

Biomarqueurs liés au microenvironnement tumoral dans les cancers du pancréas

Franck Monnien

Angélique Vienot

UMR1098 RIGHT, INSERM, EFS, UFC
CHU Besançon

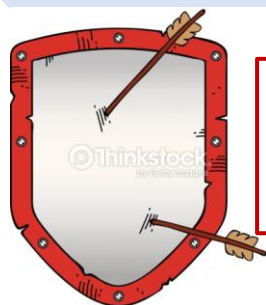
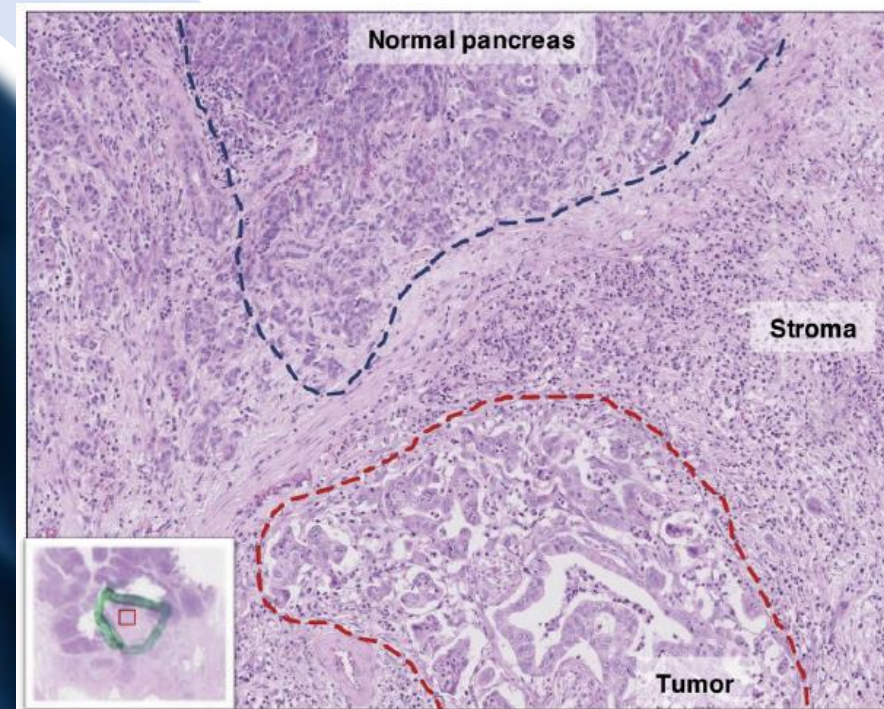
↗ Incidence et mortalité

Nombre de **nouveaux cas**
X3 en 20 ans

2^e cause de décès par
cancer en **2030**

CANCERS DU PANCREAS

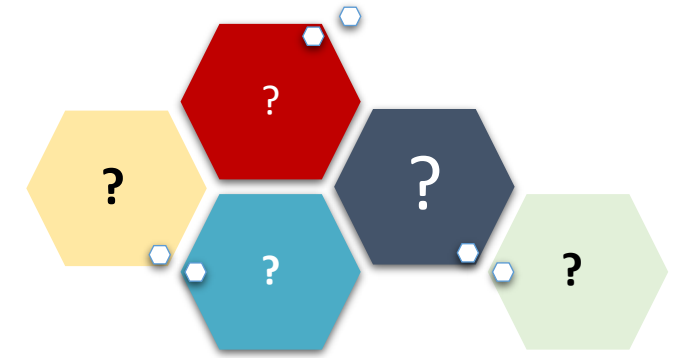
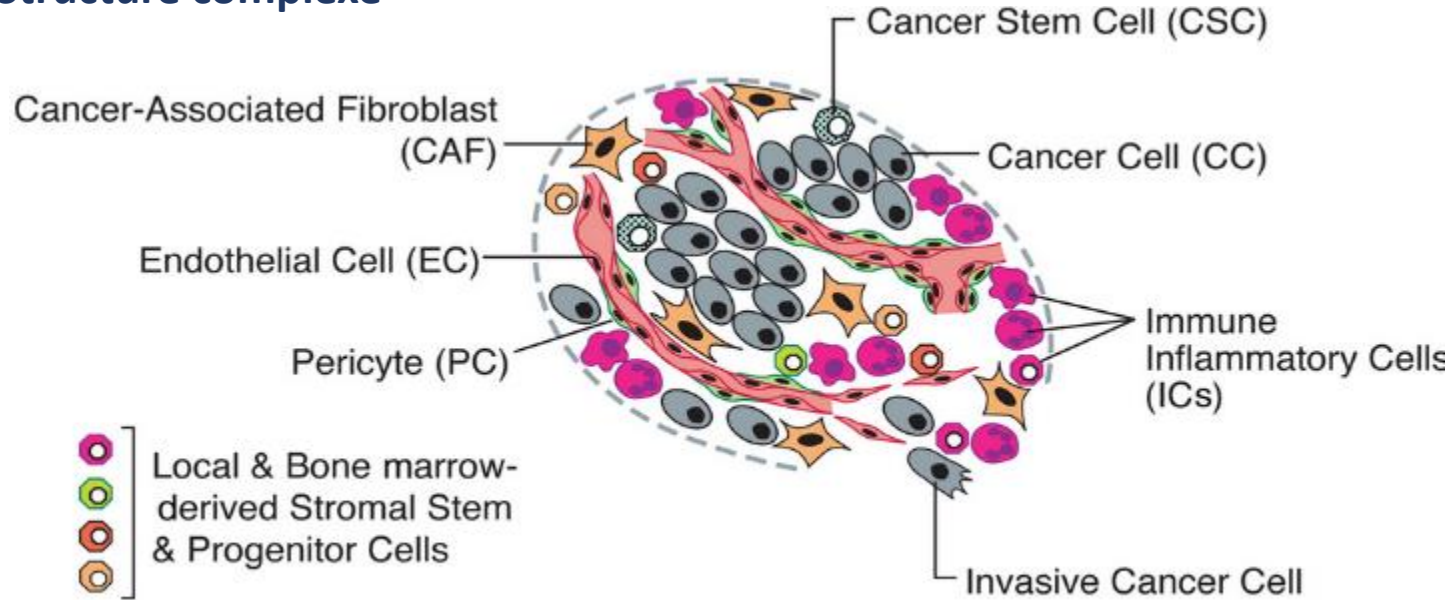
Stroma tumoral abondant



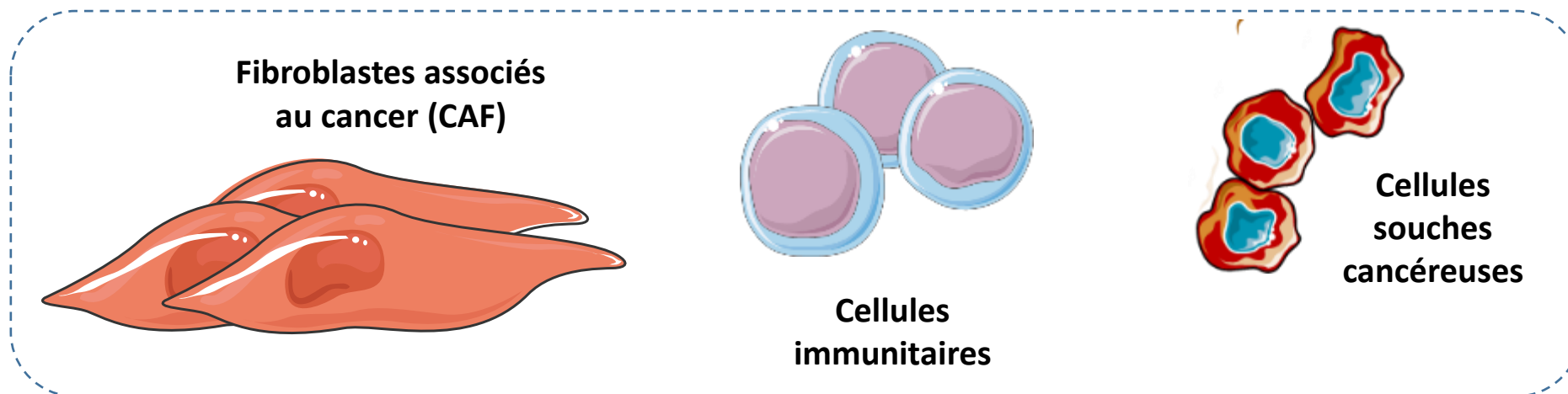
→ Résistance aux traitements
→ Difficulté de diagnostic

Stroma tumoral = microenvironnement entourant les cellules cancéreuses

→ **Structure complexe**

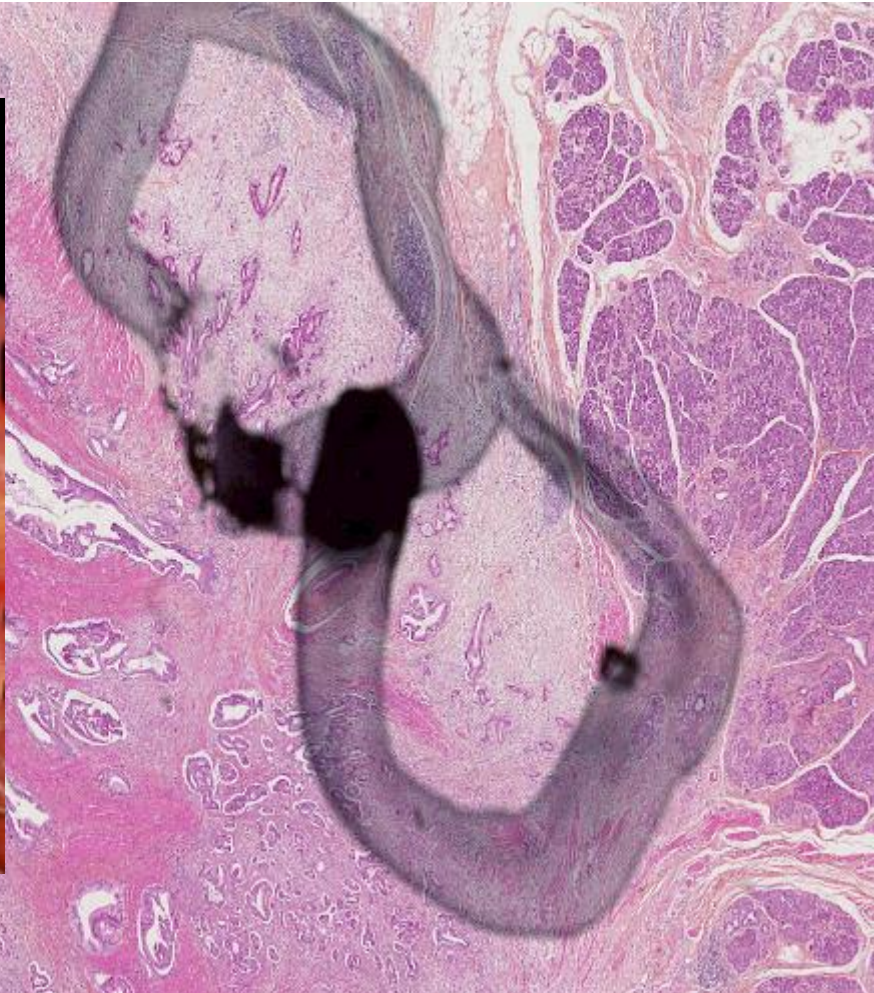
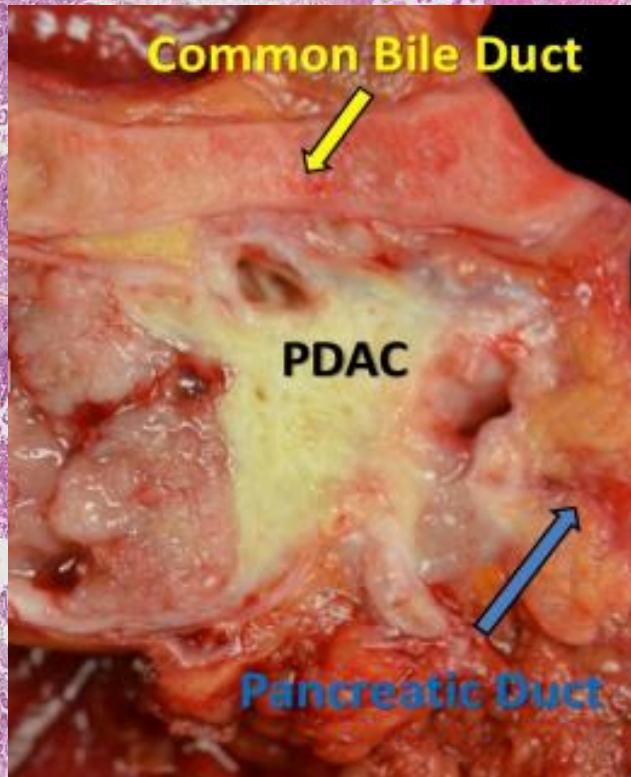


