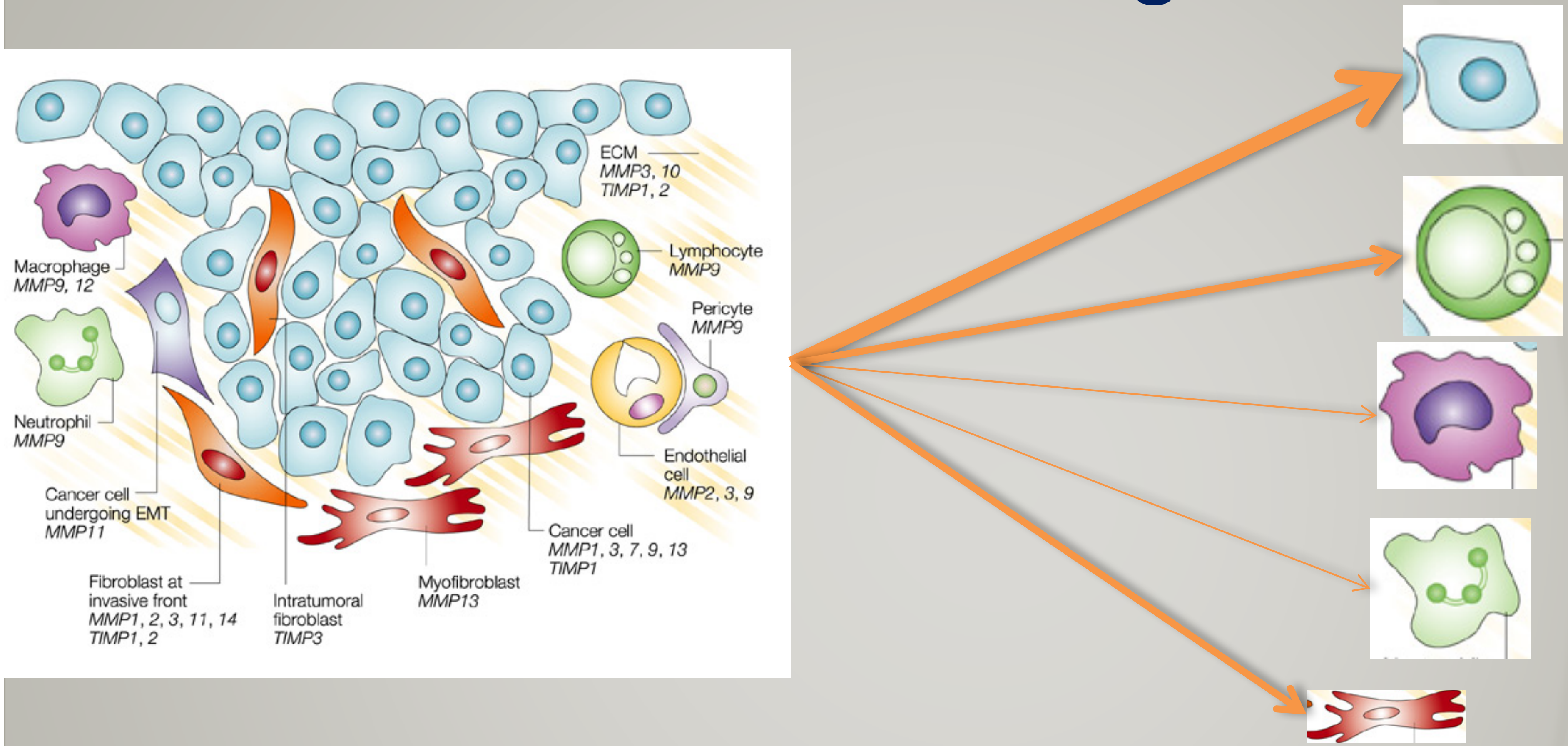
Lung cancer is heterogenous



Part 1

Part 2

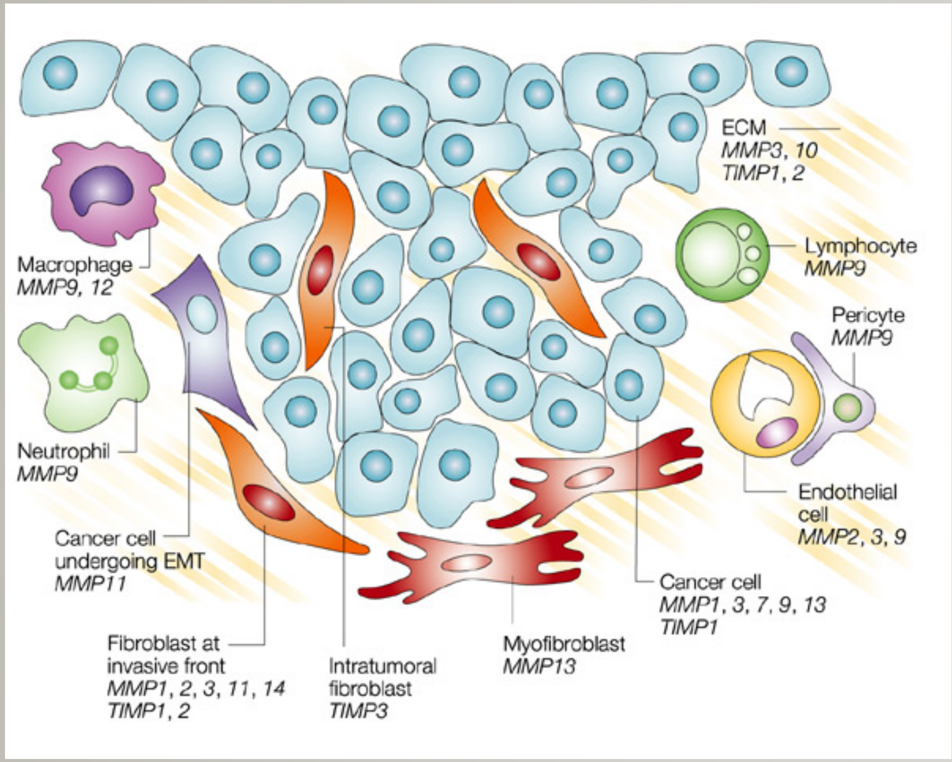
Deconvolution of signals



Is it possible to isolate individual signatures from a single pattern?

- **Math aspects: define the best model**
- **Biological aspects: reduce number of variables => meaningfull**
- **Clinical aspects: stratification, survival**

