



# Imagerie Multiplex Cell Dive pour visualiser les niches des cellules souches/progénitrices du cancer du foie

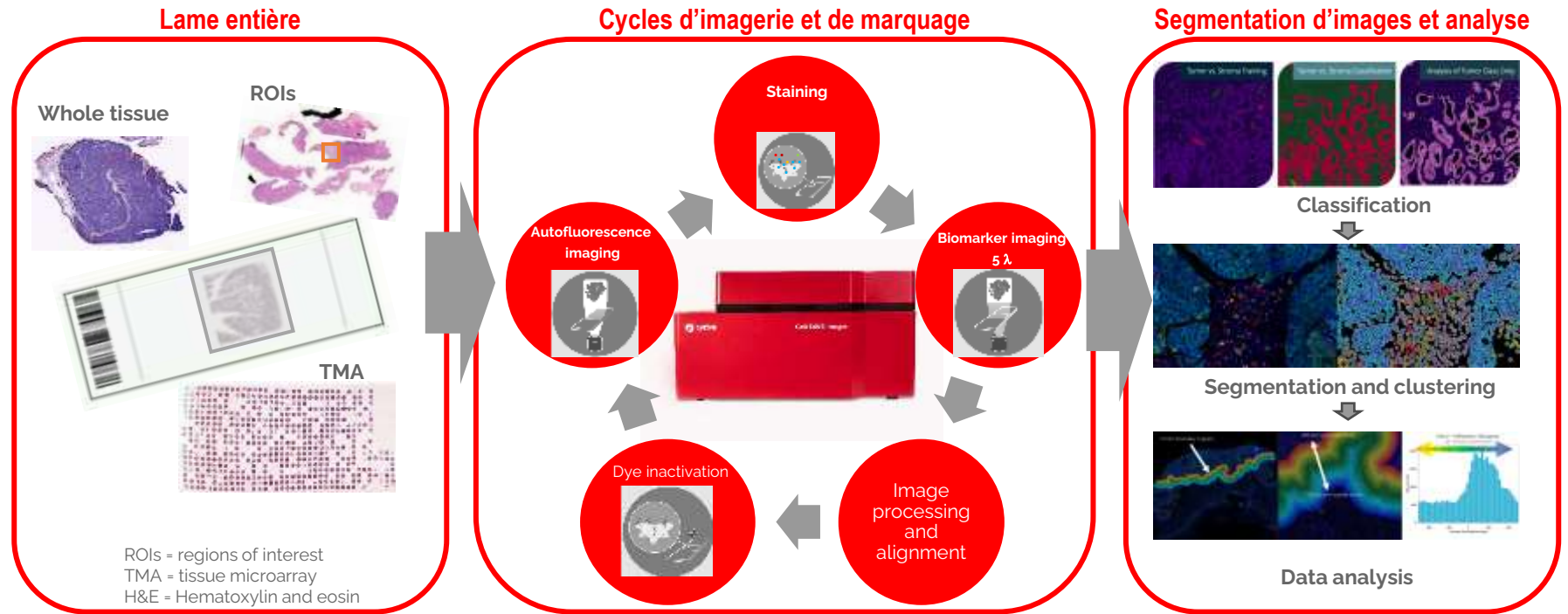
Roselyne Viel, Romain Désert, Natalia Nieto, David Pointu, Nicolas Mouchet, Anthony Sébillot, Alain Fautrel, Orlando Musso



Univ Rennes, INSERM, CNRS, UMS Biosit, Plateforme H2P2, Rennes, France  
INSERM, Univ Rennes, INRAe, Institut NuMeCan  
Department of Pathology, Univ Illinois at Chicago  
Leica Microsystems, 35578 Wetzlar, Allemagne



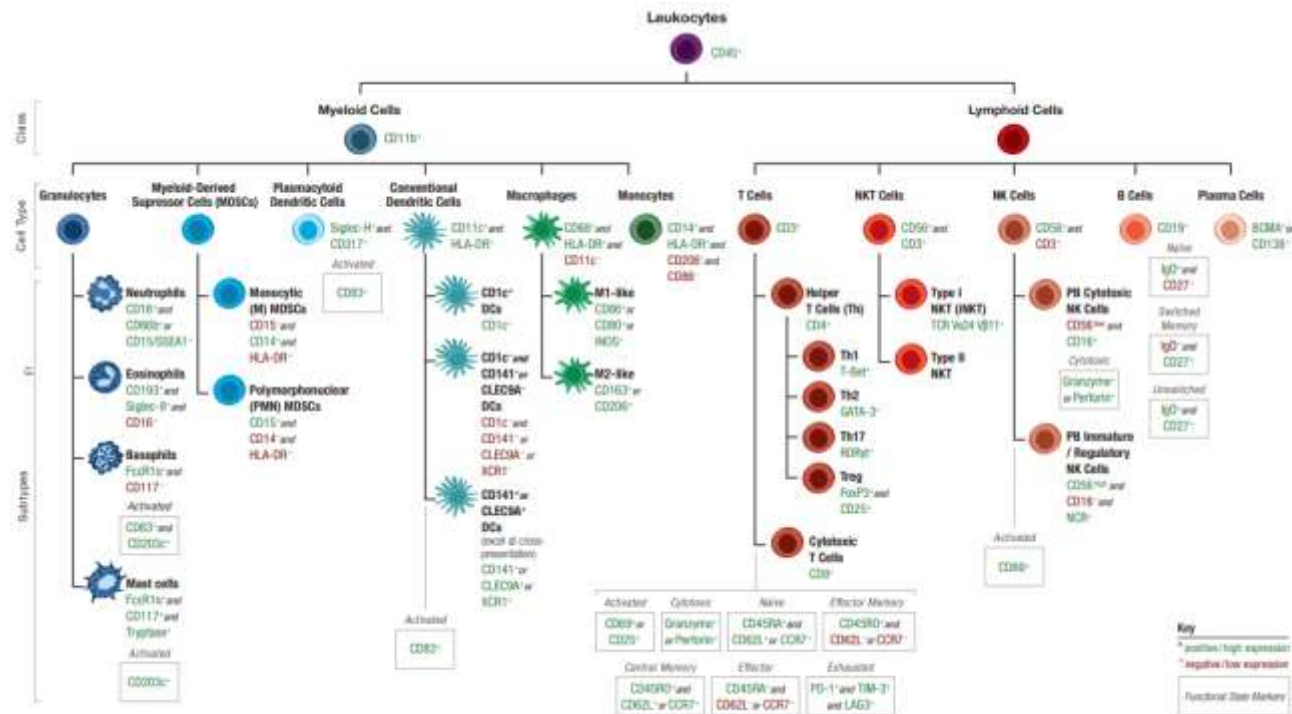
# CellDIVE™ le workflow



Marquage cyclique – image – inactivation - répétition du process pour 60+ biomarqueurs

# Les étapes de caractérisation et de validation des anticorps

IDENTIFICATION DES BIOMARQUEURS POUR LES TISSUS HUMAINS ET SOURIS



**Key**

- Positive/High expression
- Negative/Low expression
- Functional/Star Markers

## IDENTIFICATION DES ANTICORPS (ANTICORPS MONOCLONAUX ET POLYCLONAUX)

- Cellules tumorales, cellules immunes, le stroma, les cellules endothéliales,...
- Analyse de l'expression de chimiokines, étude de la prolifération