Hétérogénéité stromale

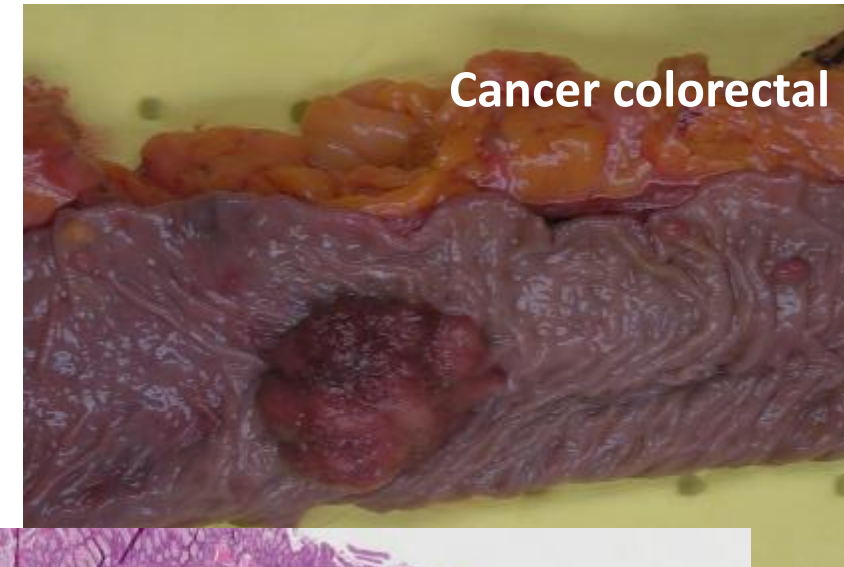


Stroma tumoral → ≠ Quantités

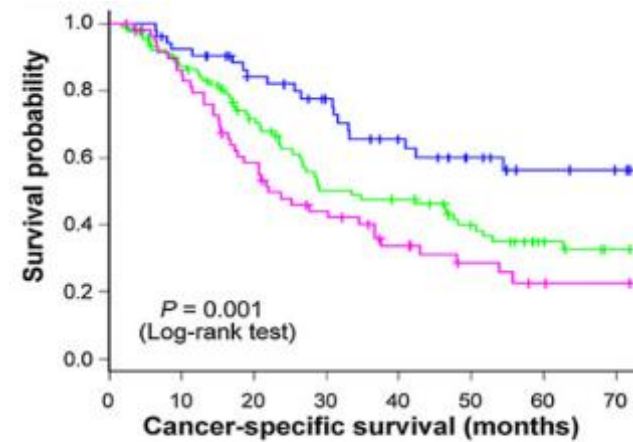
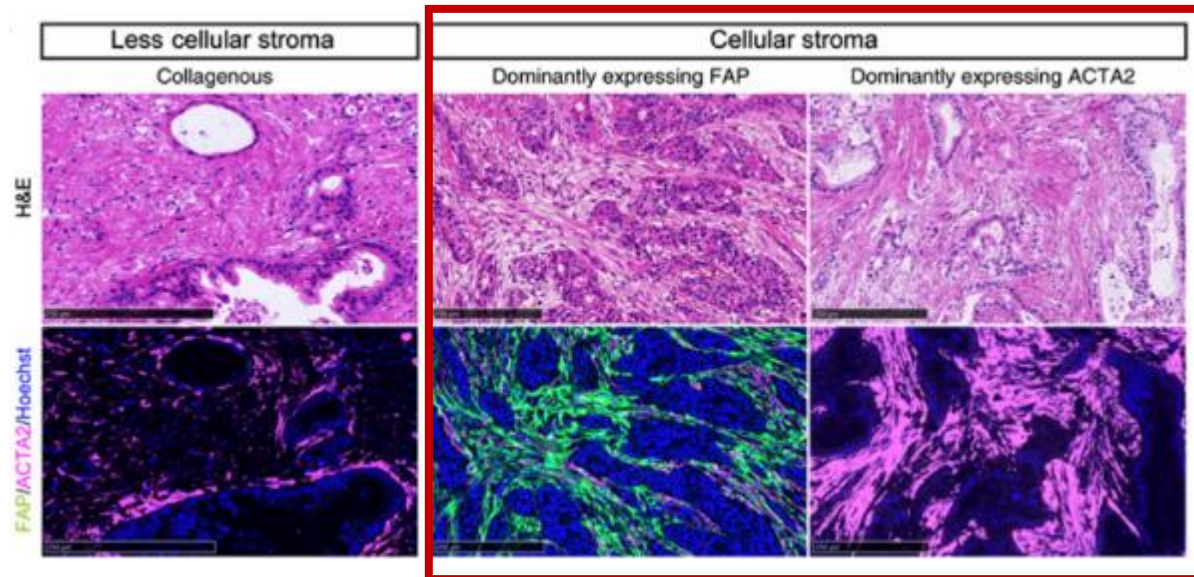
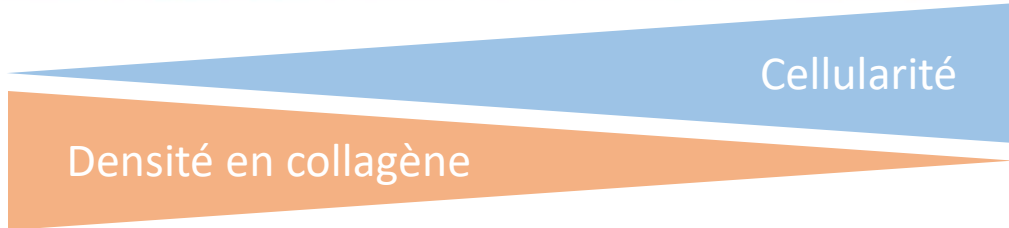
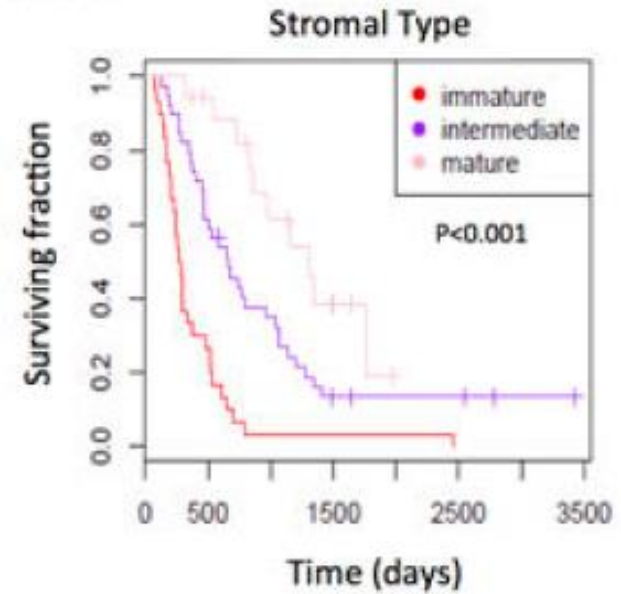
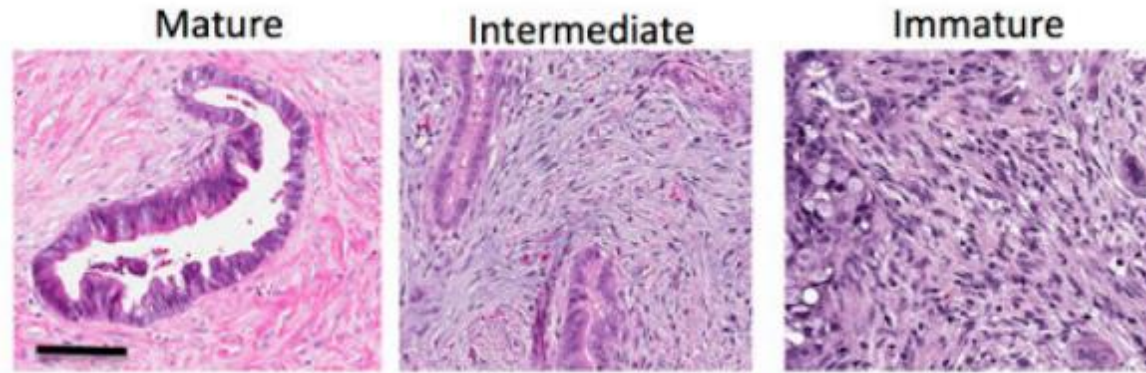
Stroma desmoplasique dense



≠



Stroma tumoral → ≠ Qualités



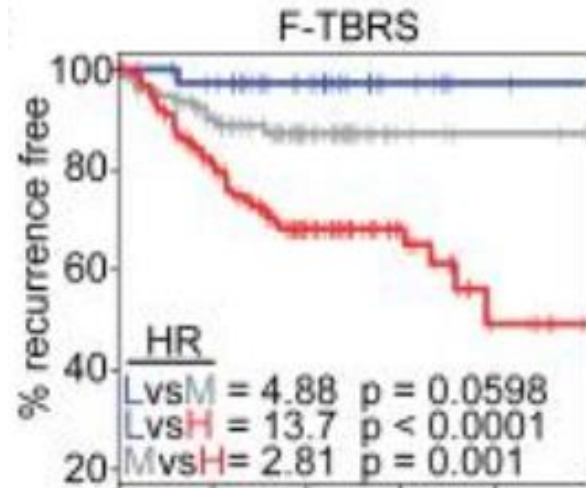
Number at risk	0	10	20	30	40	50	60	70 (months)
C-stroma	53	48	39	32	24	18	12	10
F-stroma	93	75	55	37	34	24	16	11
A-stroma	61	50	33	23	15	10	6	5