Deconvolution of signals



Cancer Patients

Kuner R, et al. *Lung Cancer* 2009

Affymetrix microarrays

Pneumocytes

Ghiabi P, et al. In Press

Lung Fibroblast

Peng R, et al. *PLoS One* 2013

Vessels

Kuner R, et al. *Lung Cancer* 2009

Blood

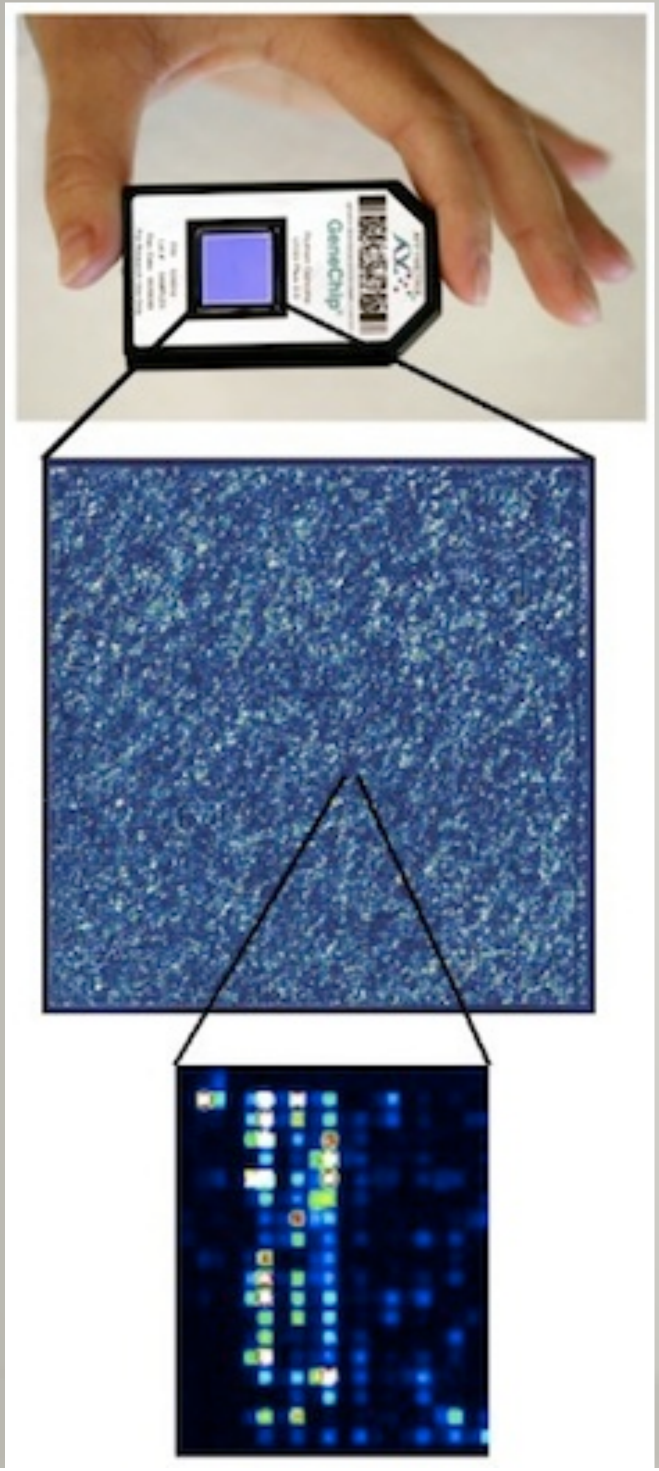
- Neutrophils
- Lymphocytes
- Monocytes

Allantaz F, et al. *PLoS One* 2012

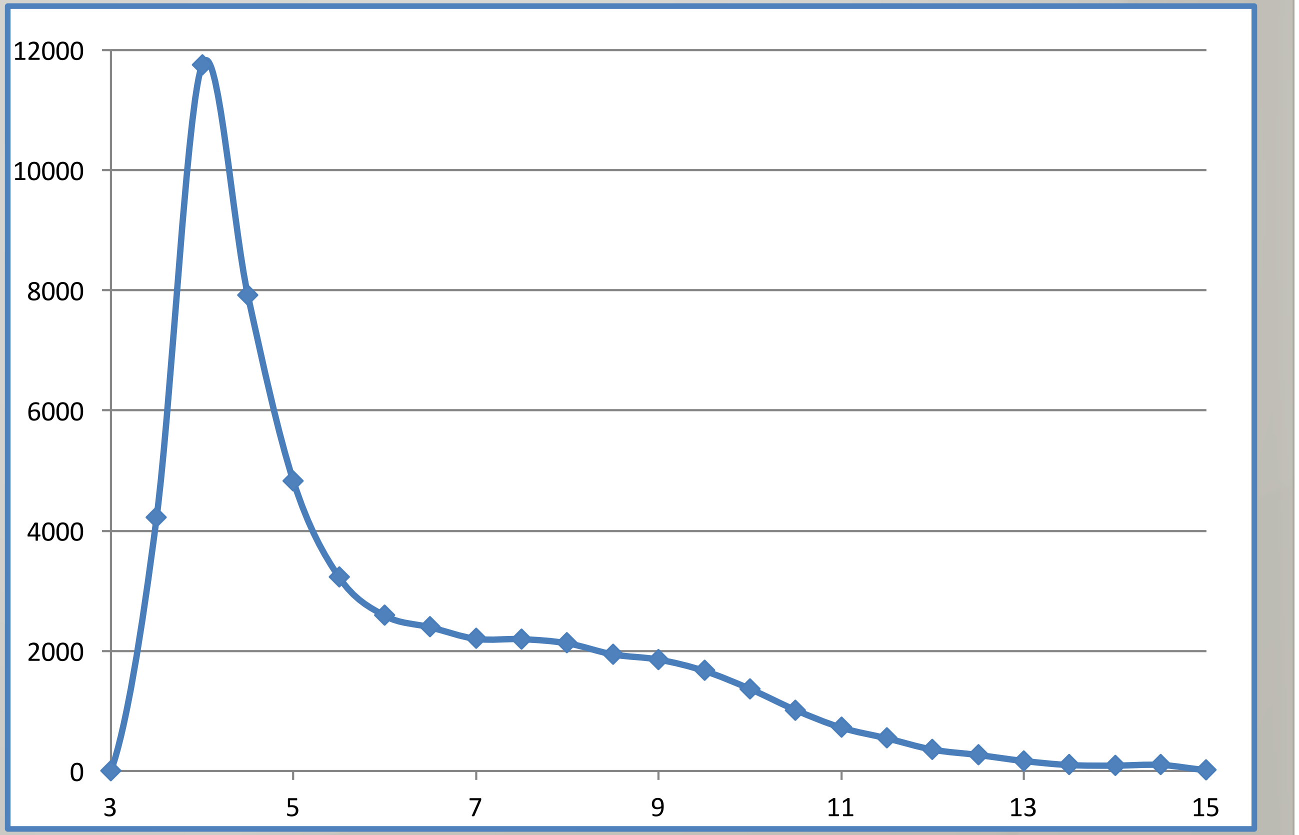
Cancer cell lines?

CCLE project

Microarray

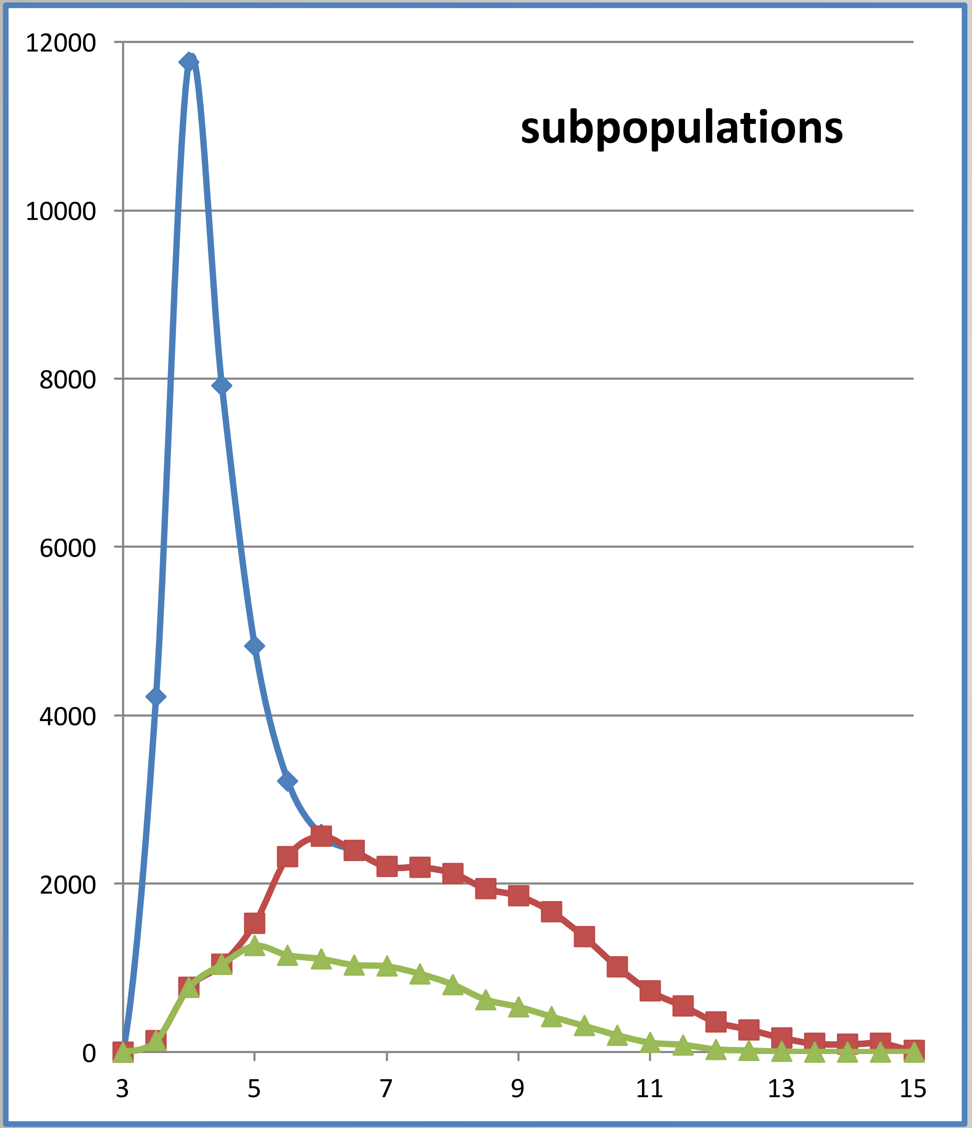


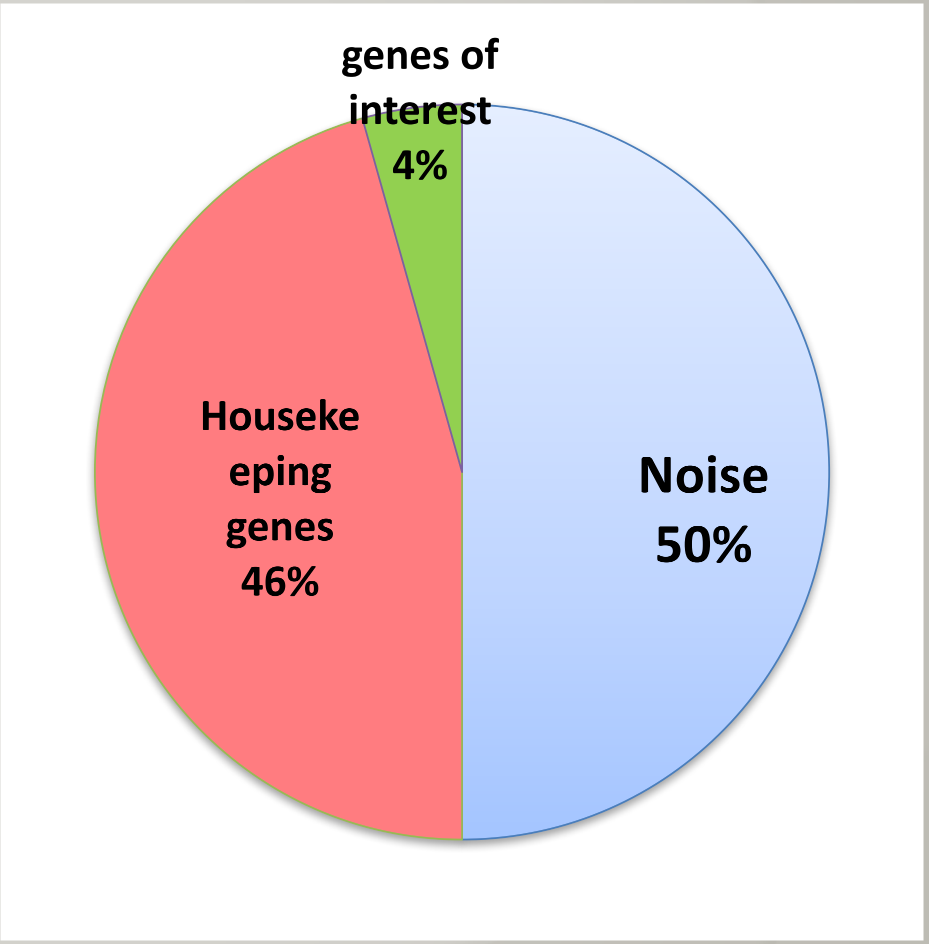
Signals expressed in 65536 levels of grey



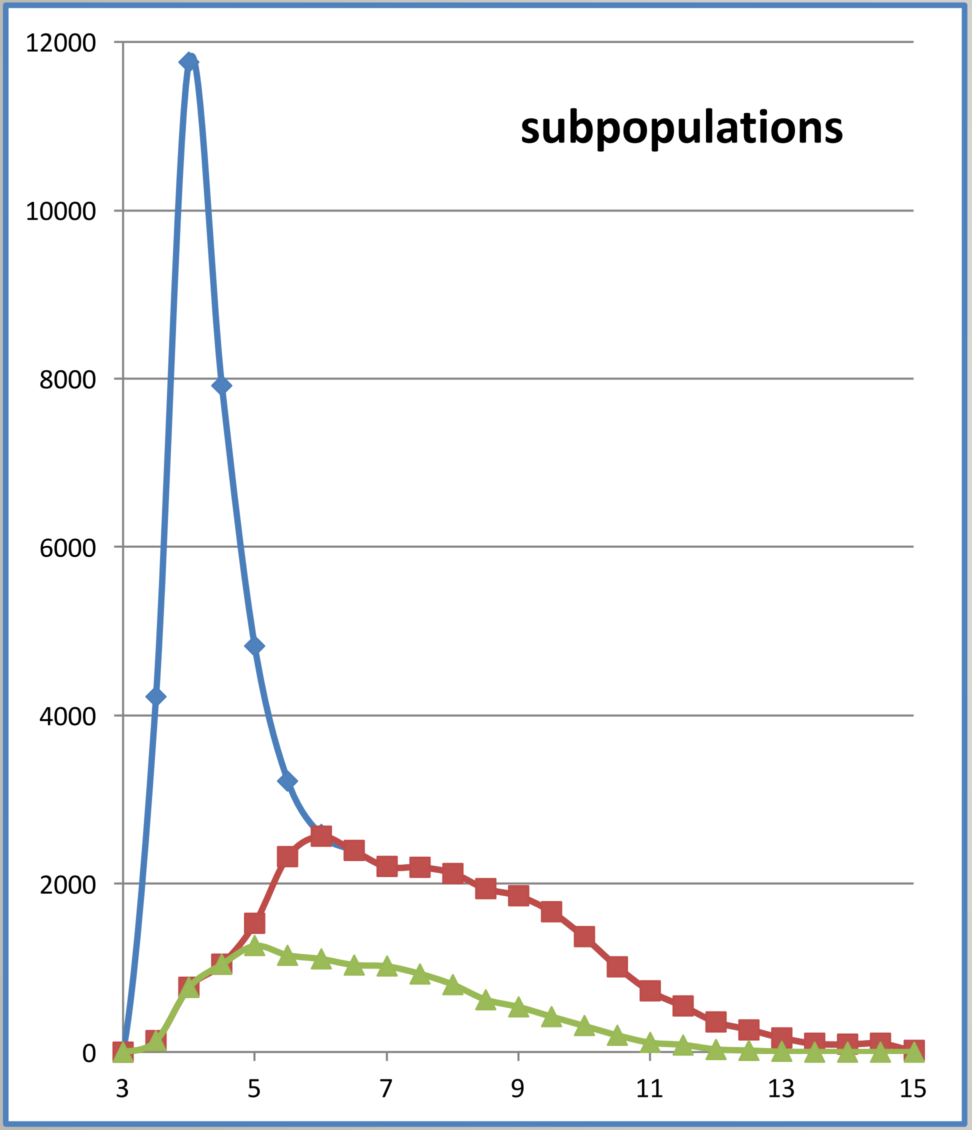
Signal intensity (log2)