## IDENTIFICATION DES BIOMARQUEURS POUR LES TISSUS HUMAINS ET SOURIS



- Validated Antibody Lists



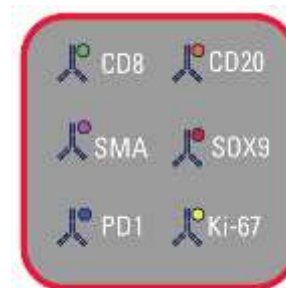
Cell Signaling  
TECHNOLOGY®

> 1400 available antibodies

350 commercially available antibodies

Antibodies Validated for Cell DIVE

- Custom Antibody Conjugation/Labeling Service



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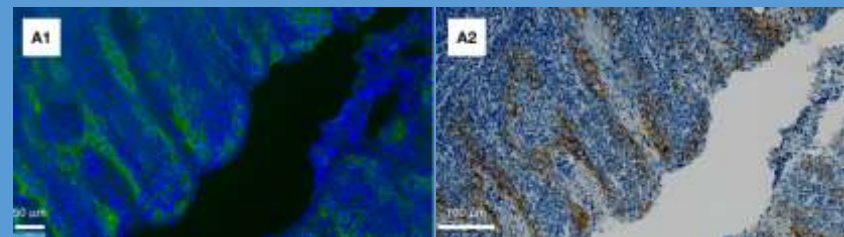
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## VALIDATION ANATOMOPATHOLOGISTE



anti-PD-L1

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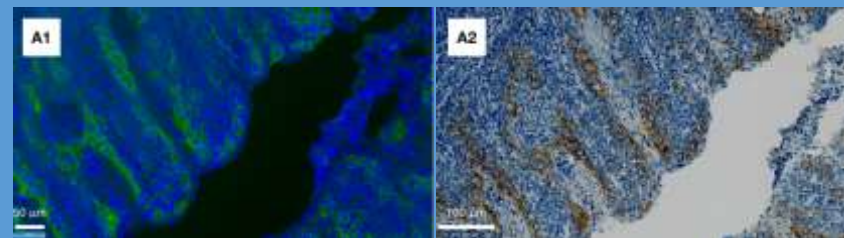
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## IDENTIFICATION DES BIOMARQUEURS POUR LES TISSUS HUMAINS ET SOURIS



## VALIDATION ANATOMOPATHOLOGISTE



anti-PD-L1

## CONCEPTION DU PANEL

	Cy2	Cy3	Cy5	Cy7
Round 1	RORyt	Fox P3	GATA3	CD274 (PD-L1)
Round 2	CD11c	CD152 (CTLA4)	CD279 (PD1)	CD3
Round 3	CD20	CD206	CD8	CD56
Round 4	PanCK	CD16	CD4	Glypican3
Round 5	CD15	CD68	WGA	
Round 6	CD64	CD31		
Round 7		HePar1		



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Department of Pathology, Univ Illinois at Chicago  
Leica Microsystems, 35578 Wetzlar, Allemagne



# Epidémiologie du Carcinome Hépatocellulaire

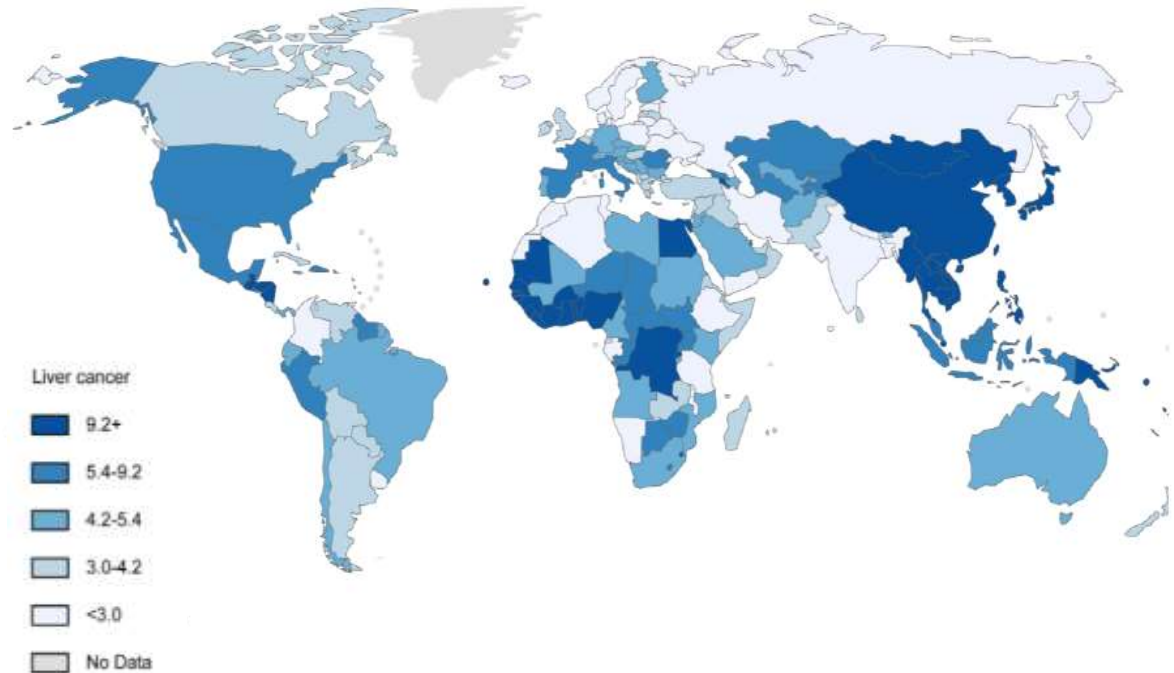
7<sup>ème</sup> cancer le plus fréquent  
3<sup>ème</sup> cancer le plus mortel