Protéines exprimées par les fibroblastes



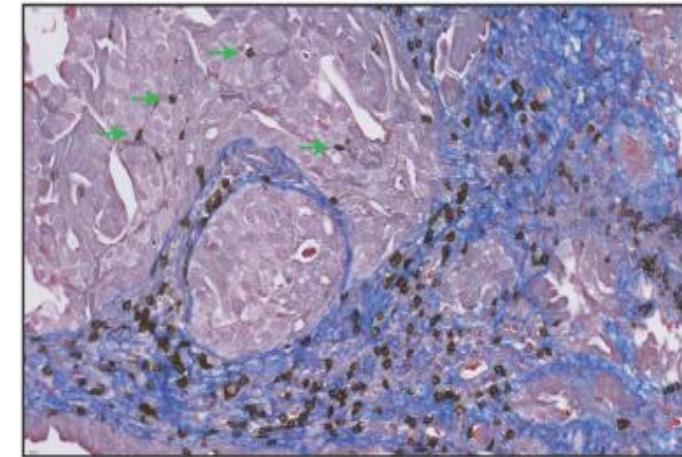
Effet amplifié par la signalisation du TGF- β

→ Signal d'activation



TGF- β contribue à un **microenvironnement immunosuppresseur**

Et à un **exclusion des lymphocytes T CD8**



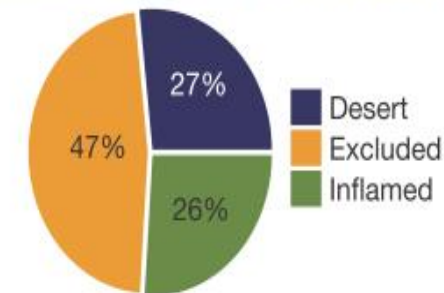
Rôle dans les fonctions des **cellules souches cancéreuses**

→ Caractère indifférencié et pluripotent

→ **Hétérogénéité tumorale**

→ Rôle de facteurs de transcription dans l'hétérogénéité stromale ?

SALL4 ?



Impliqué dans la pluripotence et l'auto-renouvellement des cellules souches embryonnaires et hématopoïétiques



Molecular Oncology



SALL4-related gene signature defines a specific stromal subset of pancreatic ductal adenocarcinoma with poor prognostic features

Angélique Vienot^{1,2,3,4}, Franck Monnier^{1,5}, Caroline Truntzer^{6,7}, Virginie Mougey^{1,4}, Adeline Bouard^{1,4}, Jean-René Pallandre¹, Chloé Mollimard⁵, Romain Loyon¹, Kamal Asgarov^{1,4}, Gerlinde Averous⁸, François Ghiringhelli^{6,7}, Frédéric Bibeau⁵, Paul Peixoto^{1,9} and Christophe Borg^{1,2,3,4}

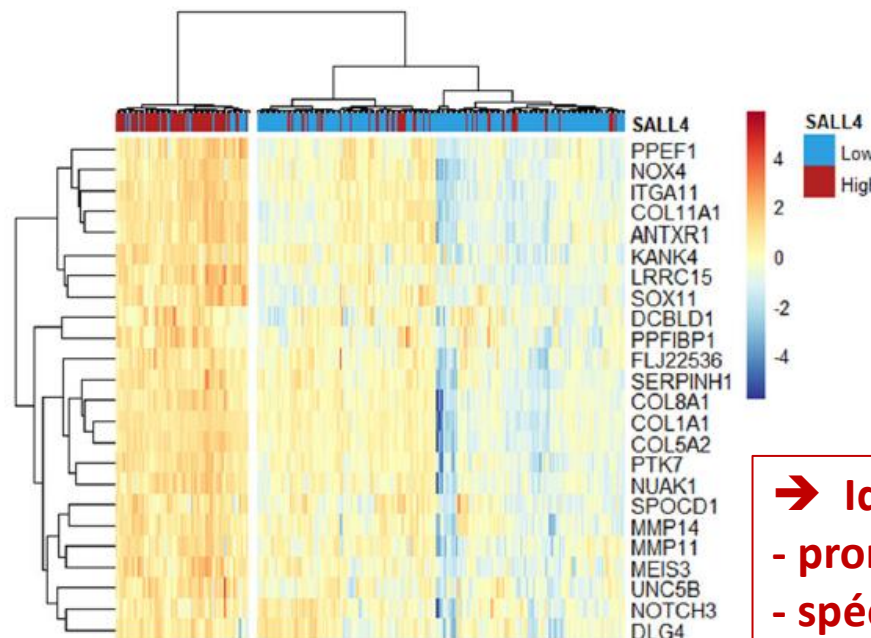
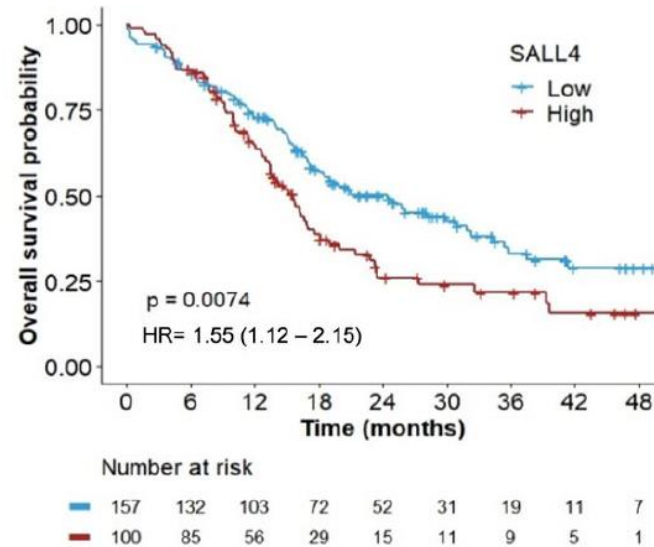
Cancers du pancréas

- Données cliniques et de survie
- Données moléculaires : transcriptome

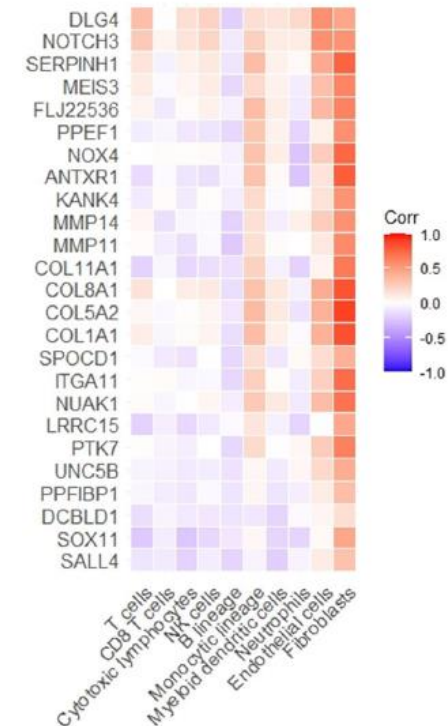
Bases de données publiques



Analyses bioinformatiques

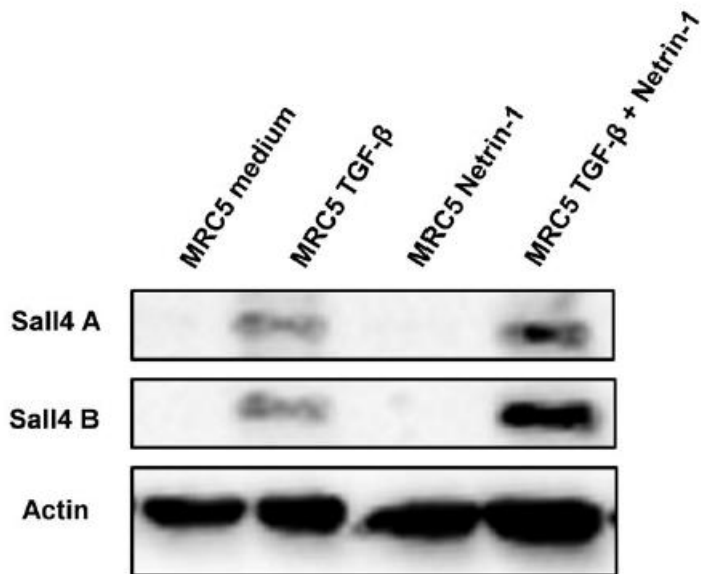
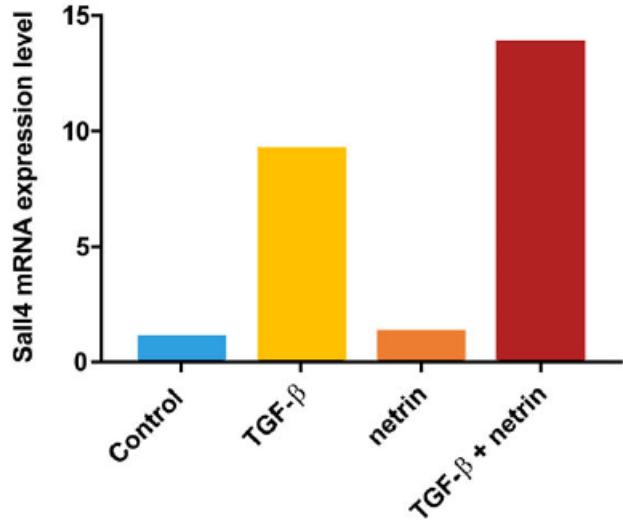