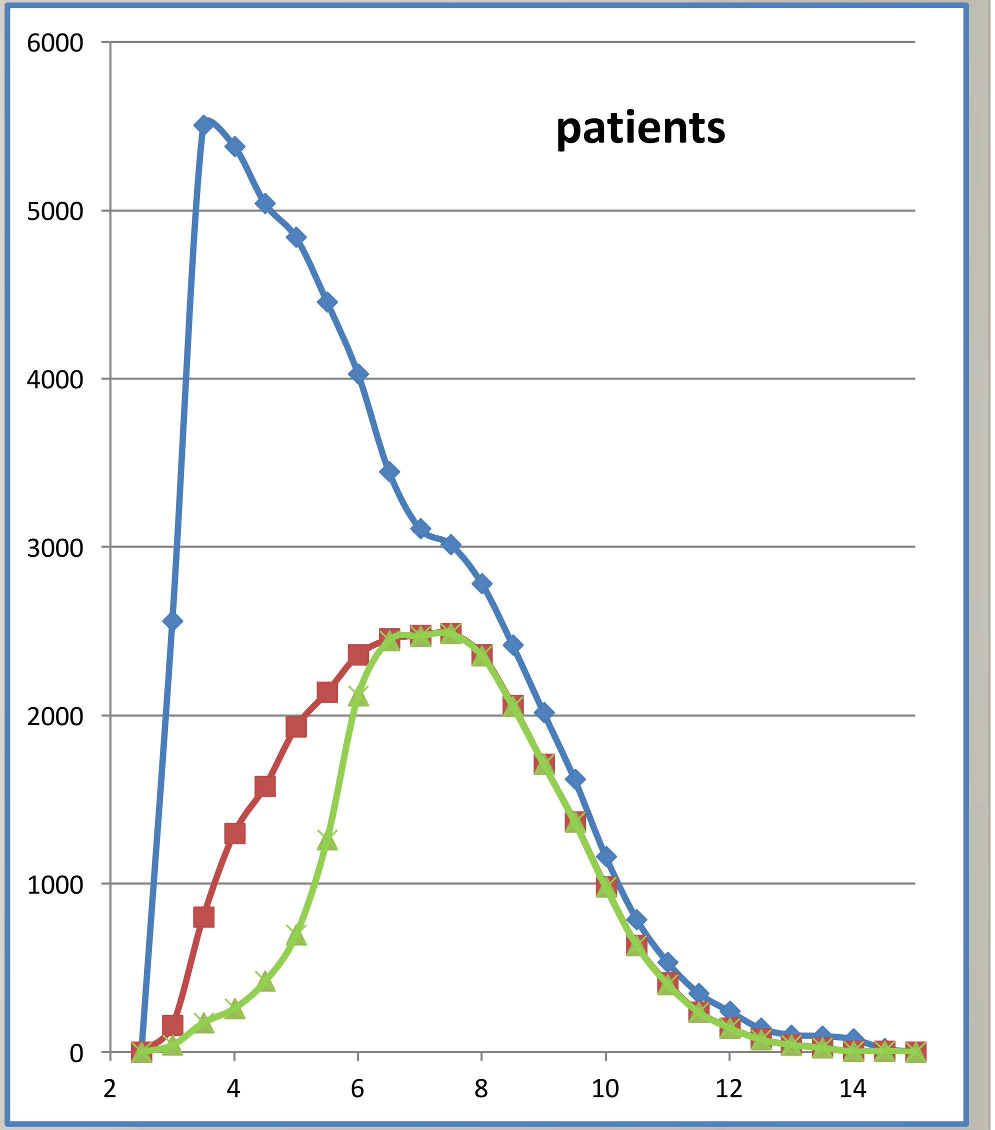
Distribution of expression



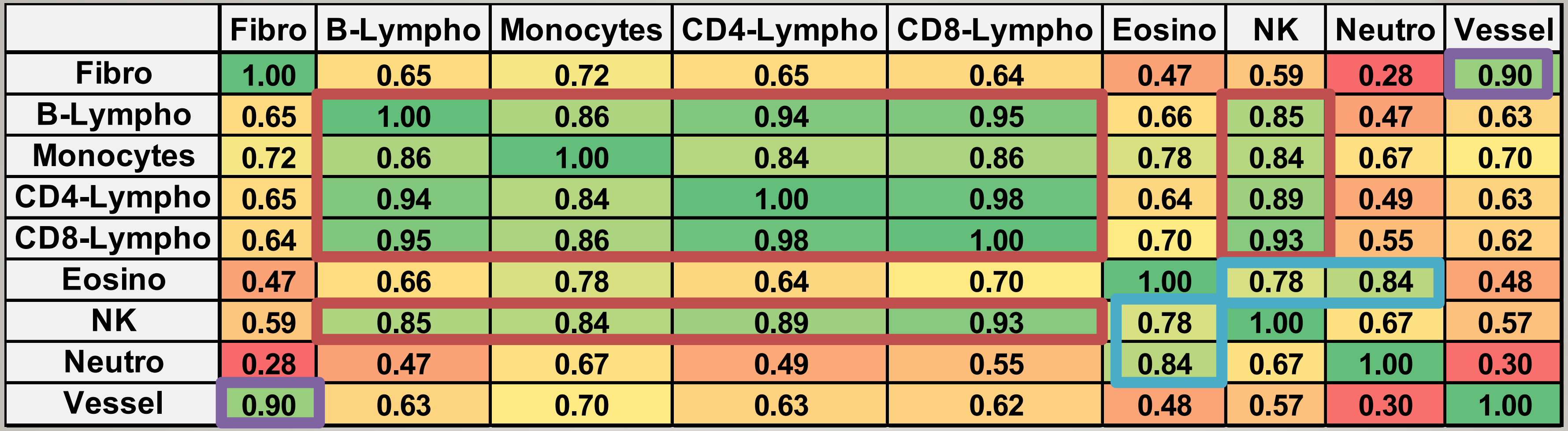


Distribution of expression





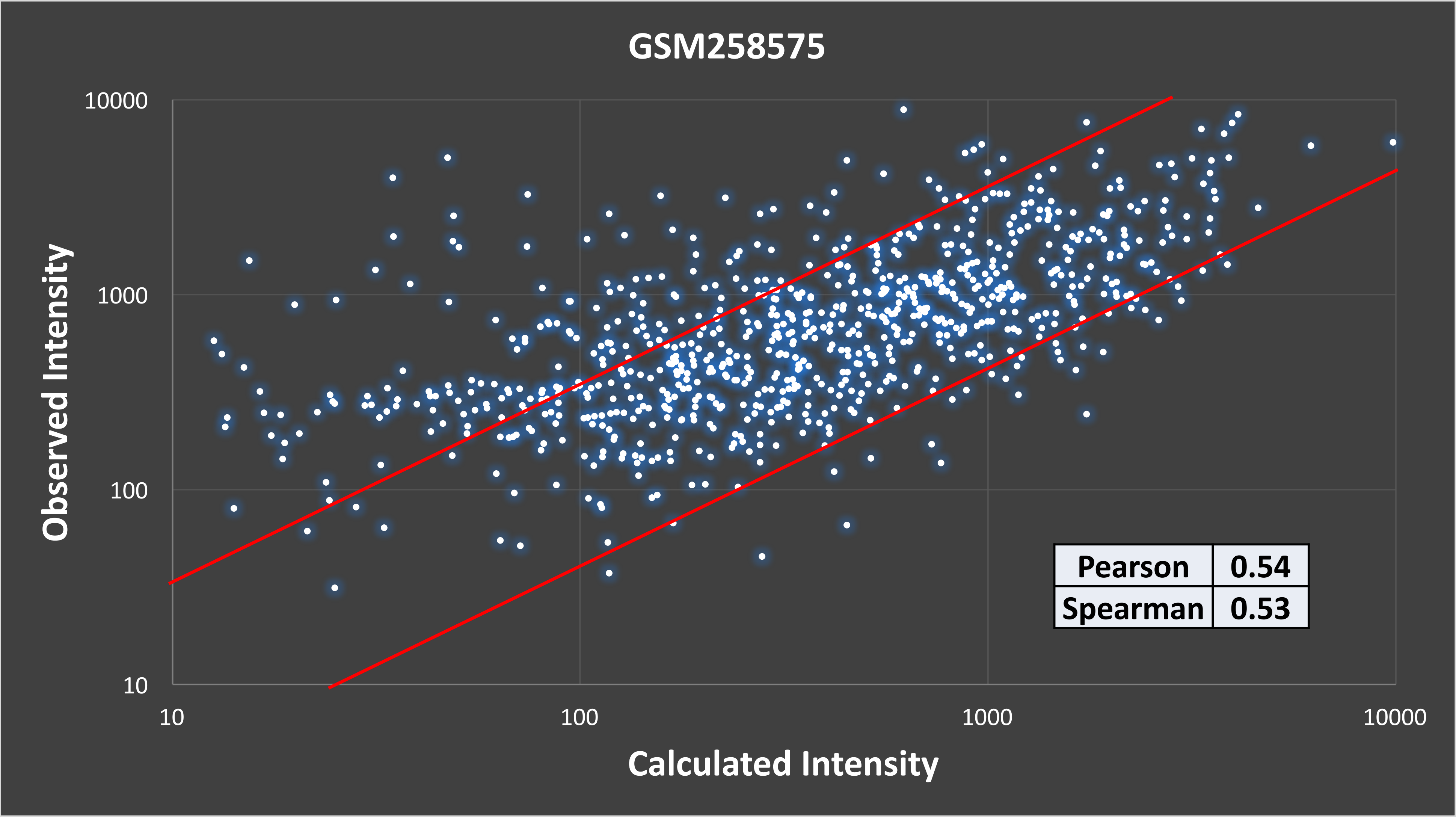
Mathematical model= Optimization



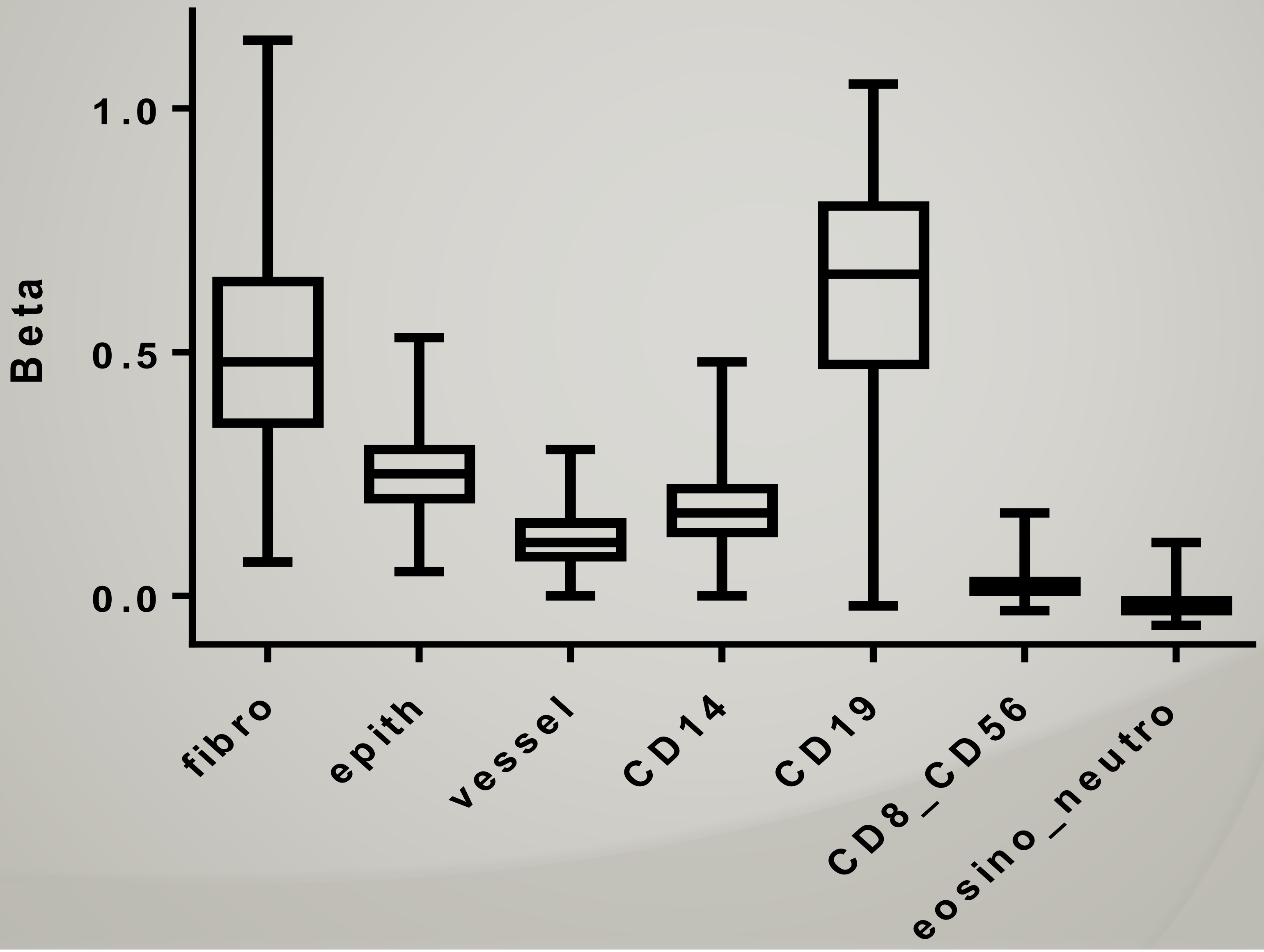
	Fibro	B-Lympho	Monocytes	CD4-Lympho	CD8-Lympho	Eosino	NK	Neutro	Vessel
Fibro	1.00	0.65	0.72	0.65	0.64	0.47	0.59	0.28	0.90
B-Lympho	0.65	1.00	0.86	0.94	0.95	0.66	0.85	0.47	0.63
Monocytes	0.72	0.86	1.00	0.84	0.86	0.78	0.84	0.67	0.70
CD4-Lympho	0.65	0.94	0.84	1.00	0.98	0.64	0.89	0.49	0.63
CD8-Lympho	0.64	0.95	0.86	0.98	1.00	0.70	0.93	0.55	0.62
Eosino	0.47	0.66	0.78	0.64	0.70	1.00	0.78	0.84	0.48