**Pas de traitement curatif sauf**

→ Chirurgie

→ Transplantation

*Sous conditions favorables*



*Incidence par âge pour 100 000 individus.*

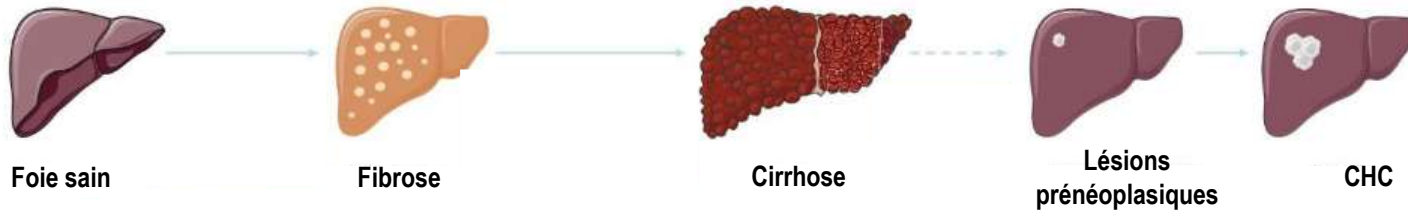


# Physiopathologie du Carcinome Hépatocellulaire (CHC)

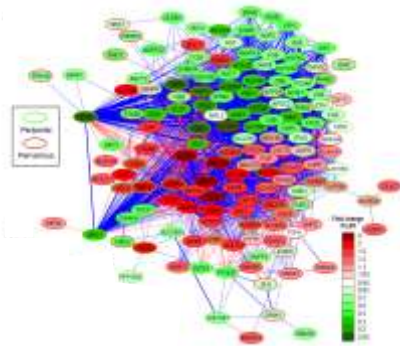
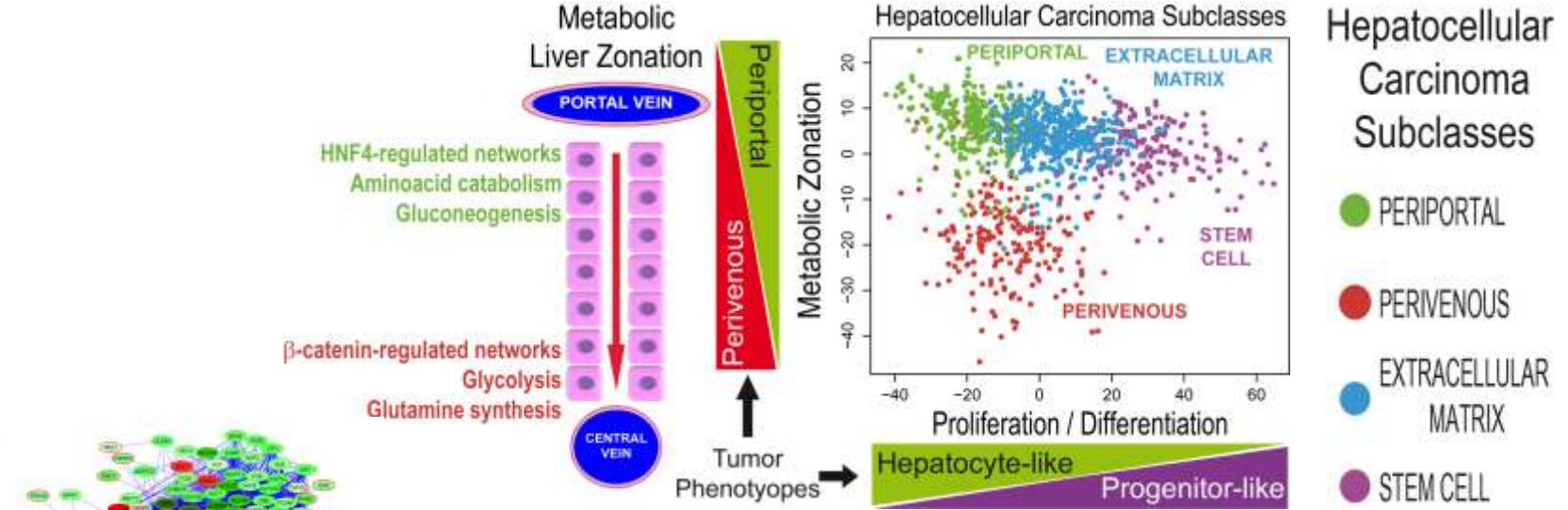
Le CHC survient dans 80% des cas sur un foie fibro-inflammatoire

Lésions chroniques fibro-inflammatoires:

- Infections virales
- Abus d'alcool
- MASLD (*Metabolic Dysfunction- Associated Steatotic Liver Disease*)
- Maladies génétiques (e.g., hémochromatose).

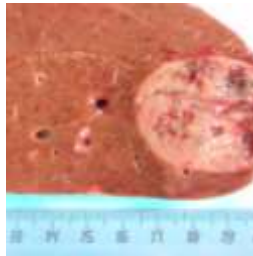
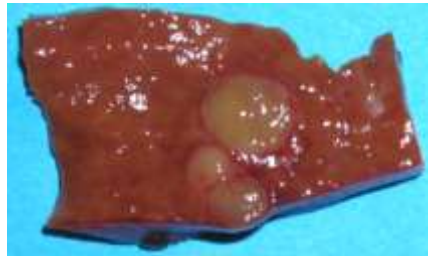
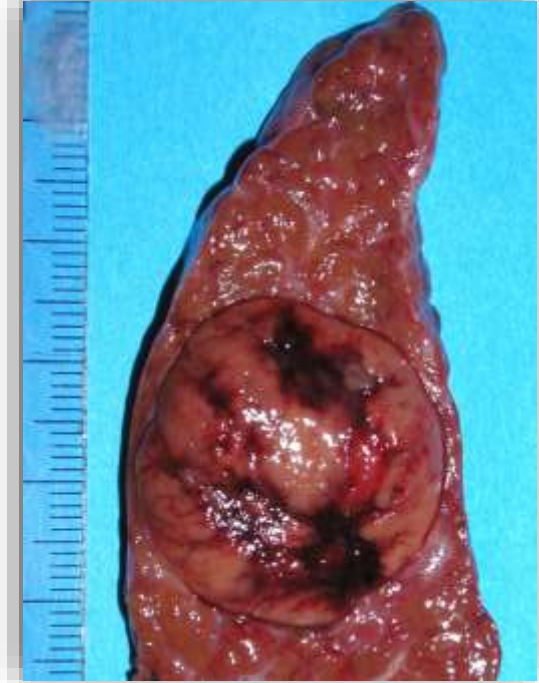
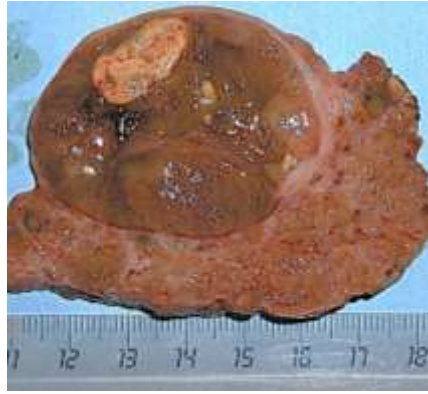


# Carcinome hépatocellulaire : hétérogénéité moléculaire

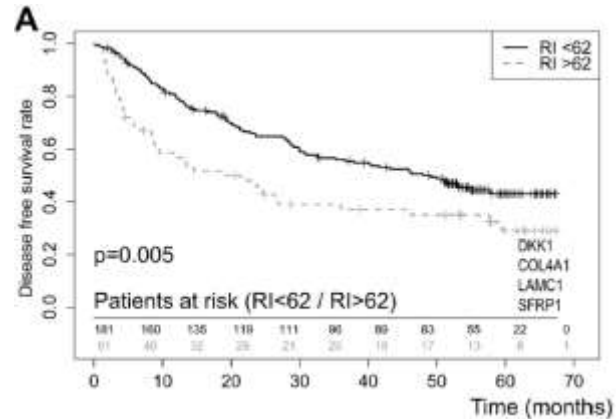
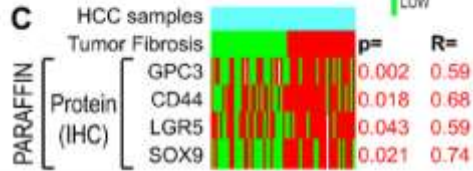
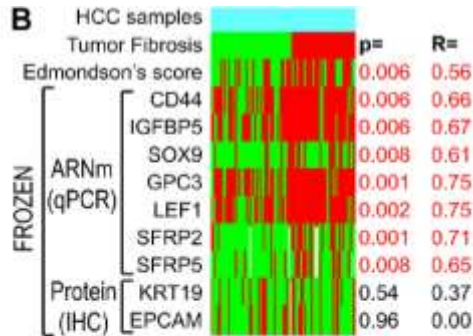
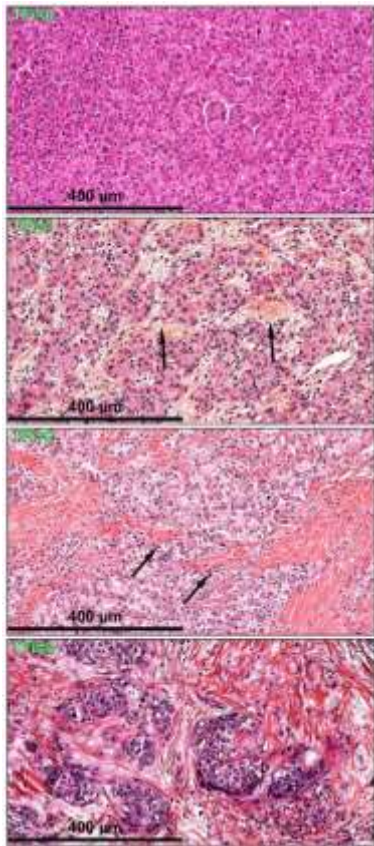