Déconvolution cellulaire

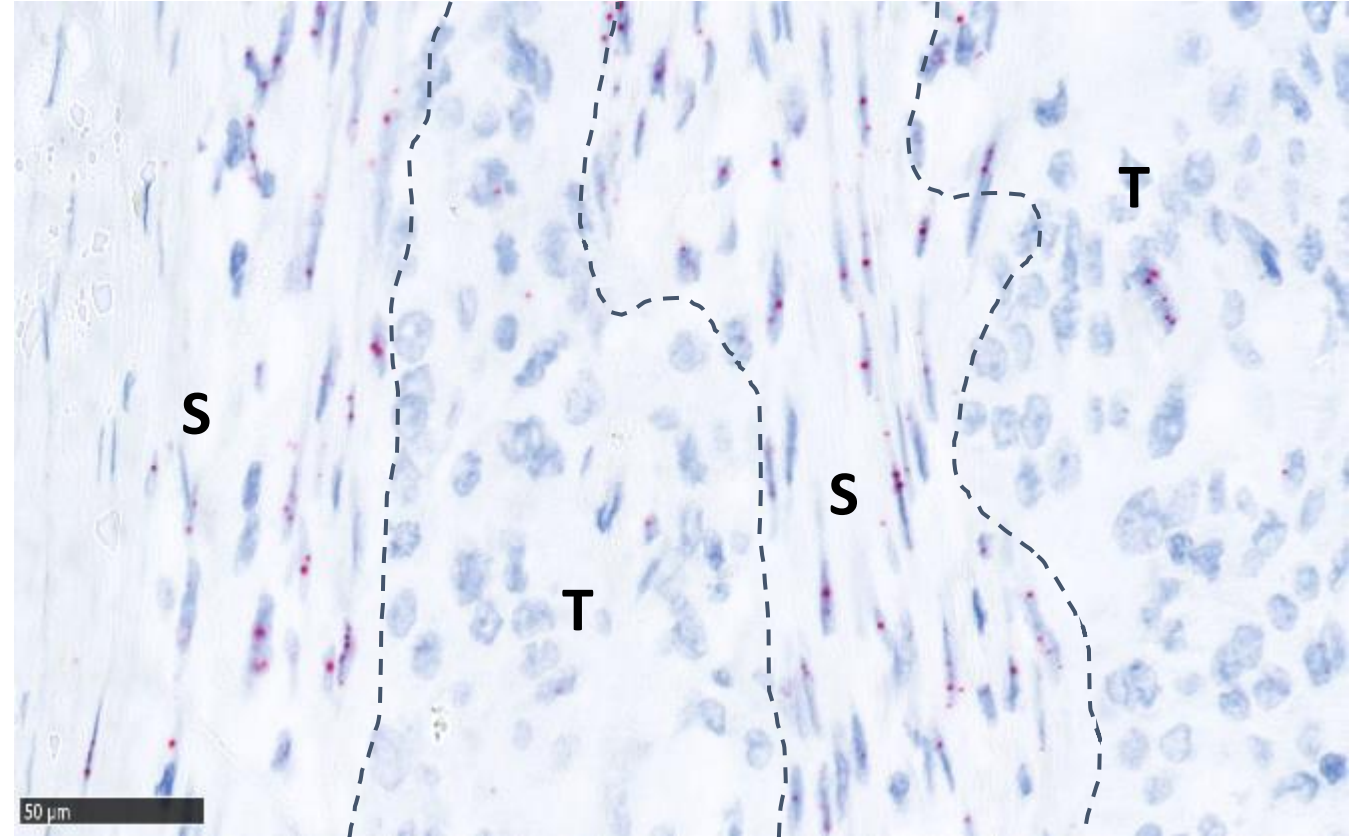


→ **Identification d'une signature**
 - pronostique
 - spécifique du stroma tumoral

Expression de SALL4 dans les fibroblastes



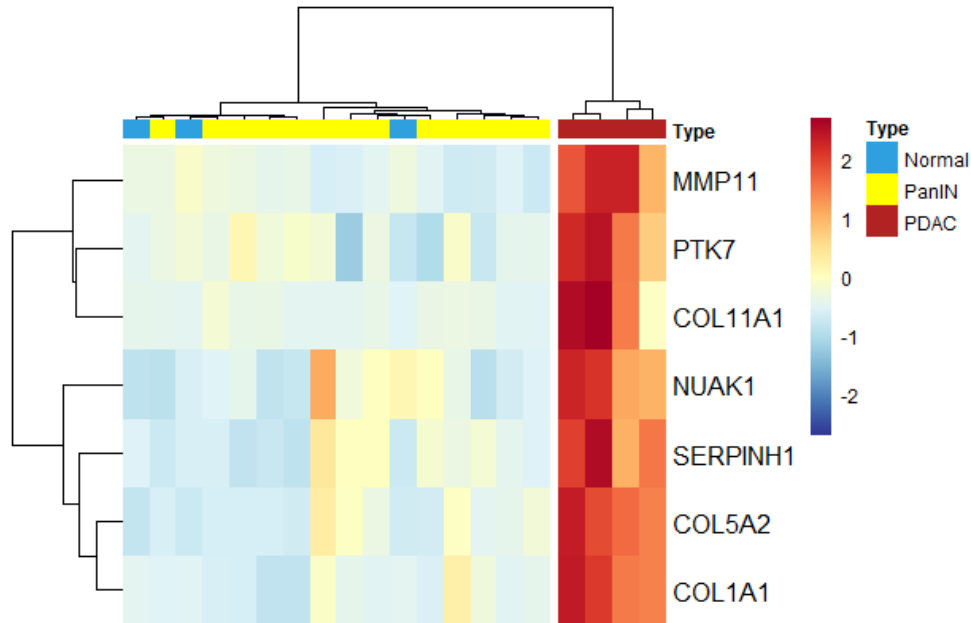
RNAscope SALL4



Expression IHC SALL4 non détectable

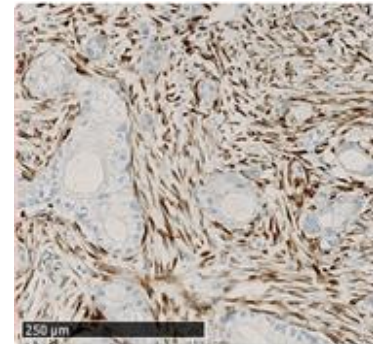
Validation en immunohistochimie de la signature stromale

Signature SALL4 spécifique aux tumeurs invasives

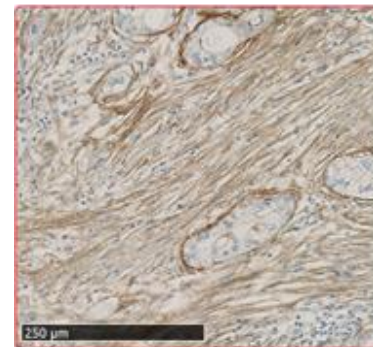


→ La signalisation liée à SALL4 contribue à définir un stroma spécifique lié au caractère invasif et un pronostic défavorable

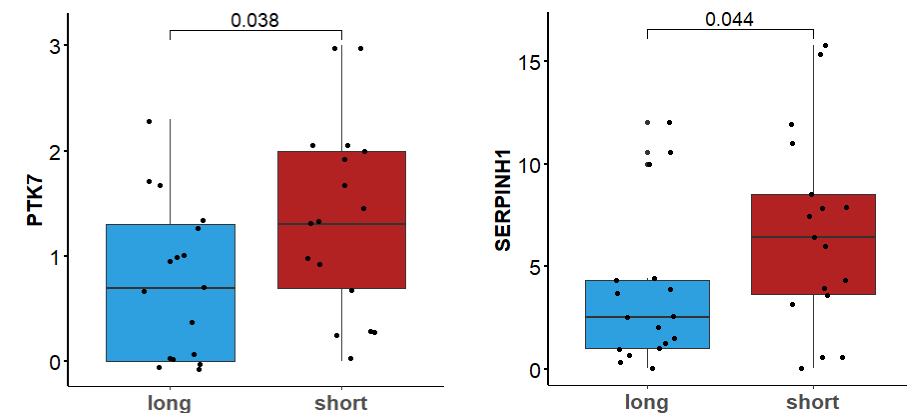
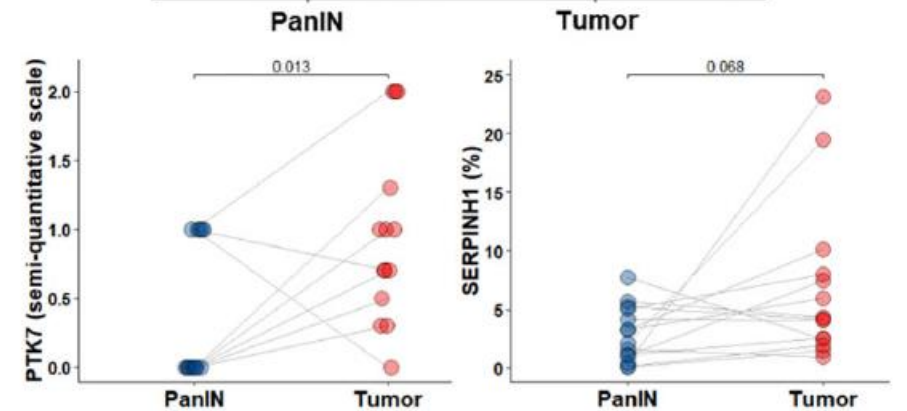
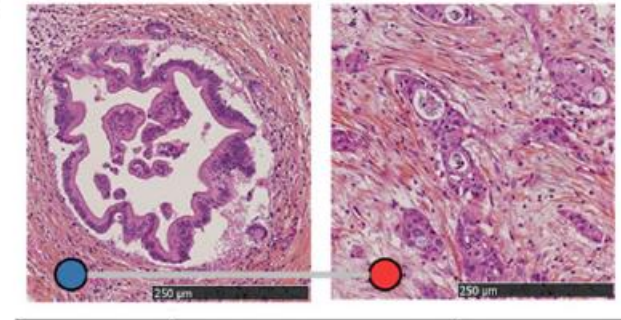
SERPINH1



PTK7



(D) matched comparison



253 patients operated for pancreatic resection between 2000 and 2017



Dr Molimard

- Blocs not found in the archives n = 7
- Without residual tumor cells n = 1
- Unknown patient n = 1
- Non adenocarcinoma tumour n = 19
- Remaining macroscopic tumor tissue after pancreatic resection n = 2
- Unknown resection statut n = 2
- Death within 30 days following surgery n = 1

Eligible for TMA construction
N = 220

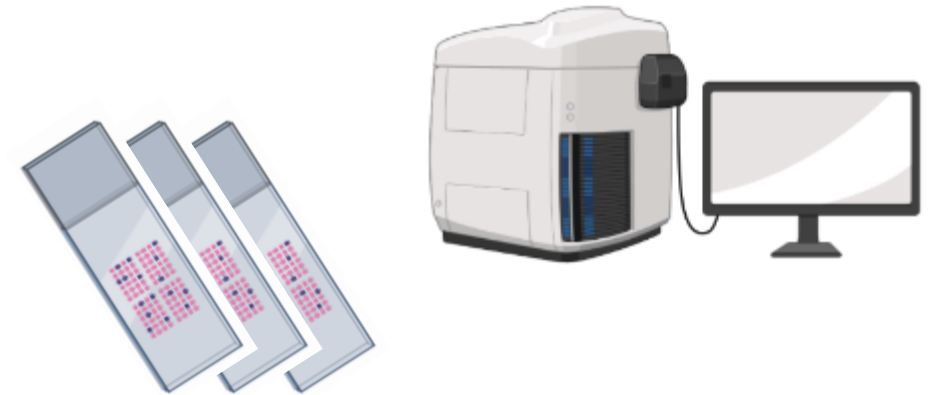
At least one exploitable spot per patient N = 217

No neoadjuvant treatment
N = 201

Neoadjuvant
N = 16



- 217 APC, patients opérés entre 2010 et 2017
- Tissue MicroArrays
- Immunohistochimie (IHC) et Colorations spéciales