NK	0.59	0.85	0.84	0.89	0.93	0.78	1.00	0.67	0.57
Neutro	0.28	0.47	0.67	0.49	0.55	0.84	0.67	1.00	0.30
Vessel	0.90	0.63	0.70	0.63	0.62	0.48	0.57	0.30	1.00

partial least squares, least squares, manual…others?

Reconstitution of signature in patients (calc vs obs)



Pearson	0.54
Spearman	0.53


Beta
1.0
0.5
0.0
fibro
epith
vessel
CD14
CD19
CD8_CD56
eosino_neutro

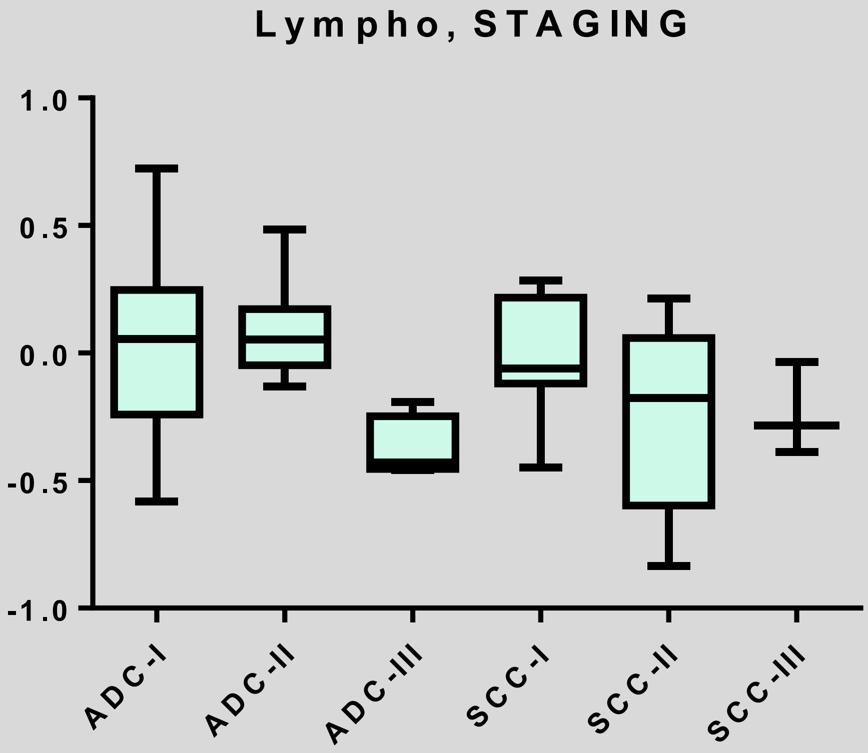
Clinical Data

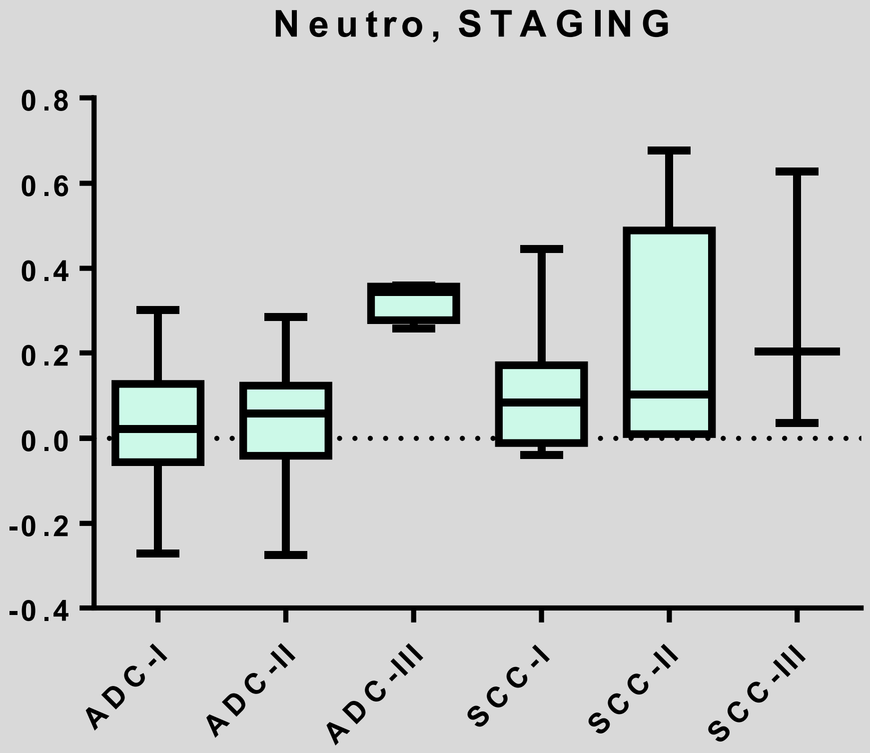
Tumor subtype (ADC, SCC)
Staging (I, II, III)
TNM-T (Tumor size)
TNM-N (Lymph node)
Time until relapse in month
Relapse (0/1)
Survival time in month
Died (0/1)
censored: follow-up < 3 years
Age (39-83y)
Sex (14F/44M)