Désert et al., *Hepatology* 2017  
 Ng et al., *Hepatology* 2017 (Editorial)  
 Désert et al., *World Journal of Gastroenterology* 2018  
 Desquilles et al., *Scientific Reports* 2022  
 Désert et al., *Hepatology* 2023  
 Kanzaki et al., *Hepatology* 2023 (Editorial)

# Carcinome hépatocellulaire: hétérogénéité macroscopique

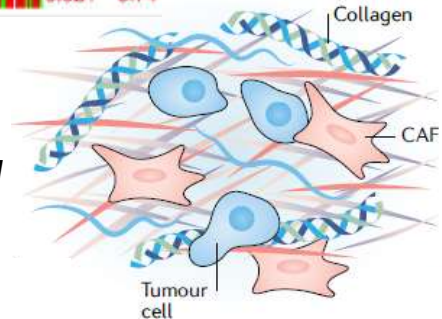


# Carcinome hépatocellulaire: hétérogénéité macroscopique



Désert et al. *Int. J. Bioch. Cell Biol.* 2016  
 Mebarki et al., *Oncotarget* 2016

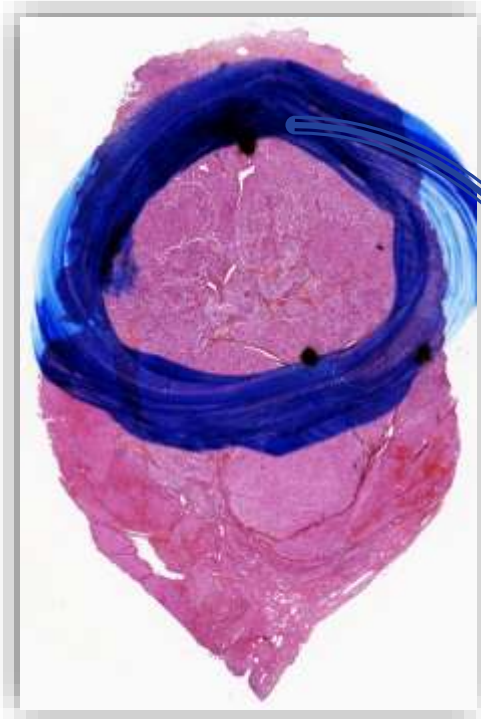
**Stroma tumoral**



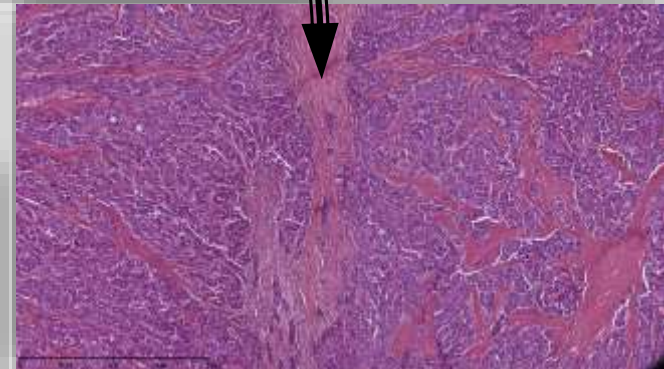
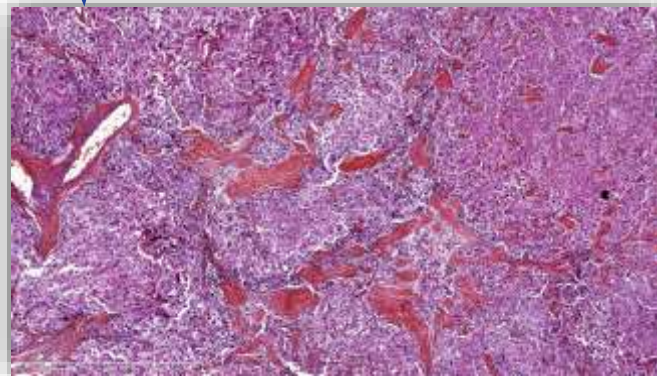
**Matrice Extracellulaire (matrisome)**

Cox, *Nat Reviews Cancer* 2021

# Carcinome hépatocellulaire : *Nids de Fibrose*



Carcinome hépatocellulaire (0.5X)

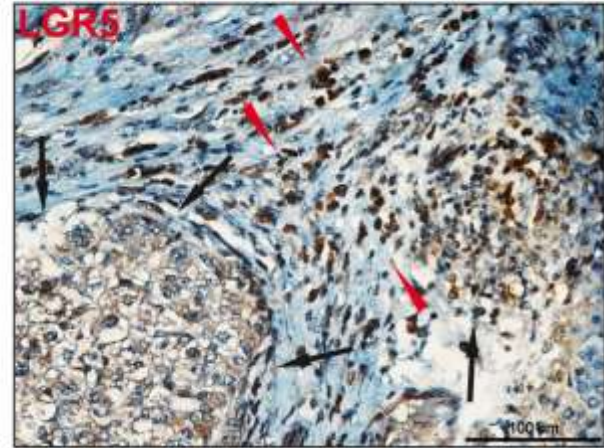
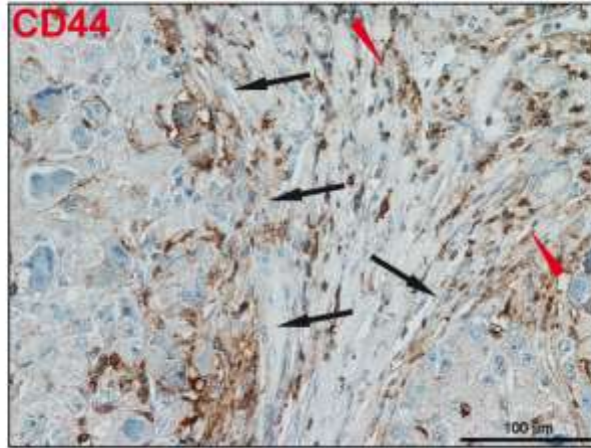
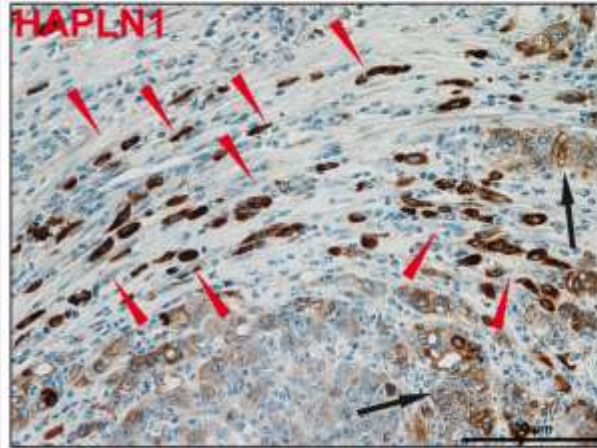