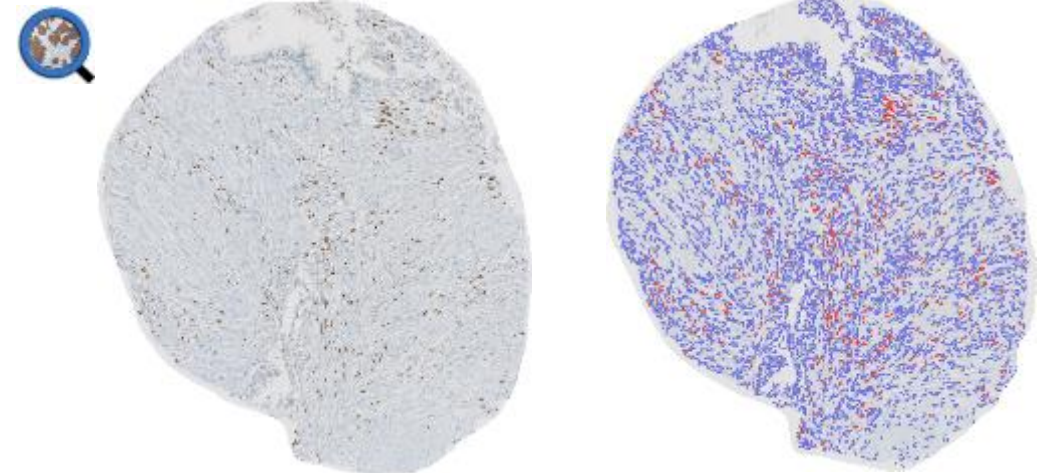


CD3	Lymphocyte T	Cellules immunitaires	Dénombrement automatique Cellules / mm²	
CD8	Lymphocyte T cytotoxiques			
CD20	Lymphocytes B			
Safran	Collagène de type I	Collagène	Classifieur de pixels % de surface marquée	
Rouge Sirius	Collagènes de type I & III			
FAP	Fibroblastes activés	Fibroblastes		
α -SMA	Fibroblastes activés			
HSP47*	Protéine chaperone du collagène			
LRRC15*	Protéine mésenchymateuse			
PTK7*	Regulating process in embryonic development	Fibroblastes et Cellules tumorales		Échelle visuelle semi-quantitative 0 1+ 2+ 3+
β -catenin	Adhésion cellulaire			