×

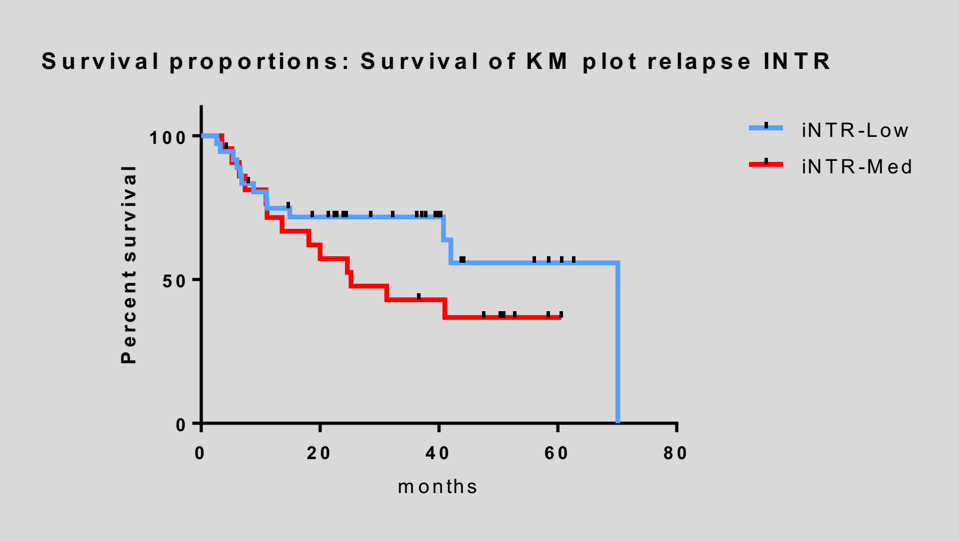
Math Models

Neutrophils are associated with progression

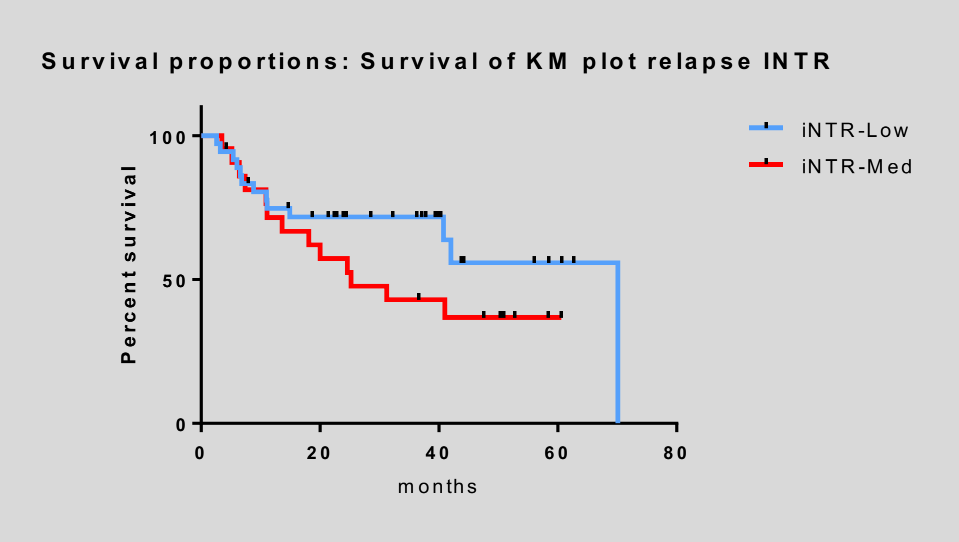




Neutrophil to Lymphocyte Ratio

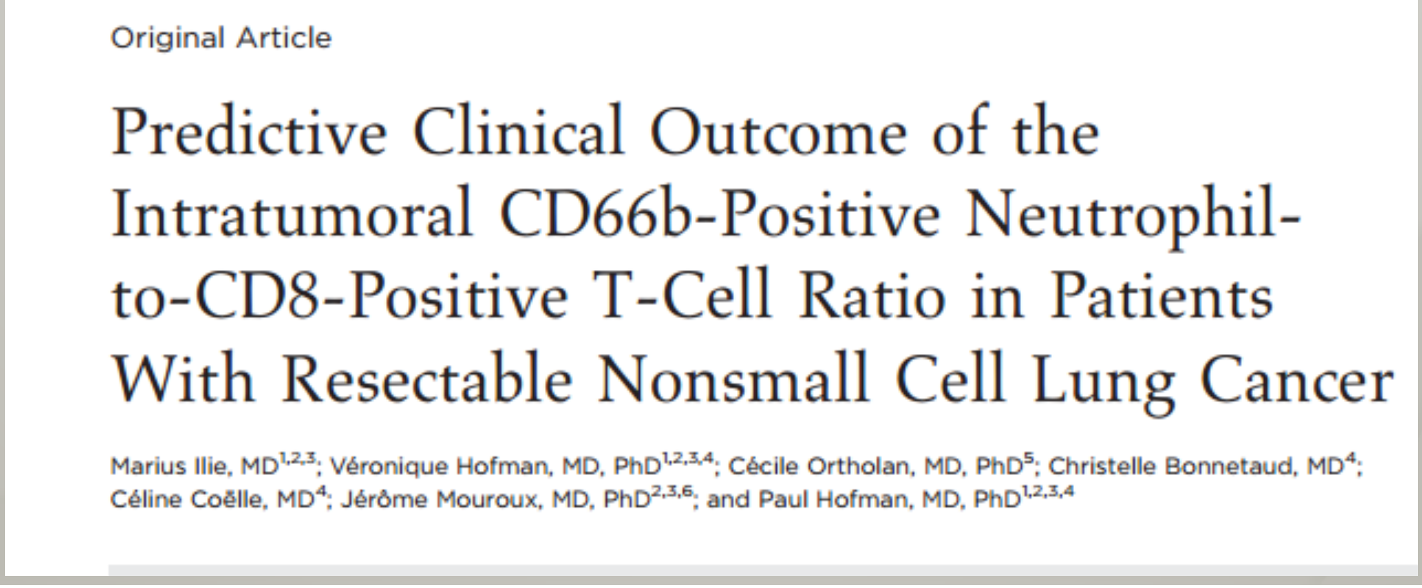


Neutrophil to Lymphocyte Ratio



Survival proportions: Survival of KM plot relapse INTR

Survival proportions: Survival of KM plot death iNTR



Original Article

Predictive Clinical Outcome of the Intratumoral CD66b-Positive Neutrophil-to-CD8-Positive T-Cell Ratio in Patients With Resectable Nonsmall Cell Lung Cancer

Marius Ilie, MD[1,2,3]; Véronique Hofman, MD, PhD[1,2,3,4]; Cécile Ortholan, MD, PhD[5]; Christelle Bonnetaud, MD[4]; Céline Coëlle, MD[4]; Jérôme Mouroux, MD, PhD[2,3,6]; and Paul Hofman, MD, PhD[1,2,3,4]

IRCAN Team 3

Paul Hofman
Baharia Mograbi
Valérie Vouret
Marius Ilie
Xavier Hébuterne
Josephine Zangari
Carole Binard
Nathalie Yazbek
Hussein Alakhrass
Sylvia Korzeniewski

IPMC-CNRS (Bioinformatique)

Kevin Lebrigand
Bernard Mari
Pascal Barbry
Chimène Moreilhon

INRA

Arlette Darfeuille- Michaud
Pierre Lapaquette

LPCE- BIOBANK

Paul Hofman
Marius Ilie
Véronique Hofman
Sandra Lassalle
Eric Selva
Virginie Gavric
Olivier Bordone
Katia Havet
Elodie Long

IAL-CNRS
TAPTAR

Annick Harel-Bellan
Mouloud Souïdi

Instituts thématiques Inserm
Institut national de la santé et de la recherche médicale

Centre Hospitalier Universitaire de Nice

Fondation Recherche Médicale

IRCAN

PACA Canceropole Provence-Alpes Côte d'Azur

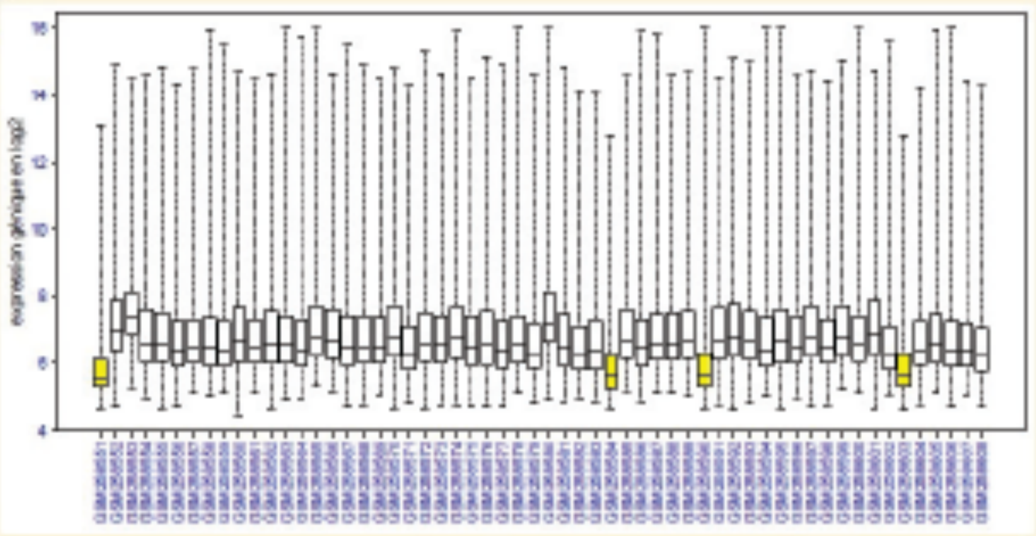
Projet financé par l'ANR - Project funded by the ANR

Association pour la Recherc sur le Cancer

Institut National du Cancer

Élaboration des bases de données

Phase 2 : Traitements des bases de données

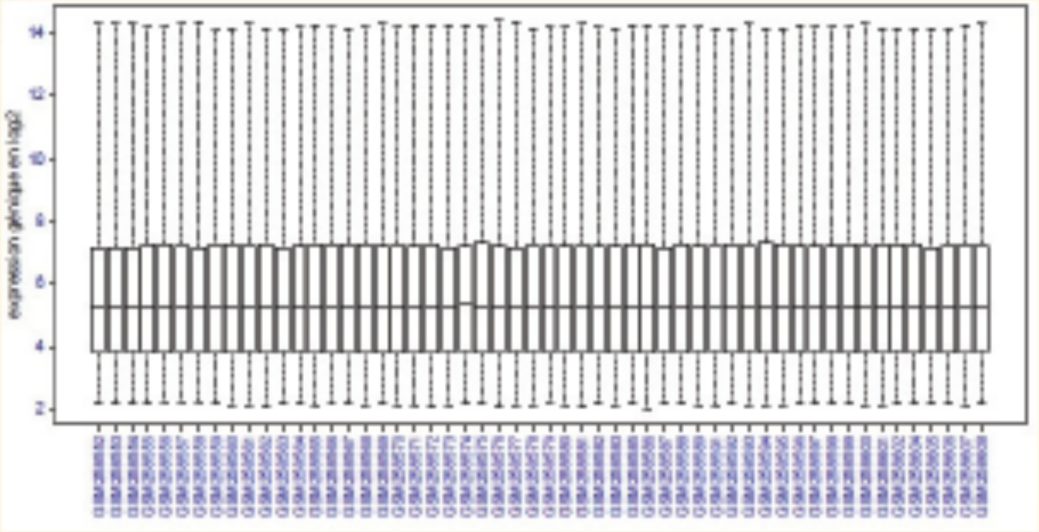


Patients cancer

Retrait patients

Élaboration des bases de données

Phase 2 : Traitements des bases de données

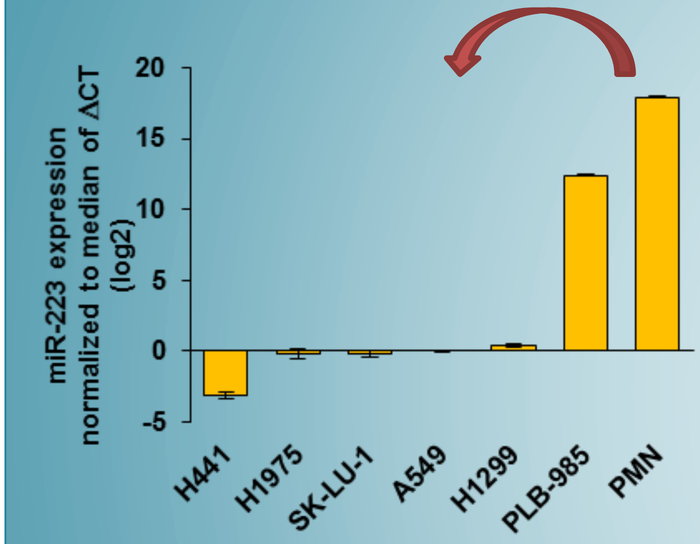


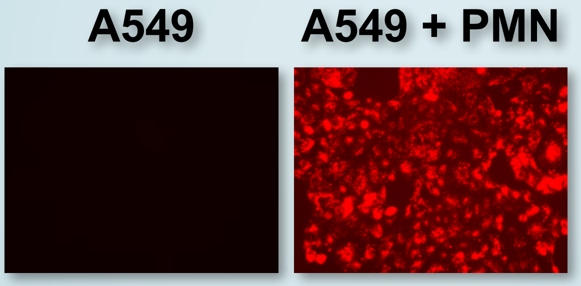
Patients cancer

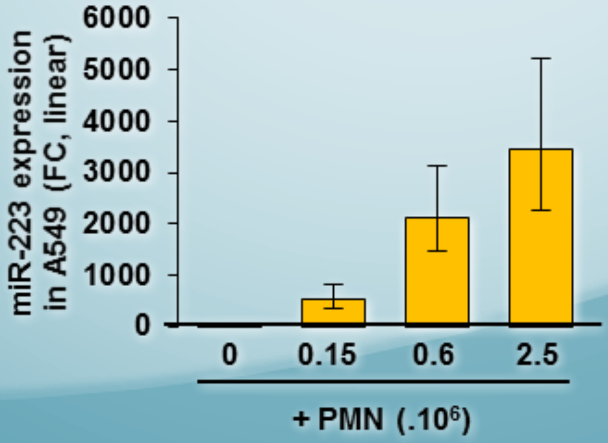
Retrait patients

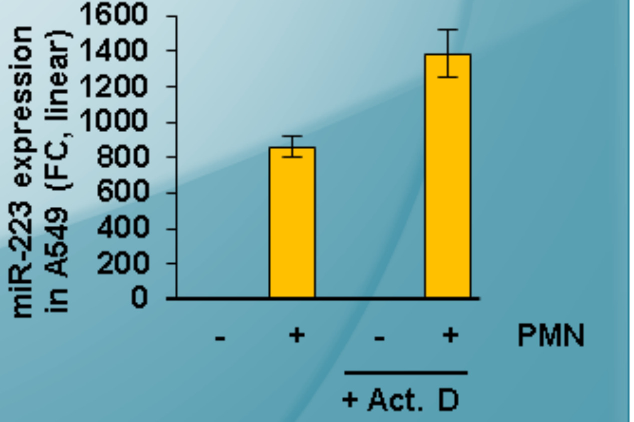
Normalisation

miR-223 is efficently transfered in epithelial cells (I)