Carcinomes hépatocellulaires (20X)  
HES

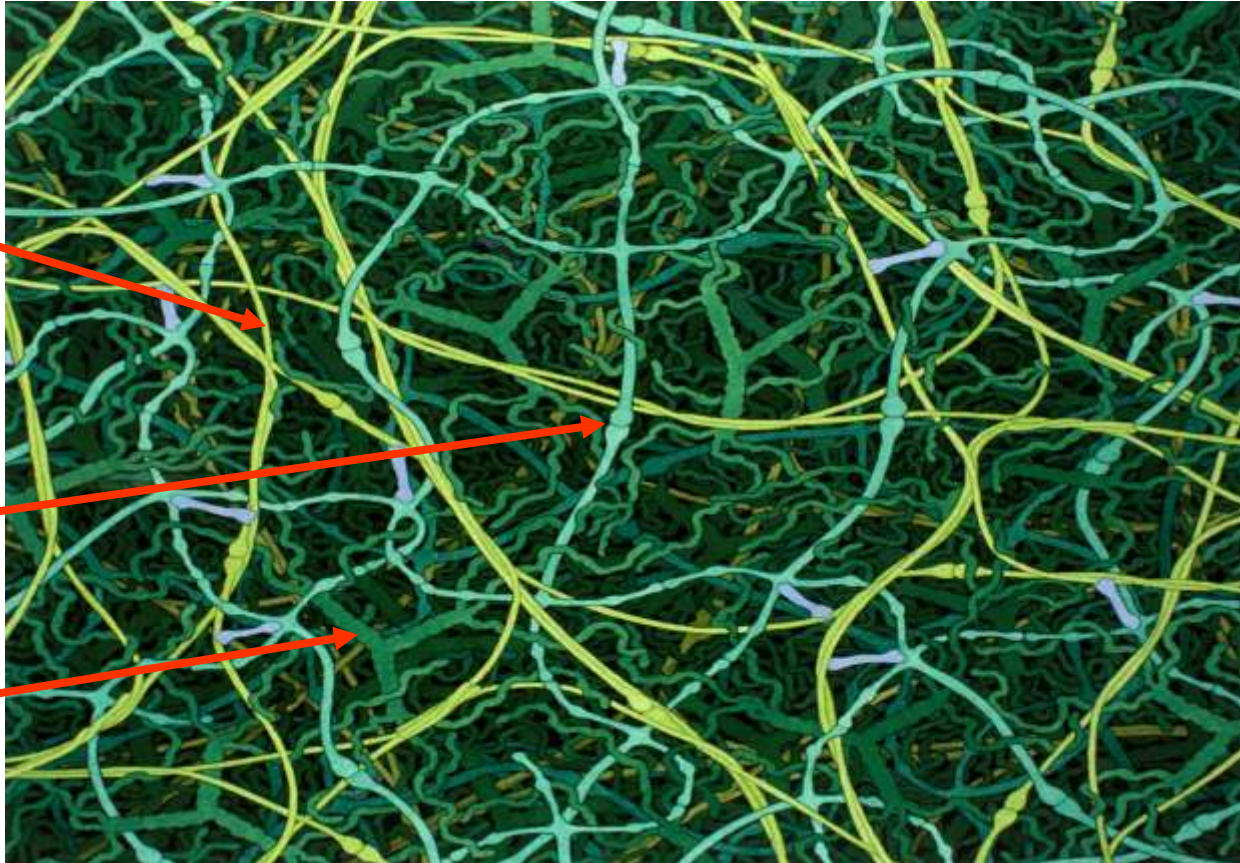
Carcinome hépatocellulaire :

***Nids de Fibrose et Marqueurs de Cellules Souches/Progénitrices du Cancer***

**A**



# La Matrice Extracellulaire



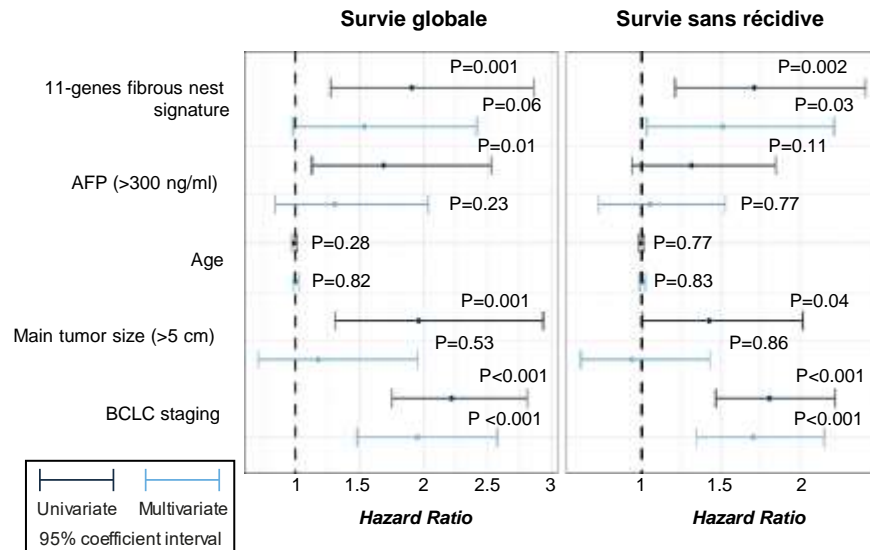
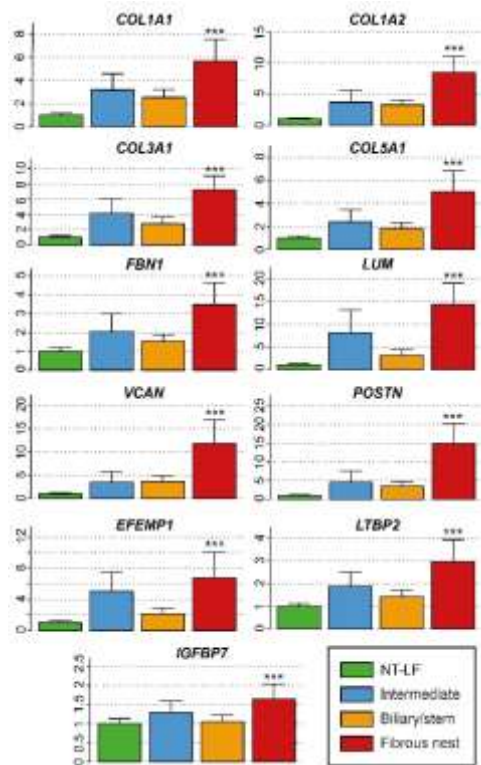
Collagènes