Densité de cellules + / mm²

Lymphocytes : exemple CD3

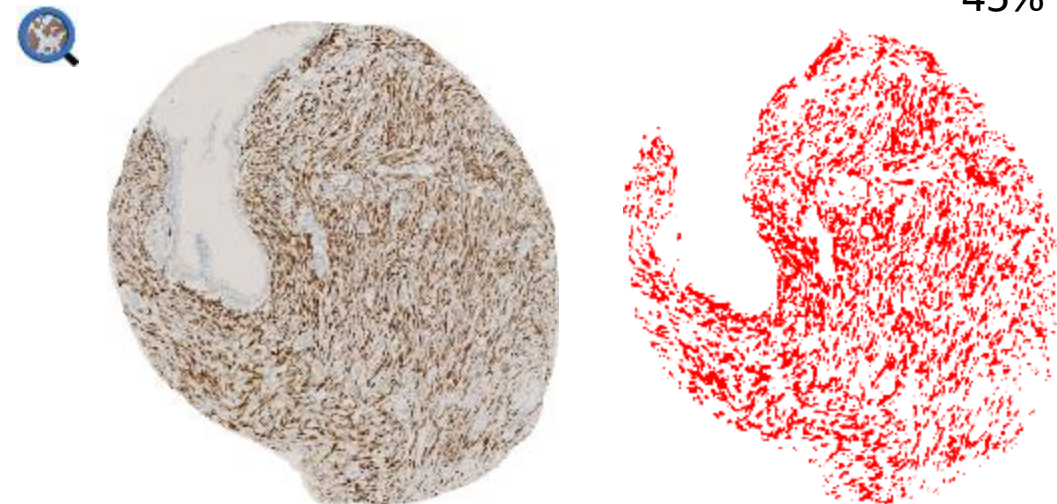
114 cells/mm²



% de surface marquée

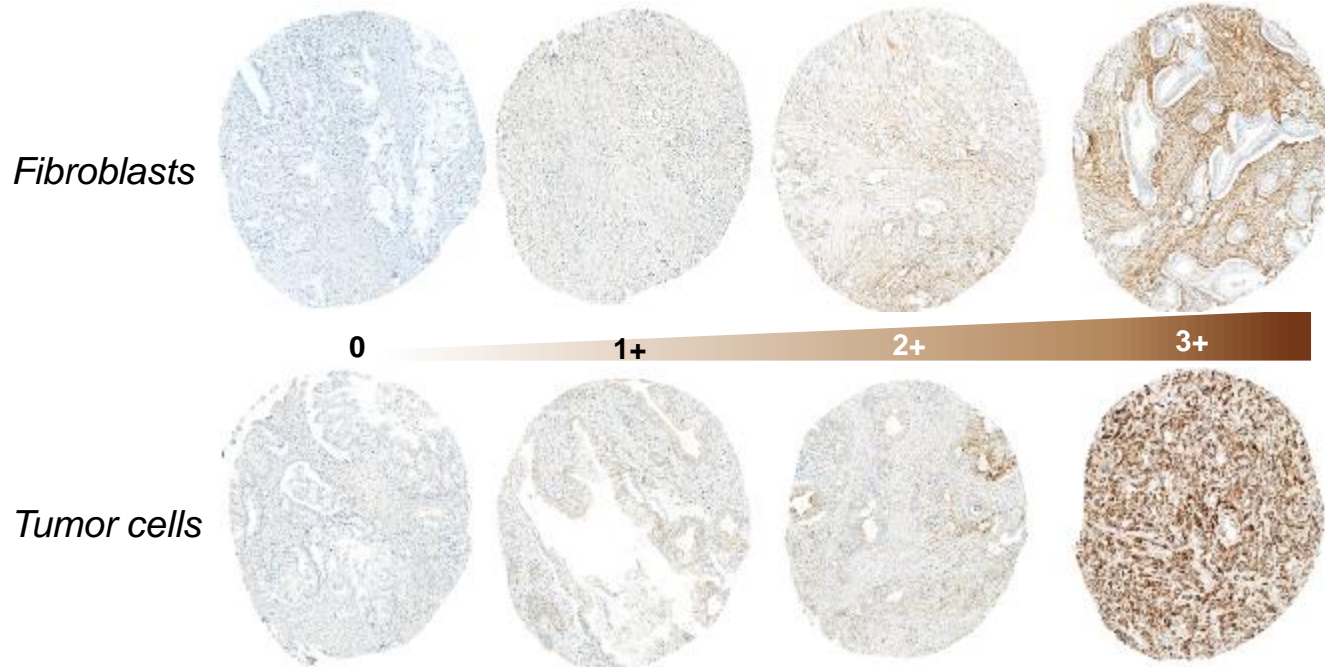
Fibroblastes : exemple FAP

45%



Échelle semi-quantitative

Exemple β -Catenin



Données brutes



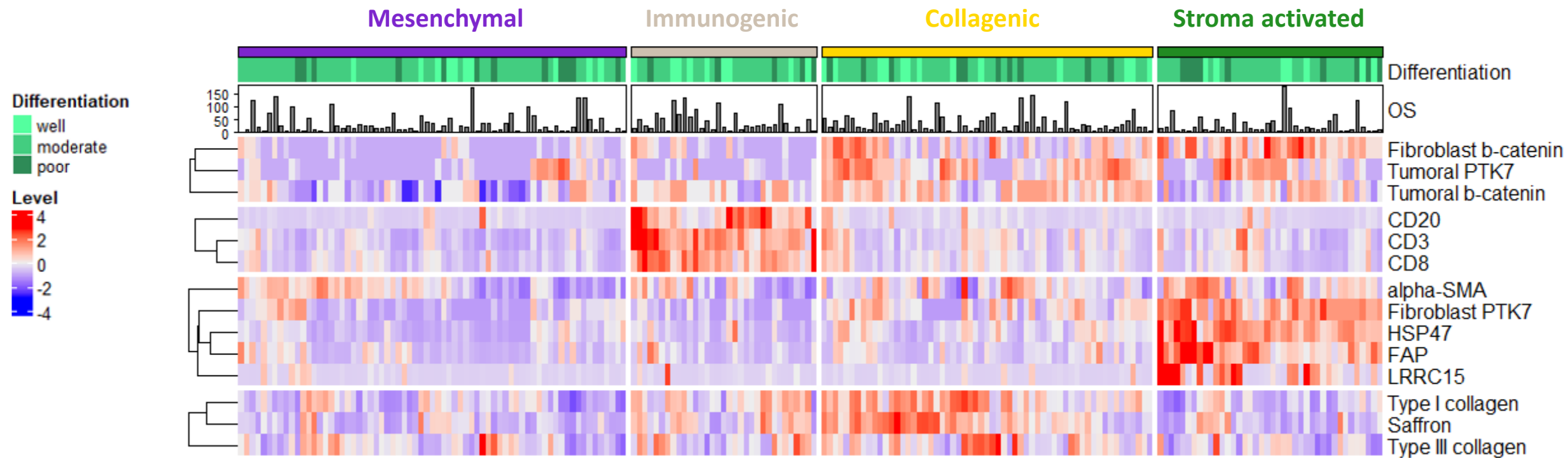
Analyse de cluster

MOYENNE des spots

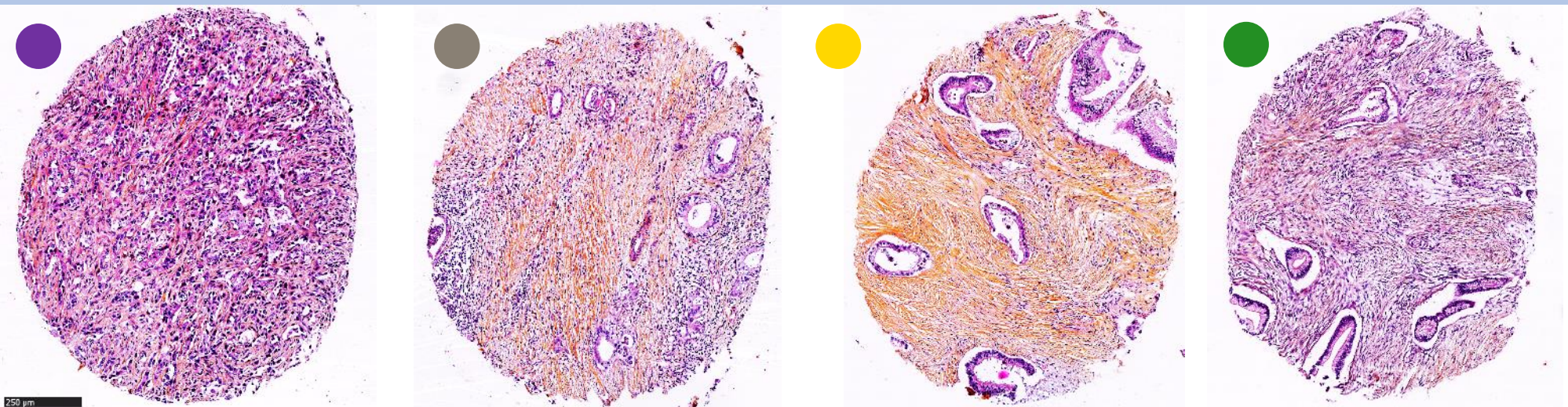
Dichotomisation



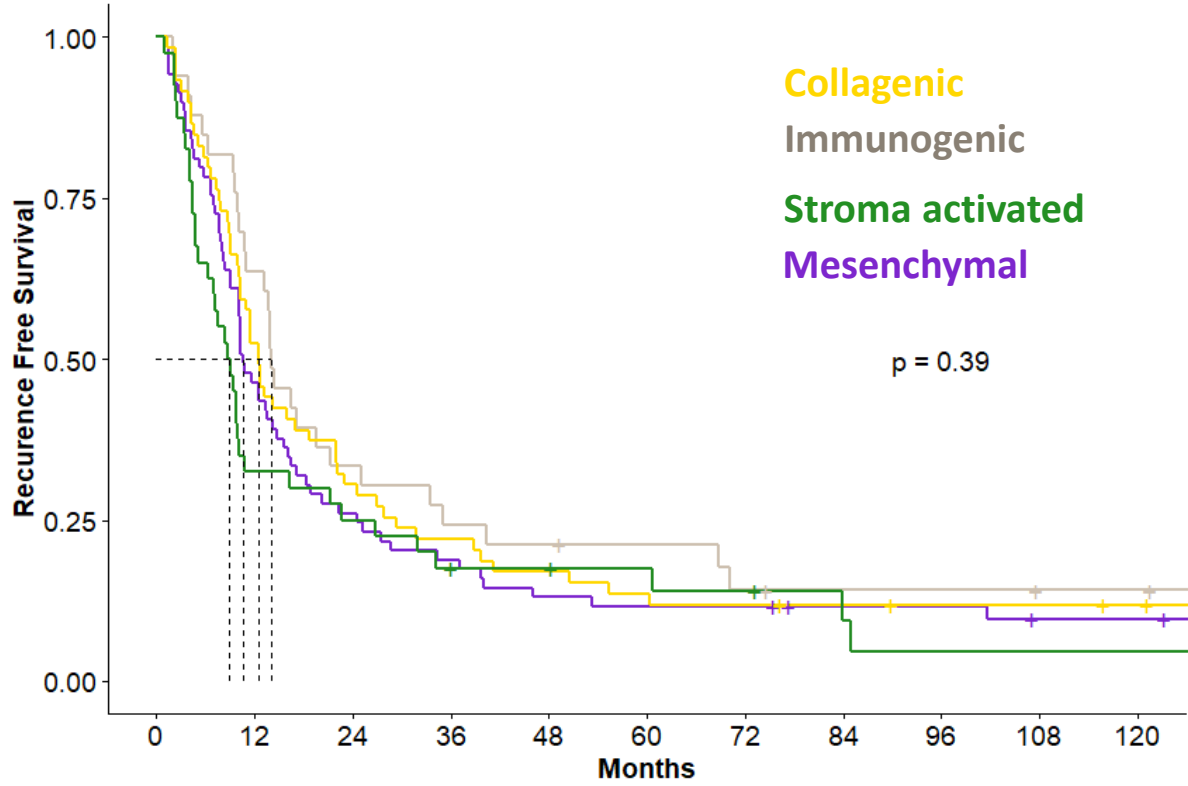
Analyse de survie



Transposable en "patterns" morphologiques des présentations observées sur HES ?



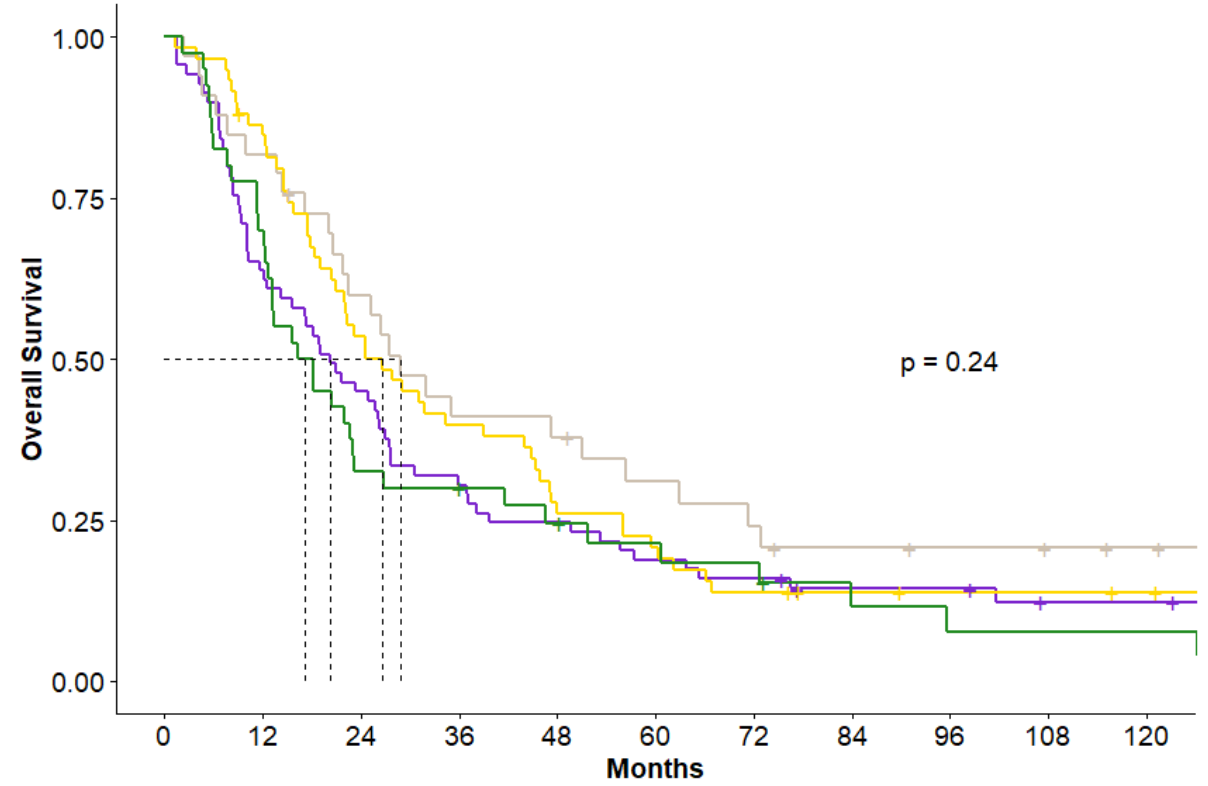
Survie Sans Progression



Number at risk

69	32	18	13	9	8	8	6	6	4	4
33	21	11	8	7	6	4	3	3	2	2
59	31	18	13	10	8	7	6	5	5	4
40	13	10	7	6	5	4	2	1	1	1

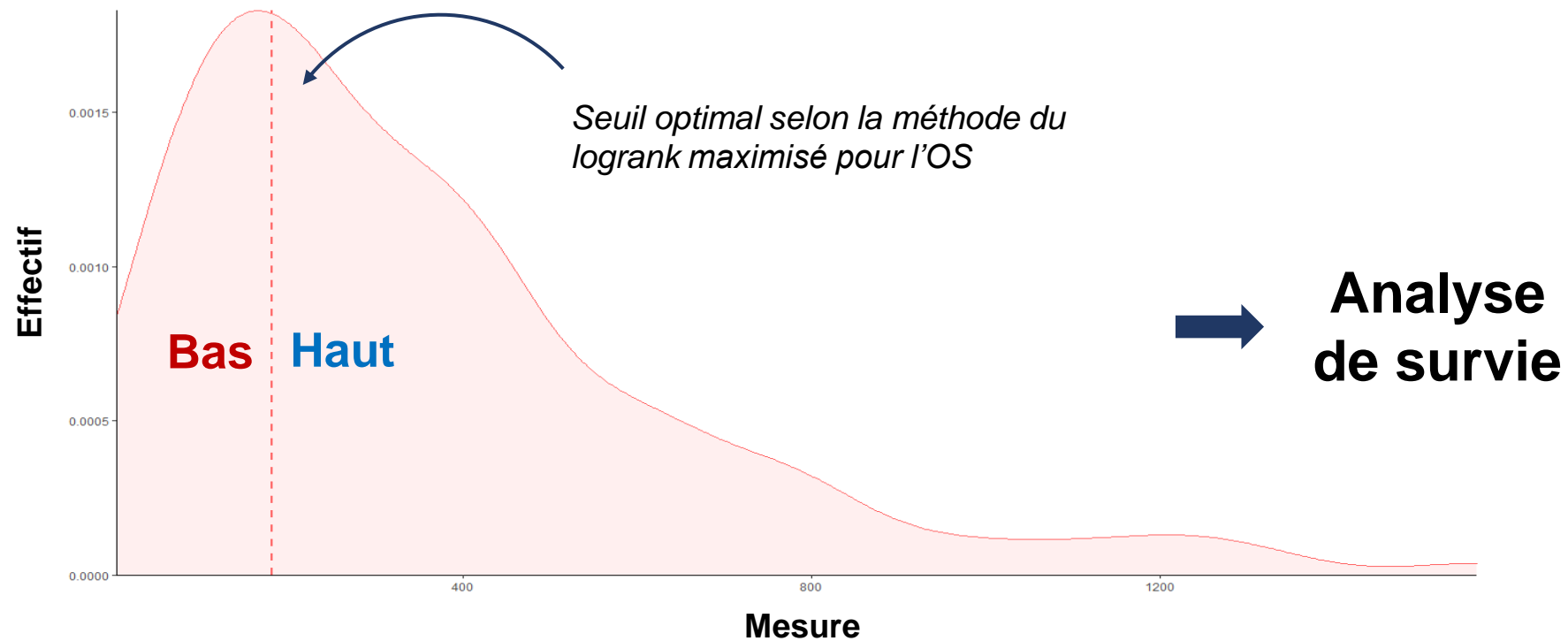
Survie Globale



Number at risk

69	44	31	21	17	13	11	8	8	5	5
33	27	19	13	12	9	7	5	4	3	2
59	50	31	23	16	12	8	6	5	5	4
40	28	13	12	9	7	6	3	2	2	2

SEUILLAGE de la moyenne des mesures des spots pour chaque patient



		N	HR (95% CI)	p
Age	65 or under	91	0.71 (0.53 – 0.96)	0.03
Sex	Male	102	0.77 (0.57 – 1.03)	0.08
Tumor site	Head	168	0.98 (0.66 – 1.47)	0.93
Adjuvant therapy	Yes	151	0.60 (0.41 – 0.86)	<0.01
Resection margin	R1	40	1.22 ((0.85 – 1.76)	0.29
Differentiation	Moderate vs well	126	1.27 (0.85 – 1.88)	0.24
	Poor vs well	36	2.11 (1.30 – 3.42)	<0.01
pT	2 vs 1	112	1.52 (0.97 – 2.47)	0.09
	3 vs 1	41	1.26 (0.73 – 2.19)	0.41
	4 vs 1	19	1.32 (0.69 – 2.53)	0.40
pN	1 vs 0	84	1.31 (0.86 – 2.00)	0.21
	2 vs 0	76	2.02 (1.31 – 3.10)	<0.01
Perineural invasion	Yes	175	1.78 (1.09 – 2.92)	0.02
Lymphatic invasion	Yes	96	1.07 (0.79 – 1.44)	0.7
Veinous invasion	Yes	93	1.20 (0.89 – 1.63)	0.2

		N	HR (95% CI)	p
CD3	Low	56	1.61 (1.16 – 2.23)	<0.01
CD8	Low	49	1.63 (1.15 – 2.29)	<0.01
CD20	Low	128	1.36 (0.99 – 1.86)	0.06
Saffron	Low	131	1.27 (0.93 – 1.74)	0.1
Type I Collagen	Low	148	1.17 (0.83 – 1.65)	0.4
Type III Collagen	Low	63	0.81 (0.58 – 1.12)	0.2
αSMA	Low	153	1.28 (0.90 – 1.83)	0.2
FAP	Low	60	0.73 (0.52 – 1.01)	0.06
HSP47	Low	137	0.63 (0.46 – 0.86)	<0.01
LRRC15	Low	130	1.28 (0.93 – 1.75)	0.1
Fibroblast B-Catenin	Low	119	1.19 (0.87 – 1.62)	0.3
Fibroblast PTK7	Low	149	0.95 (0.67 – 1.34)	0.8
Tumoral B-catenin	Low	160	1.73 (1.17 – 2.56)	<0.01
Tumoral PTK7	Low	121	1.17 (0.86 – 1.60)	0.3