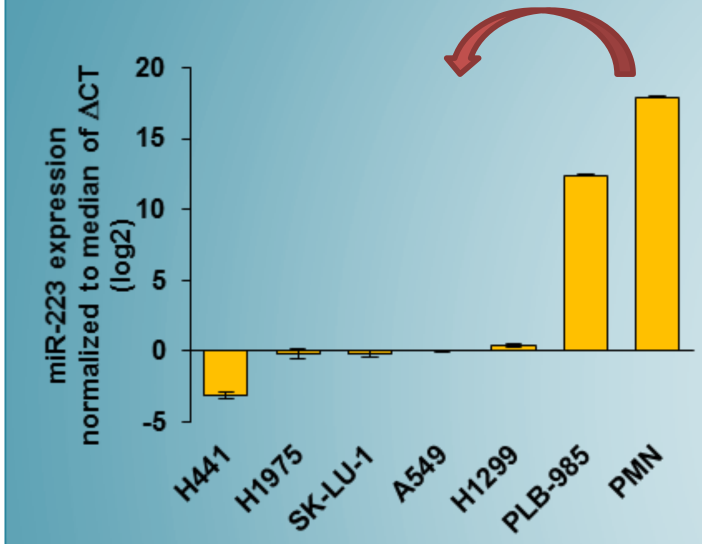


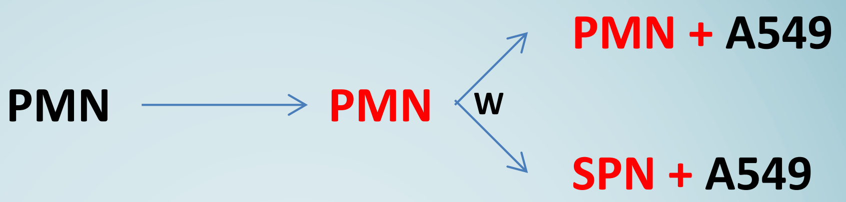




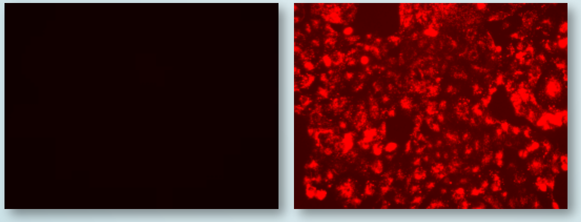


miR-223 is efficently transfered in epithelial cells (I)

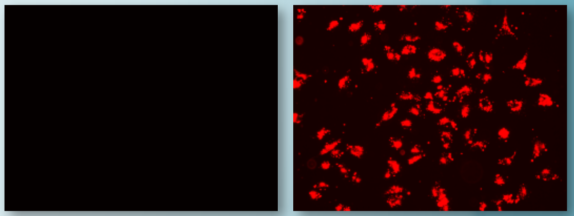


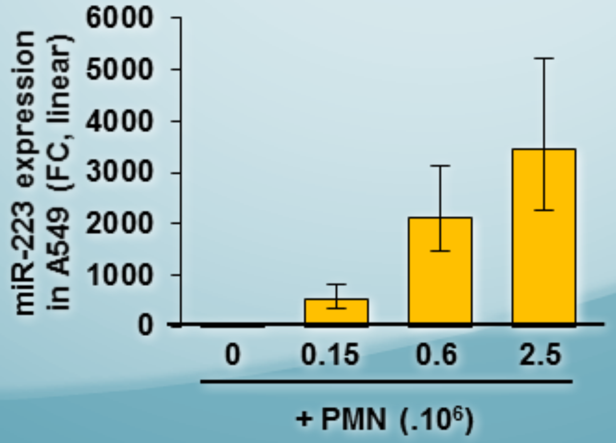


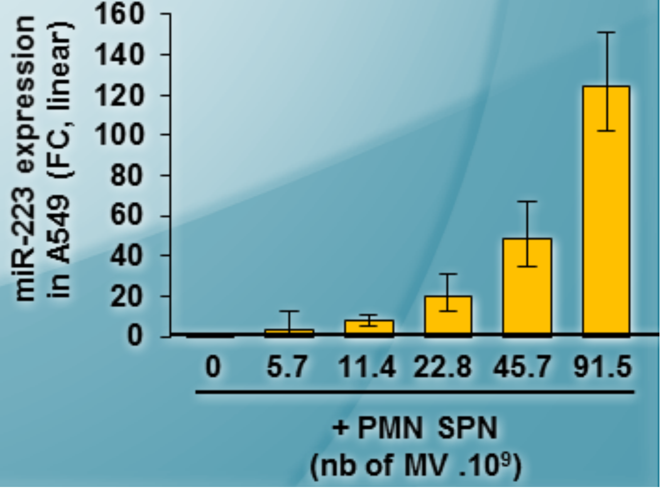
A549 **A549 + PMN**



A549 **A549 + PMN SPN**

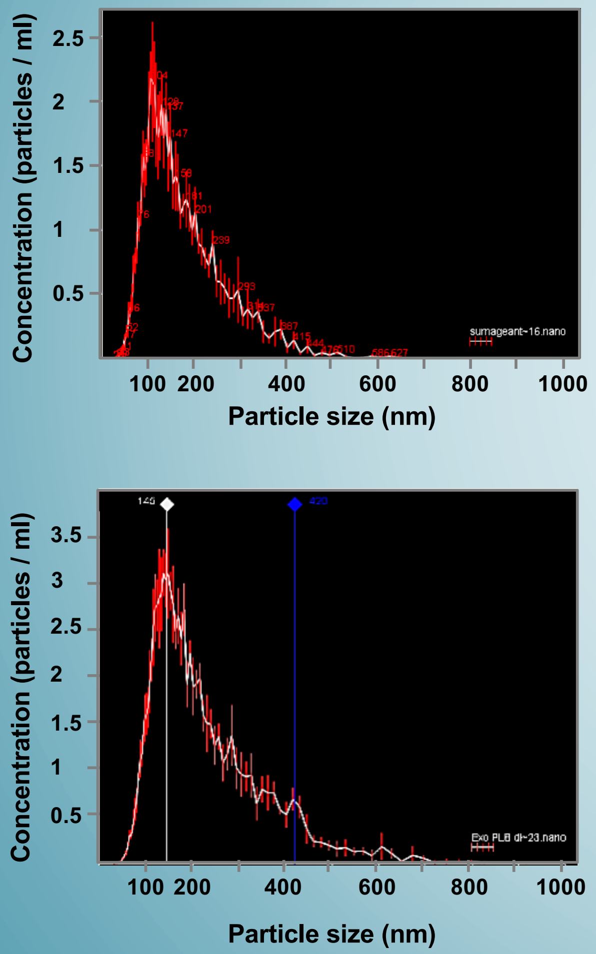


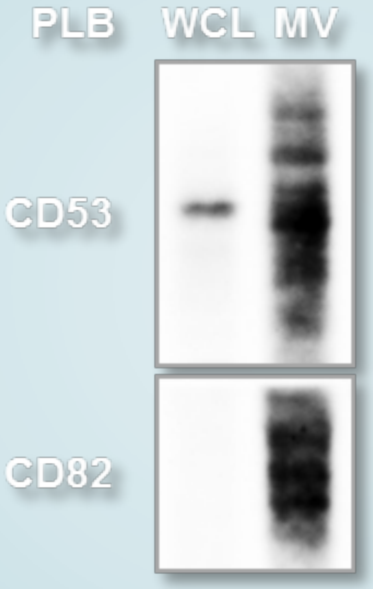


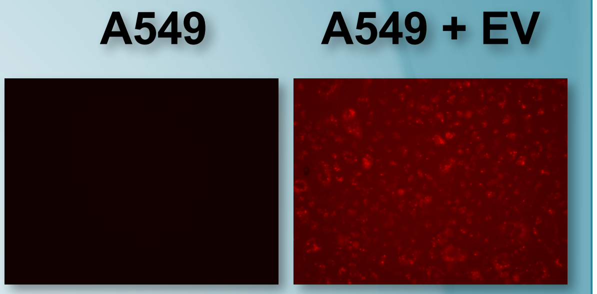


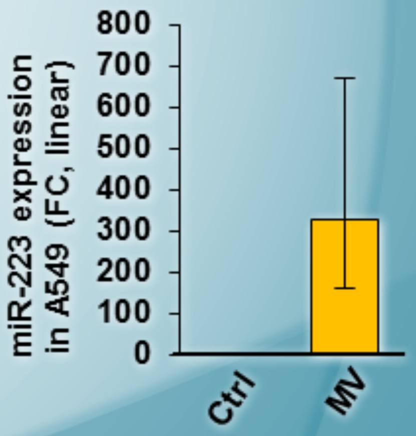
miR-223 is efficently transfered in epithelial cells (II)

Nanoparticle tracking analysis

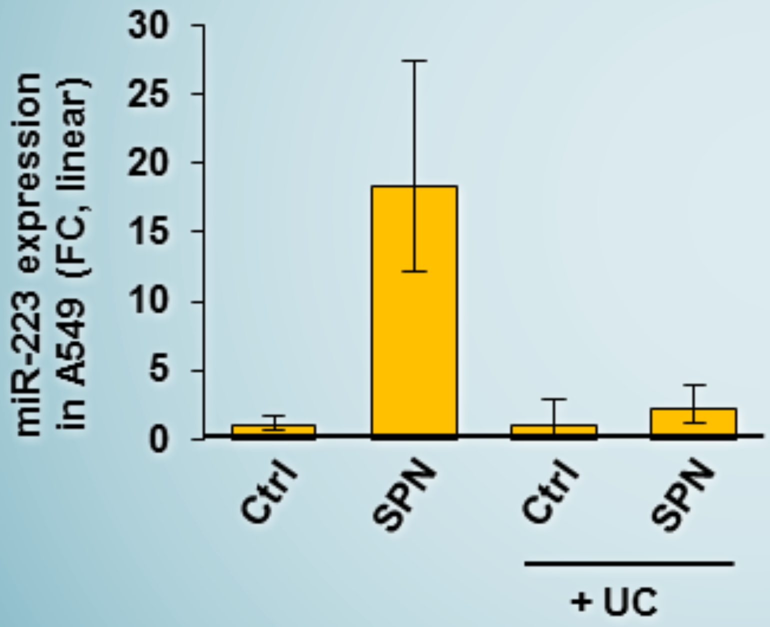


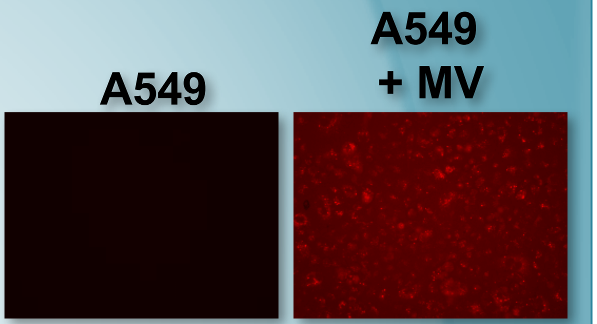


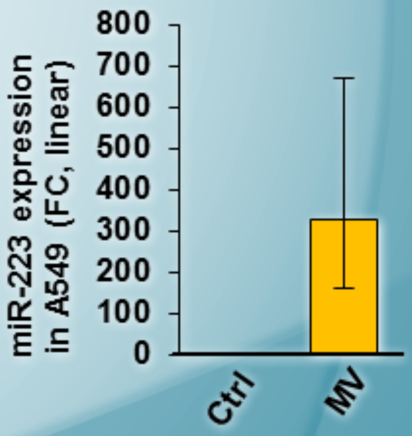




miR-223 is efficently transfered in epithelial cells (III)