Laminines

Proteoglycanes

*David Goodsell,  
Scripps Research Inst.  
La Jolla, California*

# Analyse du Matrisome des Nids de Fibrose du Carcinome Hépatocellulaire Humain

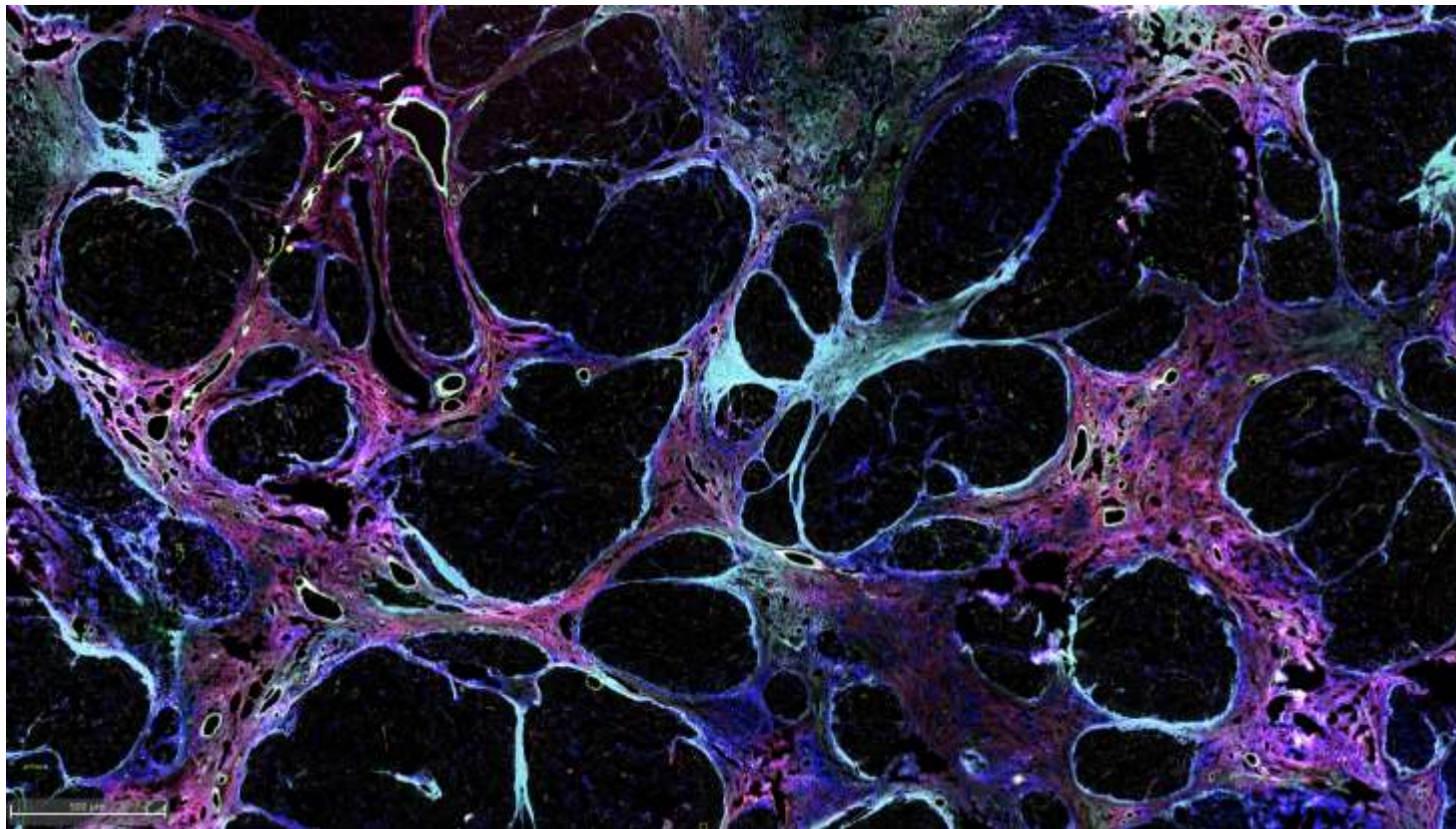


*Protéines de la matrice extracellulaire associées à la survie des patients, après résection de la tumeur*



# Visualisation du Matrisome des Nids de Fibrose du Carcinome Hépatocellulaire

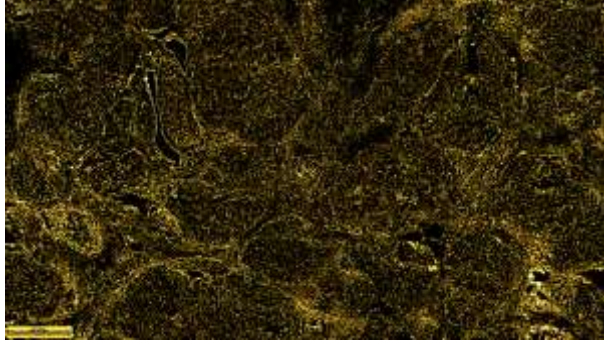
*Co-détection de protéines validées*



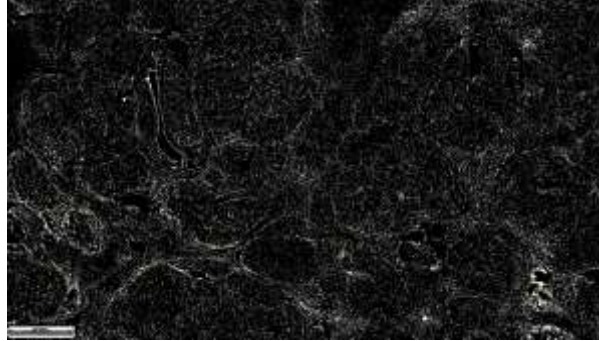
# Visualisation du Matrisome des Nids de Fibrose du Carcinome Hépatocellulaire