		N	HR (95% CI)	p
Age	65 or under	91	0.83 (0.62 – 1.12)	0.2
Sex	Male	102	0.85 (0.64 – 1.15)	0.3
Tumor site	Head	168	0.78 (0.53 – 1.16)	0.2
Adjuvant therapy	Yes	151	0.65 (0.45 – 0.93)	0.02
Resection margin	R1	40	1.30 (0.91 – 1.86)	0.2
Differentiation	Moderate	126	1.24 (0.85 – 1.83)	0.3
	Poor	36	2.28 (1.42 – 3.65)	<0.001
pT	2	112	1.45 (0.89 – 2.35)	0.1
	3	41	1.43 (0.83 – 2.48)	0.2
	4	19	1.34 (0.71 – 2.54)	0.4
pN	1	84	1.25 (0.84 – 1.88)	0.3
	2	76	1.86 (1.23 – 2.81)	<0.01
Perineural invasion	Yes	175	1.70 (1.05 – 2.75)	0.03
Lymphatic invasion	Yes	96	1.05 (0.79 – 1.41)	0.7
Veinous invasion	Yes	93	1.44 (1.07 – 1.93)	0.02

		N	HR (95% CI)	p
CD3	Low	56	1.60 (1.16 – 2.21)	<0.01
CD8	Low	49	1.64 (1.18 – 2.30)	<0.01
CD20	Low	128	1.24 (0.91 – 1.68)	0.2
Saffron	Low	131	1.25 (0.92 – 1.69)	0.2
Type I Collagen	Low	148	1.29 (0.92 – 1.81)	0.1
Type III Collagen	Low	63	0.82 (0.60 – 1.12)	0.2
αSMA	Low	153	1.43 (1.00 – 2.04)	0.05
FAP	Low	60	0.77 (0.56 – 1.07)	0.1
HSP47	Low	137	0.63 (0.46 – 0.86)	<0.01
LRRC15	Low	130	1.19 (0.88 – 1.63)	0.3
Fibroblast B-Catenin	Low	119	1.05 (0.77 – 1.41)	0.8
Fibroblast PTK7	Low	149	0.92 (0.66 – 1.30)	0.6
Tumoral B-catenin	Low	160	1.66 (1.14 – 2.43)	<0.01
Tumoral PTK7	Low	121	1.08 (0.80 – 1.47)	0.6

Multivariate Cox model for RFS (in 0.1 out 0.1)

Variable		N	Hazard ratio	p
Adjuvant therapy	no	40	Reference	
	yes	150	0.65 (0.45, 0.95)	0.024
Differentiation	well	36	Reference	
	moderate	118	1.18 (0.79, 1.77)	0.421
	poor	36	2.01 (1.23, 3.28)	0.005
pN	0	38	Reference	
	1	80	1.24 (0.81, 1.92)	0.324
	2	72	1.72 (1.11, 2.68)	0.015
Tumoral B-Catenin	high	40	Reference	
	low	150	1.42 (0.96, 2.12)	0.081
CD8	high	145	Reference	
	low	45	1.67 (1.17, 2.40)	0.005
HSP47	high	63	Reference	
	low	127	0.75 (0.54, 1.05)	0.091

Multivariate Cox model for OS (in 0.1 out 0.1)

Variable		N	Hazard ratio	p
Age	over 65	103	Reference	
	65 or under	87	0.61 (0.44, 0.85)	0.004
Adjuvant therapy	no	40	Reference	
	yes	150	0.65 (0.45, 0.95)	0.024
Differentiation	well	36	Reference	
	moderate	118	1.13 (0.75, 1.72)	0.560
	poor	36	1.80 (1.09, 2.99)	0.022
pN	0	38	Reference	
	1	80	1.35 (0.85, 2.13)	0.200
	2	72	1.93 (1.22, 3.07)	0.005
Tumoral B-Catenin	high	40	Reference	
	low	150	1.55 (1.02, 2.35)	0.038
CD8	high	145	Reference	
	low	45	1.58 (1.10, 2.28)	0.014
HSP47	high	63	Reference	
	low	127	0.68 (0.49, 0.96)	0.027

Multivariate Cox model for RFS (in 0.1 out 0.1)

Variable		N	Hazard ratio	p
Adjuvant therapy	no	40	Reference	
	yes	150	0.65 (0.45, 0.95)	0.024
Differentiation	well	36	Reference	
	moderate	118	1.18 (0.79, 1.77)	0.421
	poor	36	2.01 (1.23, 3.28)	0.005
pN	0	38	Reference	
	1	80	1.24 (0.81, 1.92)	0.324
	2	72	1.72 (1.11, 2.68)	0.015
Tumoral B-Catenin	high	40	Reference	
	low	150	1.42 (0.96, 2.12)	0.081
CD8	high	145	Reference	
	low	45	1.67 (1.17, 2.40)	0.005
HSP47	high	63	Reference	
	low	127	0.75 (0.54, 1.05)	0.091

Multivariate Cox model for OS (in 0.1 out 0.1)

Variable		N	Hazard ratio	p
Age	over 65	103	Reference	
	65 or under	87	0.61 (0.44, 0.85)	0.004
Adjuvant therapy	no	40	Reference	
	yes	150	0.65 (0.45, 0.95)	0.024
Differentiation	well	36	Reference	
	moderate	118	1.13 (0.75, 1.72)	0.560
	poor	36	1.80 (1.09, 2.99)	0.022
pN	0	38	Reference	
	1	80	1.35 (0.85, 2.13)	0.200
	2	72	1.93 (1.22, 3.07)	0.005
Tumoral B-Catenin	high	40	Reference	
	low	150	1.55 (1.02, 2.35)	0.038
CD8	high	145	Reference	
	low	45	1.58 (1.10, 2.28)	0.014
HSP47	high	63	Reference	
	low	127	0.68 (0.49, 0.96)	0.027