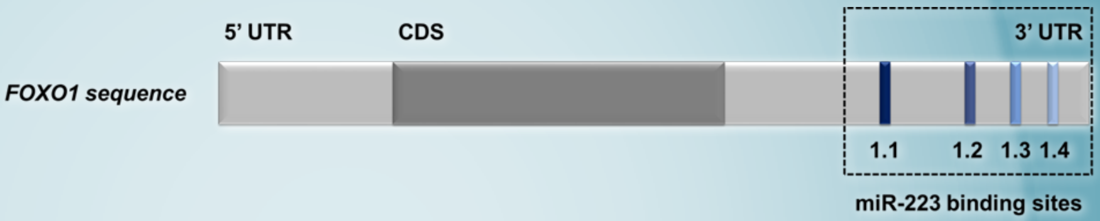


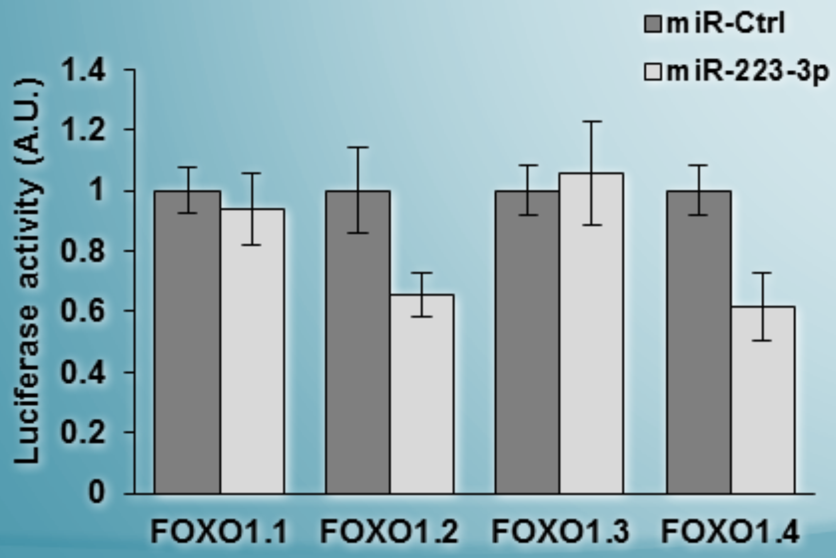




Is extracellular miR-223 functional once engulfed by recipient cells?

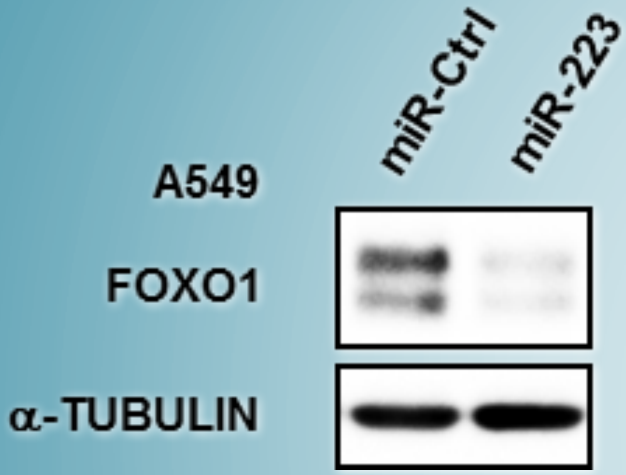
FOXO as a bone fide target of miR223

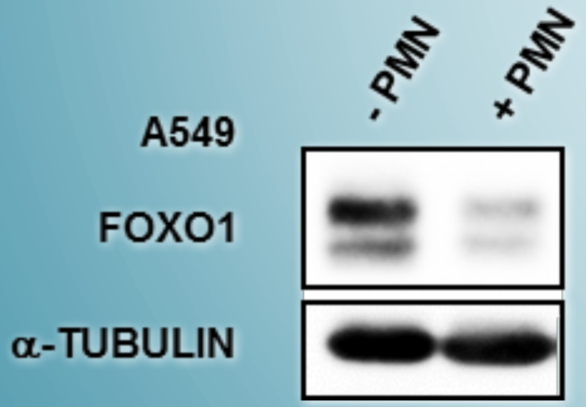


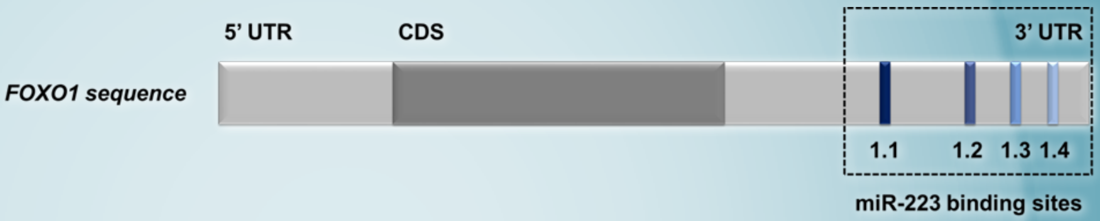


miRNA	Gene	Position	Energy	Alignment
hsa-miR-223-3p	FOXO1	2841	-16.1	5' C UCU G G 3' mRNA UGUUU GCG AACUGAC AUAAA UGU UUGACUG 3' ACCCC C U 5' µRNA
hsa-miR-223-3p	FOXO1	4065	-15.4	5' C U 3' mRNA UG AACUGACA AC UUGACUGU 3' ACCCCAUAA UGU 5' µRNA
hsa-miR-223-3p	FOXO1	4842	-14.8	5' A AU U 3' mRNA GA ACUGAC CU UGACUG 3' ACCCCAUAAA GUU U 5' µRNA
hsa-miR-223-3p	FOXO1	5089	-26.2	5' C 3' mRNA UUUGACAAACUGACA AAACUGUUUGACUGU 3' ACCCCAU 5' µRNA

FOXO as a bone fide target of miR223

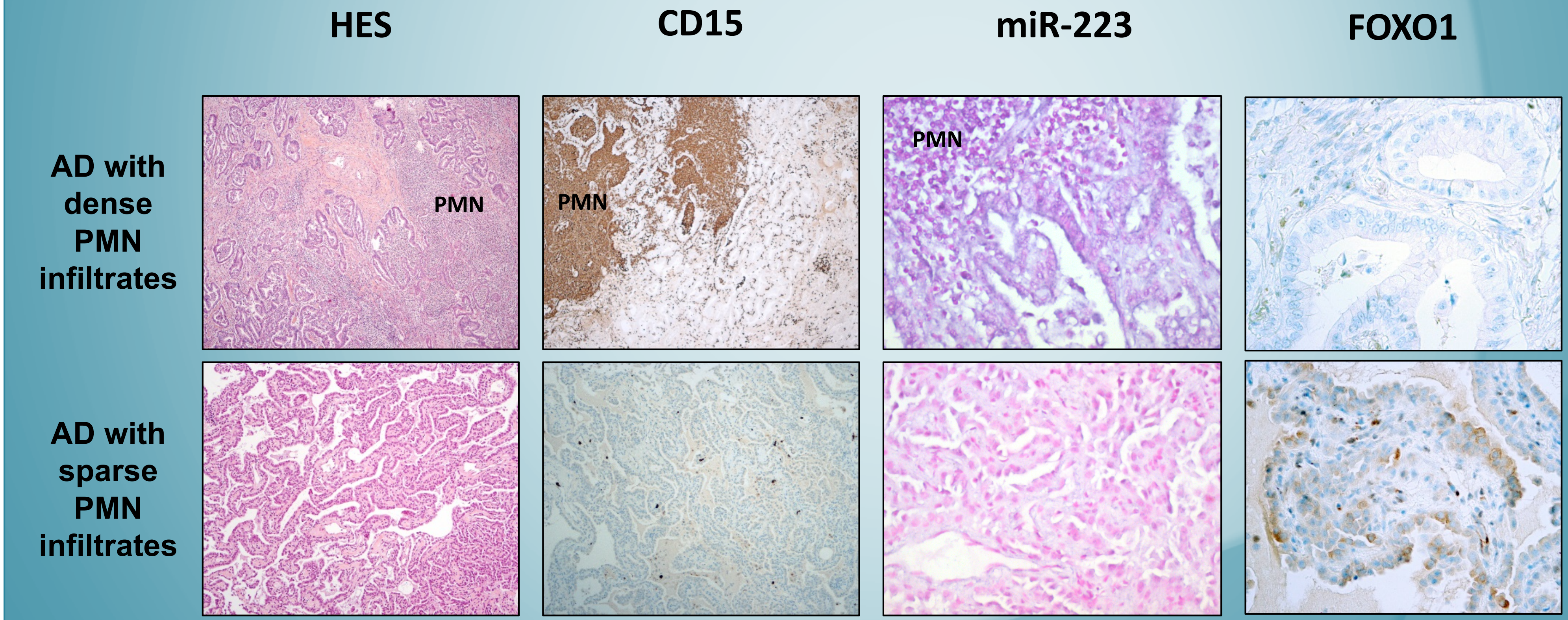




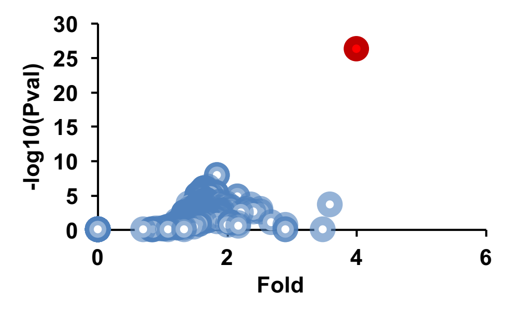


miRNA	Gene	Position	Energy	Alignment
hsa-miR-223-3p	FOXO1	2841	-16.1	5' C UCU G G 3' mRNA UGUUU GCG AACUGAC AUAAA UGU UUGACUG 3' ACCCC C U 5' µRNA
hsa-miR-223-3p	FOXO1	4065	-15.4	5' C U 3' mRNA UG AACUGACA AC UUGACUGU 3' ACCCCAUAA UGU 5' µRNA
hsa-miR-223-3p	FOXO1	4842	-14.8	5' A AU U 3' mRNA GA ACUGAC CU UGACUG 3' ACCCCAUAAA GUU U 5' µRNA
hsa-miR-223-3p	FOXO1	5089	-26.2	5' C 3' mRNA UUUGACAAACUGACA AAACUGUUUGACUGU 3' ACCCCAU 5' µRNA

A dense PMN infiltrate is associated with miR-223-3p expression and decreased FOXO1 expression in lung cancer patients