Co-détection de protéines validées

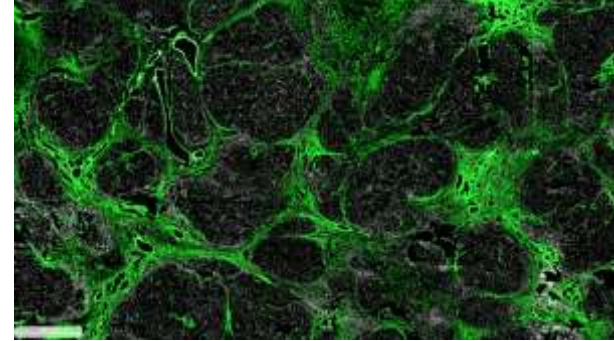
CD31



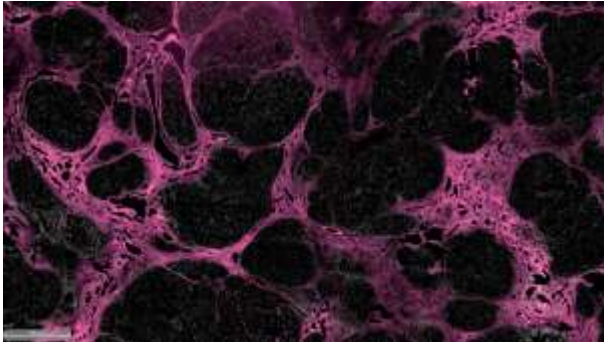
CD45



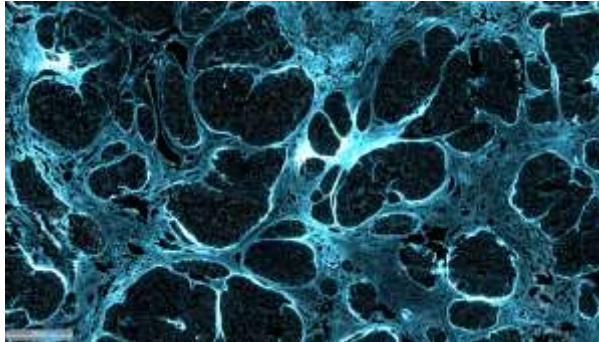
ACTA2



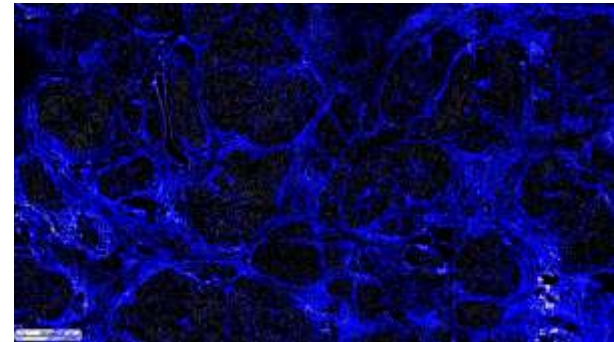
ELN



POSTN

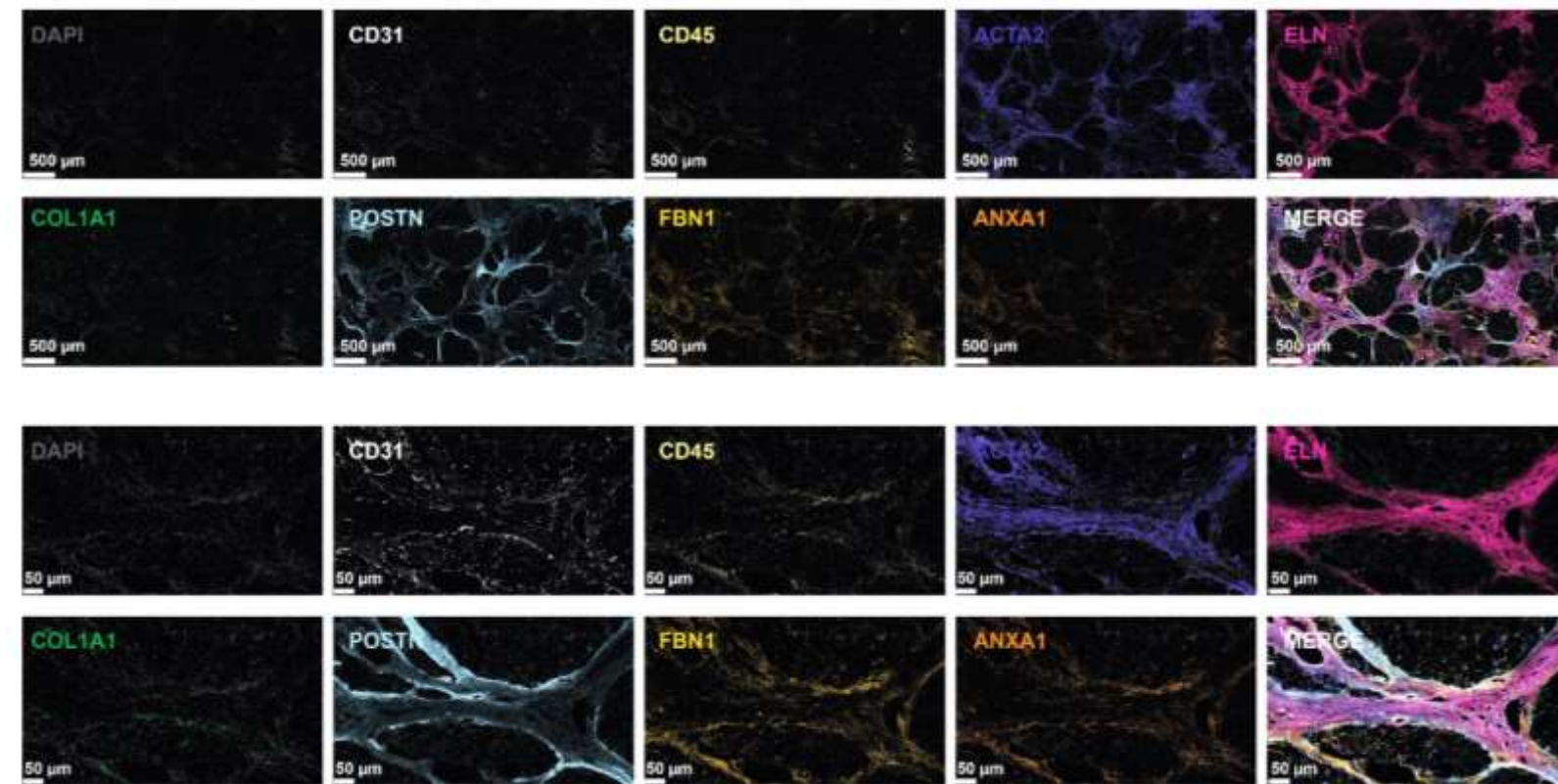


FBN1



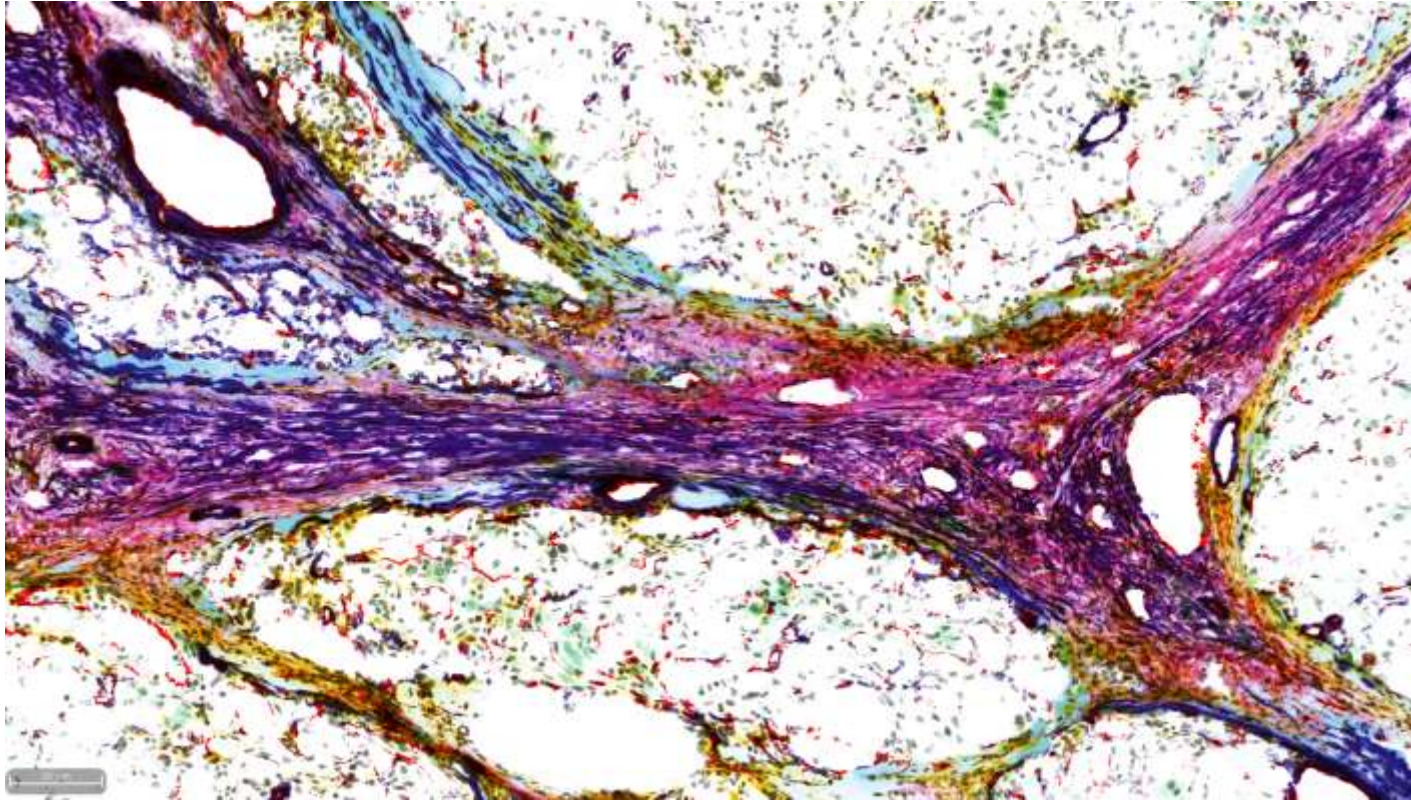
# Visualisation du Matrisome des Nids de Fibrose du Carcinome Hépatocellulaire