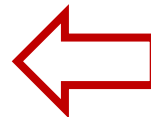
IHC panc-Score

β-caténine élevée → 1 point

CD3 élevé → 1 point

HSP47 bas → 1 point

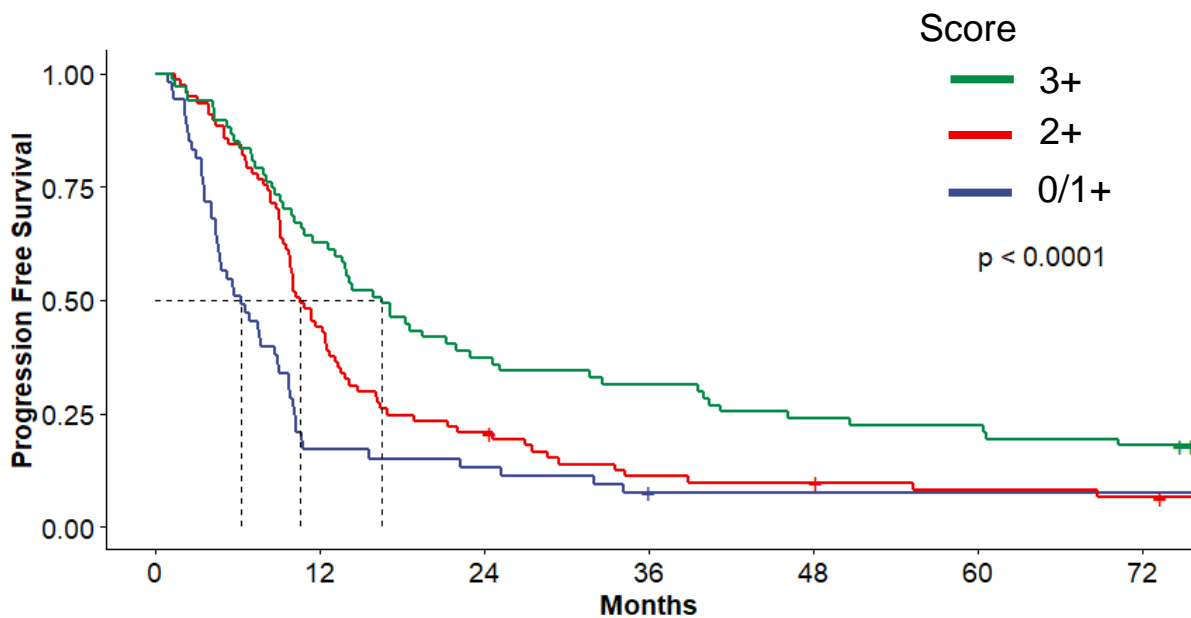
Σ



Combinaison des marqueurs → Score de 0 à 3

- β-caténine élevée → 1 point
- CD3 élevé → 1 point
- HSP47 bas → 1 point

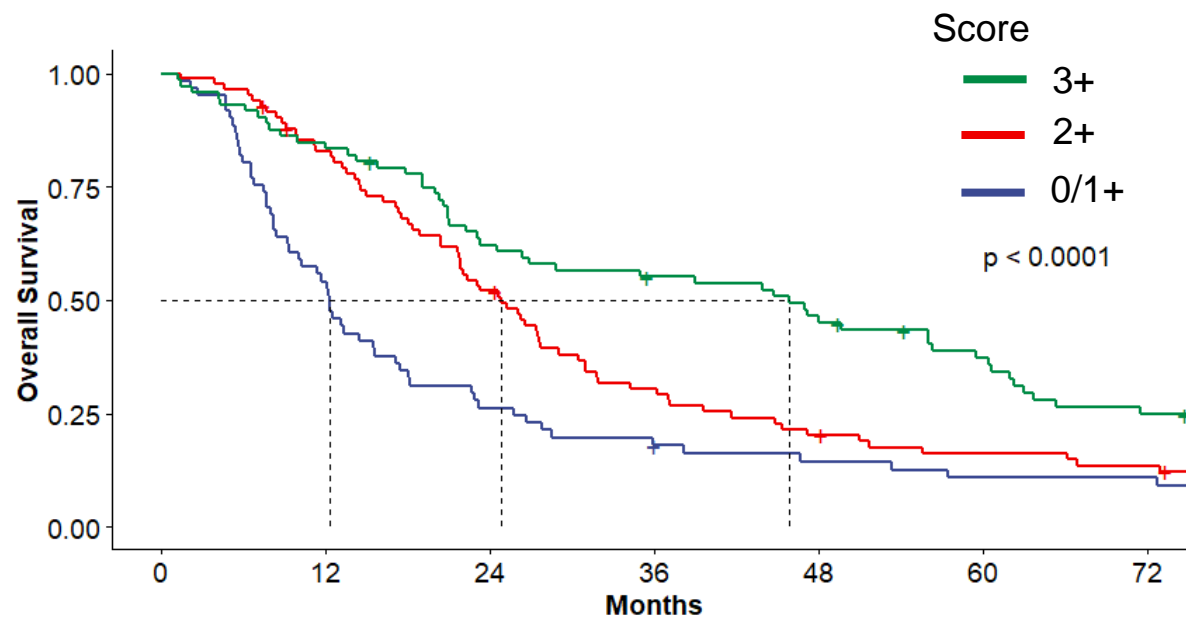
Survie sans progression



Number at risk

3+	53	9	7	4	3	3	3
2+	77	34	16	8	7	5	4
0/1+	67	42	25	21	16	15	12

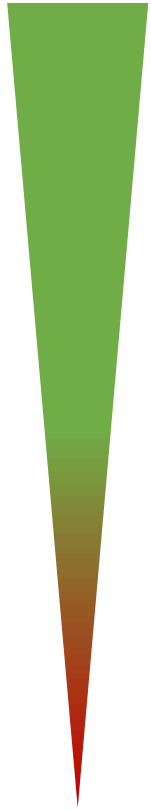
Survie globale



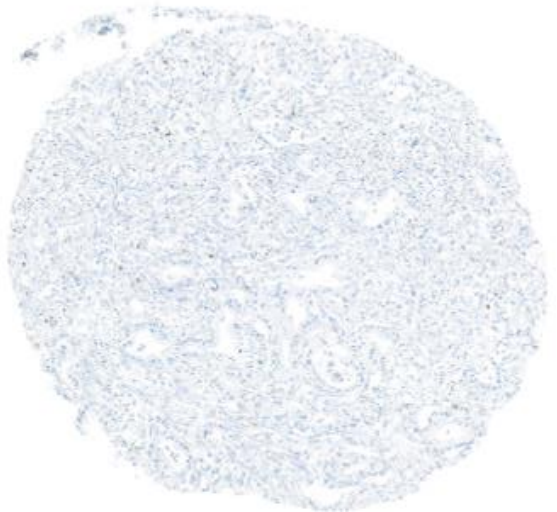
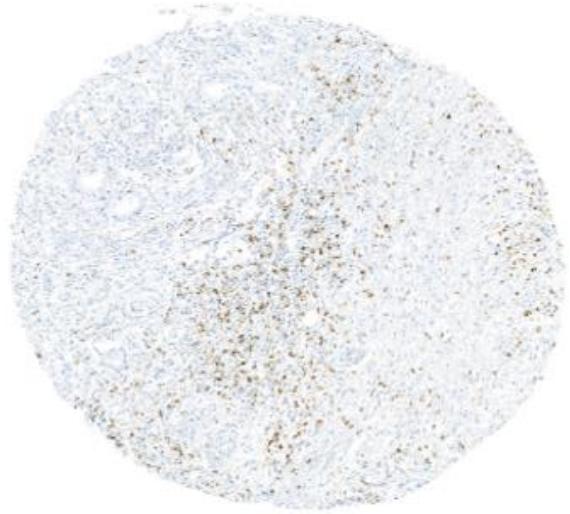
Number at risk

3+	61	33	16	11	8	6	6
2+	83	67	42	24	16	12	10
0/1+	72	61	44	38	32	24	16

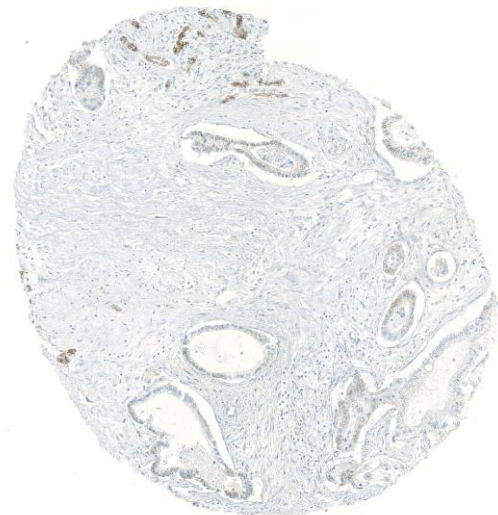
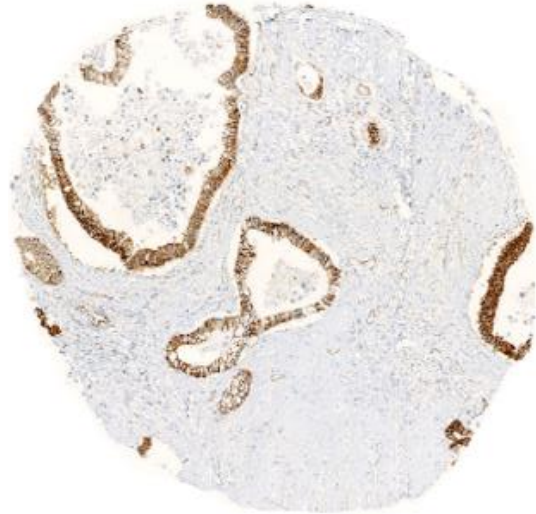
Survival



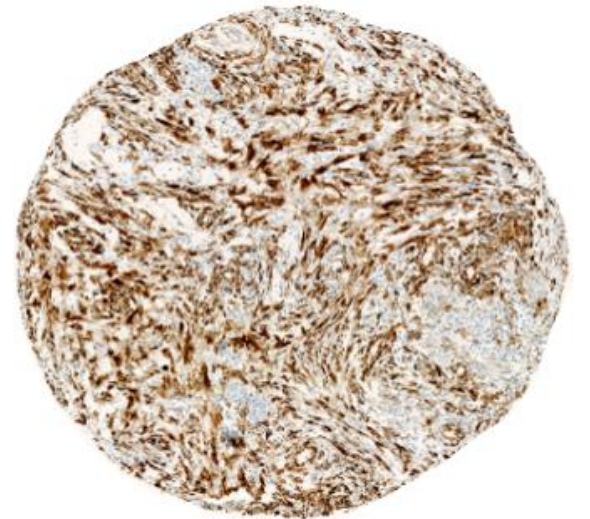
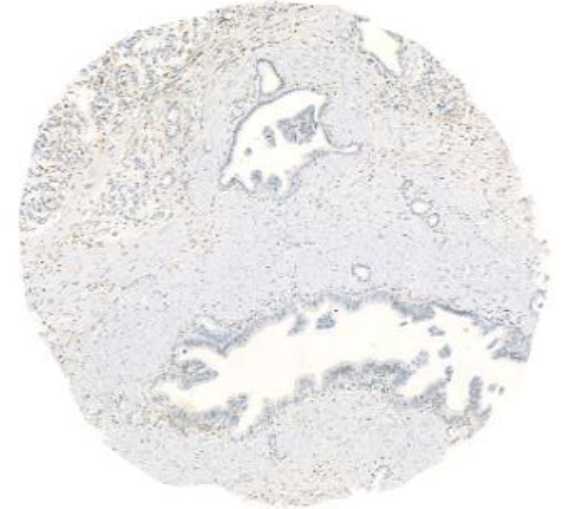
CD8



B-Caténine



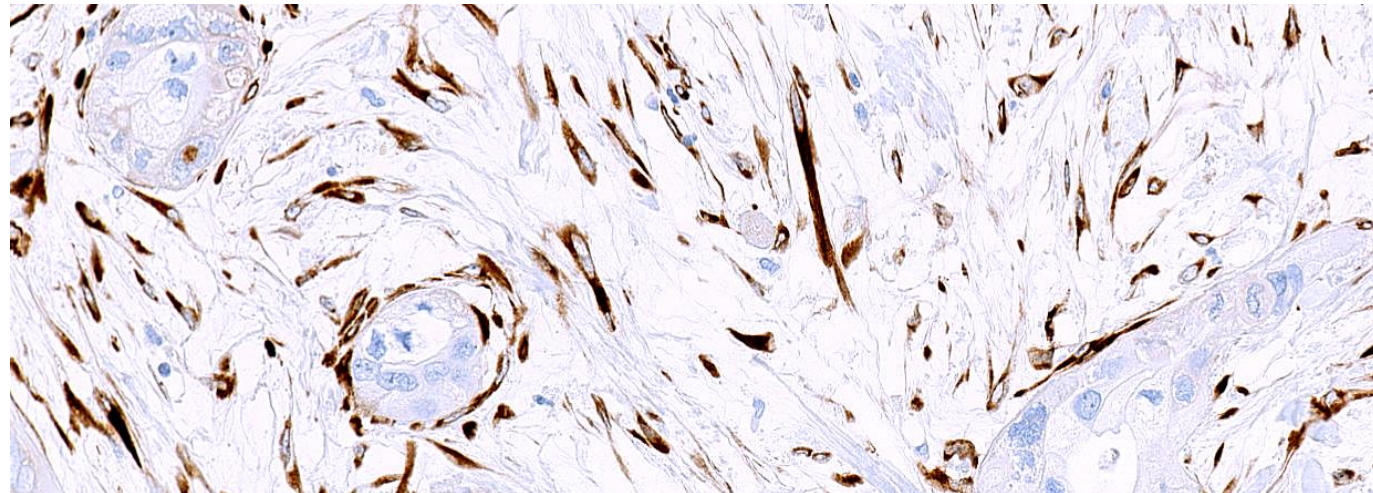
HSP47 (*SERPINH1*)



Intérêt de caractériser dans les APC **simultanément** les 3 compartiments **épithéliaux**, **immunitaires** et **fibroblastiques**

Impact pronostique objectivé pour la première fois de **HSP47** et confirmé pour la **β -caténine**

Illustration de la faculté des CAFs actifs à créer une matrice défavorable



Confirmation de la valeur pronostique :

→ À valider sur une cohorte de validation externe

Caractère **prédictif** de cette classification ?

→ À analyser sur une cohorte de patients traités en néoadjuvant

Approche tissulaire d'un panel immunohistochimique sur **pieces opératoires** : premier pas vers une **applicabilité sur biopsies** ?

IRFC – Oncologie médicale

Christophe Borg
Olivier Adotevi
Julien Viot
Emeline Orillard
Laura Mansi
Elodie Klajer
Stefano Kim

Pathologistes
Frédéric Bibeau
Chloé Molimard
Tumorothèque



Service de chirurgie digestive

Bruno Heyd
Zaher Lakkis
Alexandre Doussot

Service de hépatologie

Vincent Di Martino
Thierry Thevenot

Inserm CIC 1431 Biothérapies

Marion Jacquin
Magali Rebucci-Peixoto

Equipes paramédicale,
ARC et administrative

UMR1098 Inserm / UFC / EFS

Jean-René Pallandre Romain Lyon
Marjorie Labesse Marie Kroemer
Clara Tissier Syrine Abdeljaoued
Laurie Spehner Morgane Lopez
Adeline Bouard Marie Perchaud
Virginie Mougey Ugo Chartral
Kamal Asgarov Elodie Renaude

Service de gastro-entérologie

Lucine Vuitton
Stéphane Koch

Plateforme de biomonitoring

UMQVC

Dewi Vernerey
Julie Henriques
Aurélia Meurisse

fmonnien@chu-besancon.fr
a3vienot@chu-besancon.fr

Plateforme EPIGENExp

Paul Peixoto
Eric Hervouet



Cancéropole Est

Philippe Bachellier
Gerlinde Averous
Florence Schaffner



CGFL - Dijon

François Ghiringhelli
Caroline Truntzer
Valentin Derangere



IGBMC - GenomEast

Gabriel Malouf
Irwin Davidson
Christelle Thibault-Carpentier
Céline Keime



CIT – Ligue contre le Cancer

Aurélien de Reynies



GERCOR

Cindy Neuzillet
Anthony Turpin