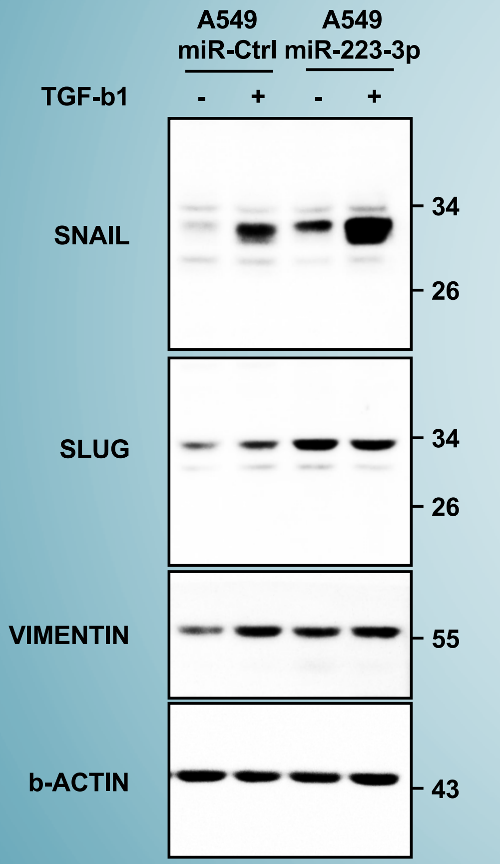
Microarray analysis

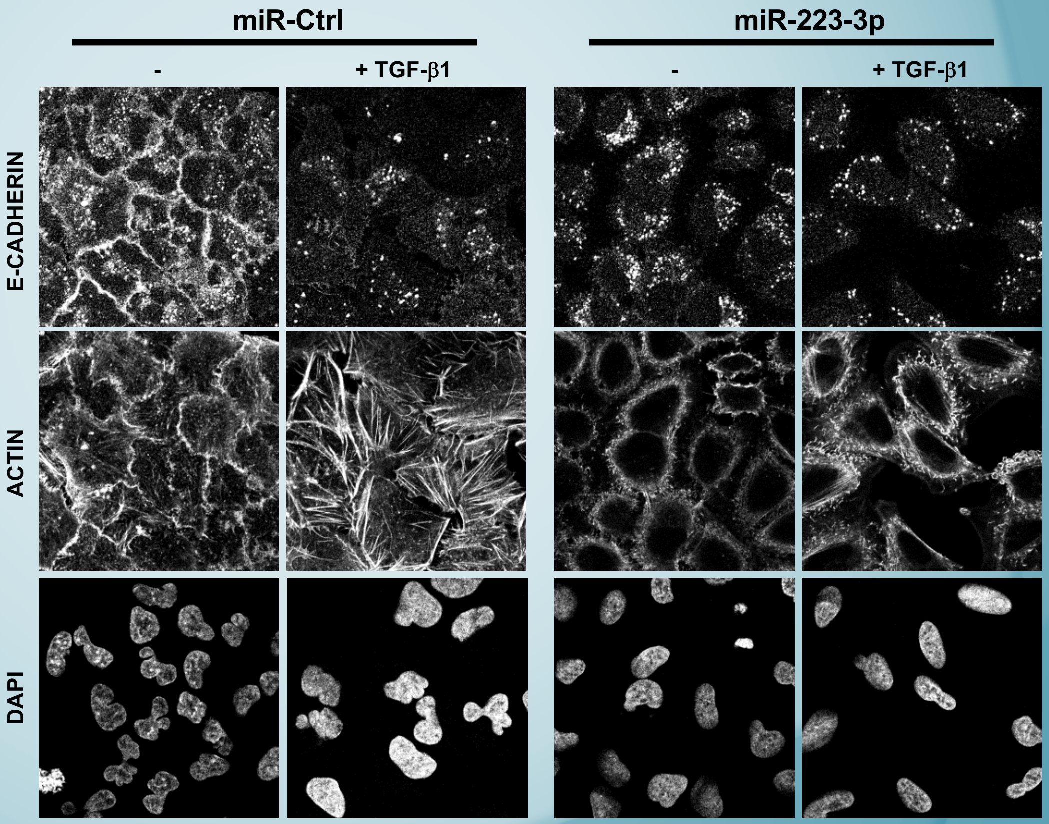


Term	Overlap	Adjusted P-value	Z-score	Combined Score	Genes
ANCHORING JUNCTION (GO:0070161)	34/419	1,15E-05	-2,37	27,00	SCARB2;KRT80;RALA;AHNAK;SDC4;CTNND1;TWF1;CIB2;TENC1;NDRG1;CSRP1;CDH2;EPB41L2;G3BP1;DAG1;FLNB;CD59;RAC1;PDLIM7;TGM2;TNS1;ITGA3;CAV1;ITGA1;RRAS2;PLEKHA7;ABI2;DLG5;PKP2;EVL;NF2;AJUBA;LIMS2;ARF6
ADHERENS JUNCTION (GO:0005912)	33/405	1,15E-05	-2,35	26,71	SCARB2;RALA;AHNAK;SDC4;CTNND1;TWF1;CIB2;TENC1;NDRG1;CSRP1;CDH2;EPB41L2;G3BP1;DAG1;FLNB;CD59;RAC1;PDLIM7;TGM2;TNS1;ITGA3;CAV1;ITGA1;RRAS2;PLEKHA7;ABI2;DLG5;PKP2;EVL;NF2;AJUBA;LIMS2;ARF6
CELL-SUBSTRATE ADHERENS JUNCTION (GO:0005924)	26/358	1,26E-03	-2,35	15,66	SCARB2;RALA;AHNAK;SDC4;CIB2;TWF1;TENC1;CSRP1;CDH2;EPB41L2;G3BP1;DAG1;FLNB;CD59;RAC1;PDLIM7;TGM2;TNS1;ITGA3;CAV1;ITGA1;RRAS2;EVL;AJUBA;LIMS2;ARF6
CELL-SUBSTRATE JUNCTION (GO:0030055)	26/362	1,26E-03	-2,34	15,64	SCARB2;RALA;AHNAK;SDC4;TWF1;CIB2;TENC1;CSRP1;CDH2;EPB41L2;G3BP1;DAG1;FLNB;CD59;RAC1;PDLIM7;TGM2;TNS1;ITGA3;CAV1;ITGA1;RRAS2;EVL;AJUBA;LIMS2;ARF6
FOCAL ADHESION (GO:0005925)	25/352	1,77E-03	-2,29	14,52	SCARB2;RALA;AHNAK;SDC4;TWF1;TENC1;CSRP1;CDH2;EPB41L2;G3BP1;DAG1;FLNB;CD59;RAC1;PDLIM7;TGM2;TNS1;ITGA3;CAV1;ITGA1;RRAS2;EVL;AJUBA;LIMS2;ARF6
CELL-CELL JUNCTION (GO:0005911)	24/335	1,88E-03	-2,25	14,11	KRT80;AHNAK;HEG1;MAGI2;CTNND1;KIAA1462;TWF1;ANK3;MPP5;ATP1B1;CLDN2;NDRG1;PLEKHA7;SIRT2;CLDN4;CDH2;ABI2;PODXL;DLG5;PKP2;DAG1;SYNPO;AJUBA;CGNL1

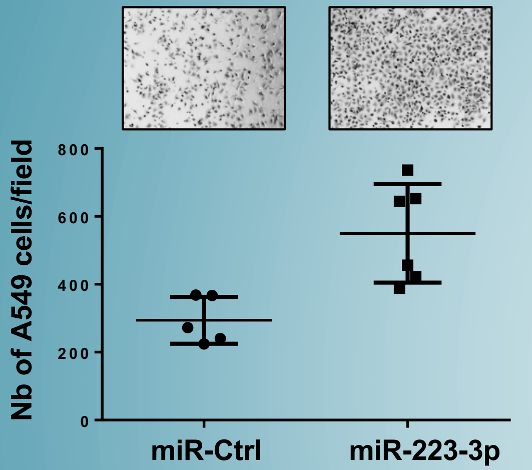
EMT/Migration/Invasion ?

miR-223-3p promotes EMT phenotype

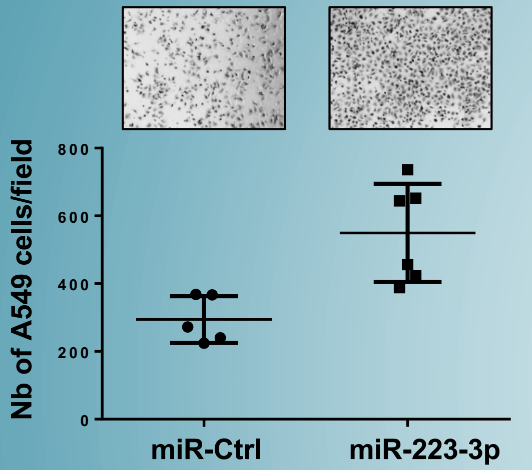


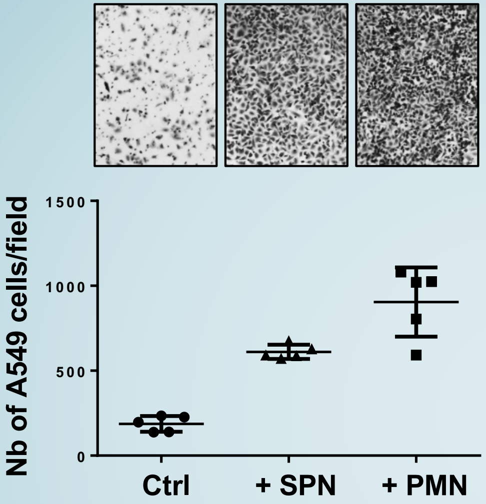


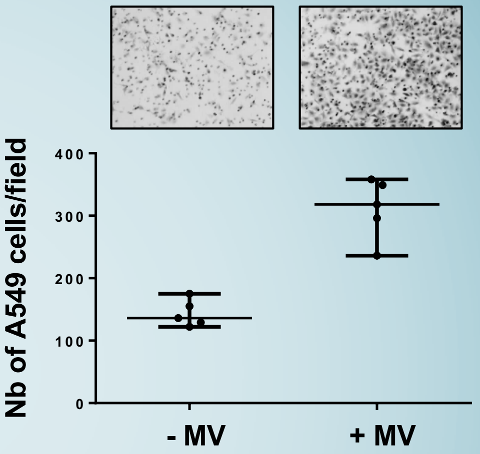
Invasion as a bone fide endpoint of phenotypic impact of miR-223

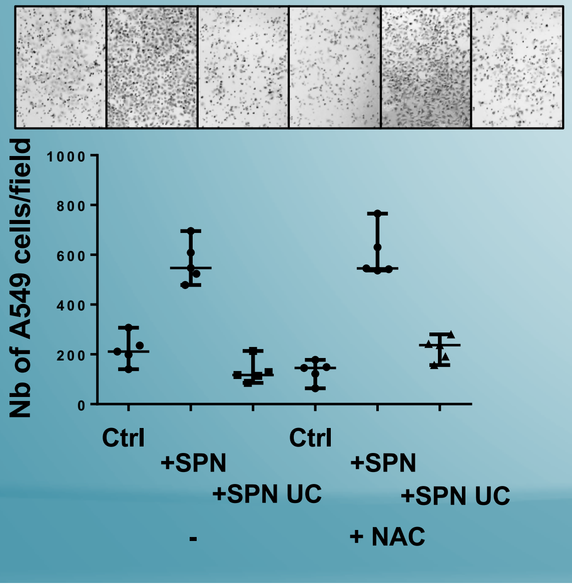


Invasion as a bone fide endpoint of phenotypic impact of miR-223









Invasion as a bone fide endpoint of phenotypic impact of miR-223