Co-détection de protéines validées



# Visualisation du Matrisome des Nids de Fibrose du Carcinome Hépatocellulaire

*Co-détection de protéines validées*



DAPI

ANAXA1

CD31

CD45

COL1A1

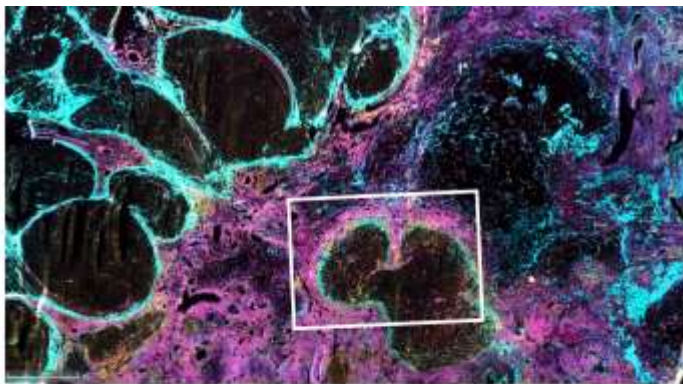
ELN

FBN1

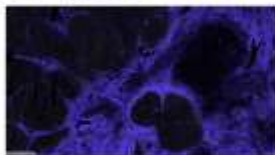
POSTN

ACTA2

# Matrisome Analysis of hepatocellular carcinoma fibrous nests



DAPI ACTA2 CD45 CD31 ELN POSTN FBN1



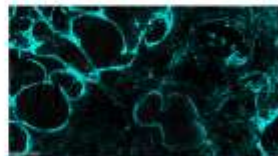
ACTA2



ELN



CD45



POSTN



CD31



FBN1

## Antibodies

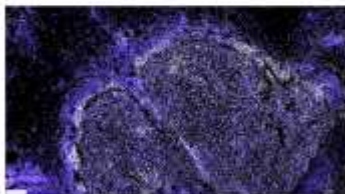
Name	Company	Species	Reference	Clone
VCAN-CF488	Clinisciences	Rb	ORB13754-CF488A	poly
ANXA1-DL680	Biotechne	Ms	NBP2-70174FR	OT3A8
FBN1-A750	Clinisciences	Rb	BS-1157R-A750	Poly
CXCL13-Y2	Novusbio	Rb	NBP2-16041G	Poly
POSTN-Y7	Clinisciences	Ms	SC-390631 AF790	F-10
COL5A1-Y3	Novusbio	Ms	NBP1-05118R	1E2-E4/Col5
DPT-Y5	Santa Cruz	MS	sc-376863 AF680	F-4
ELN-Y7	Biotechne	Ms	NBP3-088891R	ELN/2069
COL1A1-Y2	Biotechne	Rb	NBP1-77457G	Poly
CD45-Y3	Biotechne	Rb	NBP3-08910R	PTPRC/1975R
ACTA2-Y5	Biotechne	Ms	NBP2-34522FR	1A4/asm-1
CD31-Y3	Abcam	Rb	ab279331	EPR309
GDF15-A555	CliniSciences	Rb	bs-3818R-A555	poly

Versican  
Annexin 1  
Fibrillin  
CXCL13  
Periostin  
Collagen V  
Dermatopontin  
Elastin  
Collagen I  
CD45  
Alpha-SMA  
CD31  
GDF15

# Visualisation du Matrisome des Nids de Fibrose du Carcinome Hépatocellulaire



DAPI ACTA2 CD45 CD31 COL1A1 COL5A1 DPT VCAN



COL1A1



COL5A1



DPT



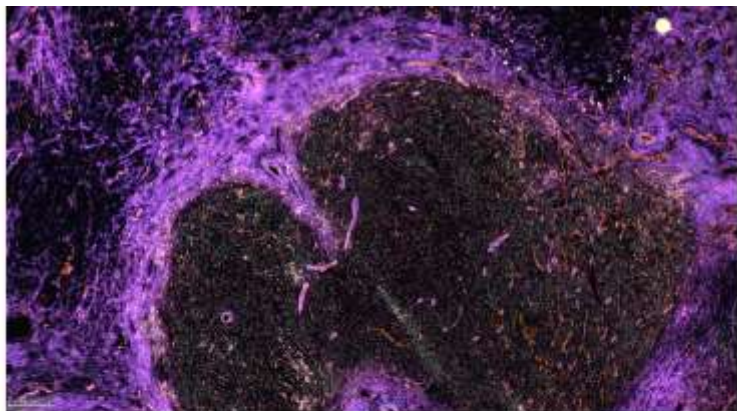
VCAN

## Antibodies

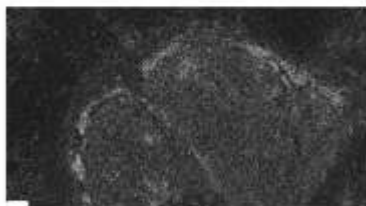
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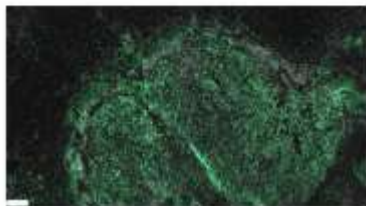
DAPI ACTA2 CD45 CD31 ANXA1 CXCL13 GDF15



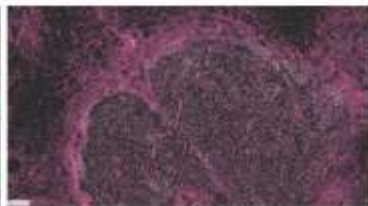
DAPI



ANXA1



CXCL13



GDF15

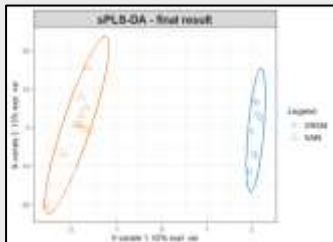
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CD45  
Alpha-SMA  
CD31  
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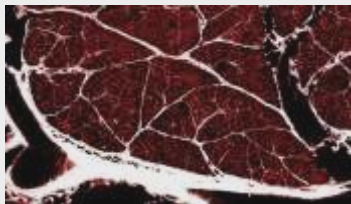
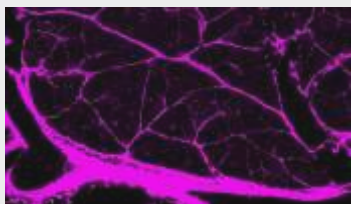
## Microscopie multimodale sans marquage

### Spectroscopie Raman



### SPLS-DA analysis

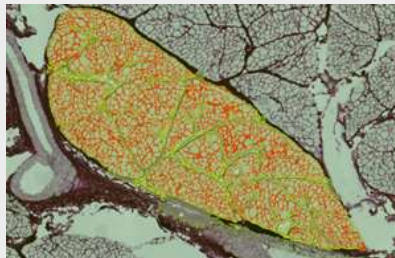
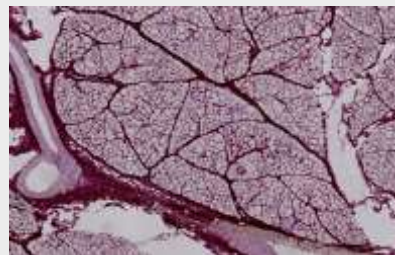
### Second harmonic generation (SHG)



*K. Rouger : Physiopathologie Animale et bioThérapie du muscle et du système nerveux - UMR 703 (PANTher)*

*Laurence Dubreil : APEX PANTher INRAE/Oniris Ecole nationale vétérinaire de Nantes*

### Coloration Picrosirius



### Quantification Picrosirius

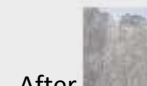
## Multiplexing Cell DIVE



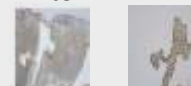
### MICRODISSECTION PAR CAPTURE LASER



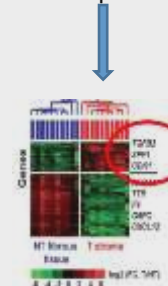
Before



After



Capture



SRS Stimulated Raman Scattering

CARS Coherent Anti-Stokes Raman Spectroscopy





Bruno TURLIN  
Marie-Dominique GALIBERT

Anthony SÉBILLOT

Roselyne VIEL  
Pascale BELLAUD  
Gevorg GHUKASYAN  
Marine SEFFALS  
Maëlle GUILLOUT

**Dr Musso Orlando** : *Principal Investigator at the Institute Nutrition, Metabolism and Cancer (NuMeCan), Inserm.*

**NuMeCan** - UMR-1317 INSERM – INRAe

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