Evaluation de l'infiltrat immunitaire dans le microenvironnement de l'adénocarcinome pancréatique : **nouvelle approche de quantification cellulaire**

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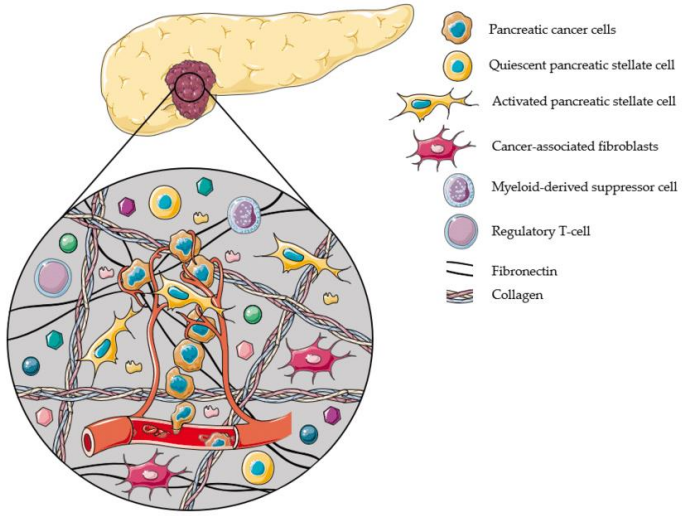
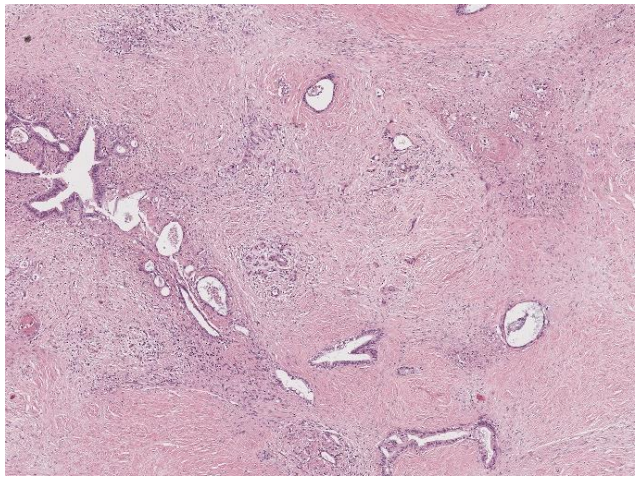
Arthur BENEDIC– *CHU Strasbourg*
Gerlinde AVEROUS – *CHU Strasbourg*

Adénocarcinome pancréatique

- Survie à 5 ans : 8,5%
- 2ème cancer le plus mortel en 2050
- 90% de type canalaire (ACP)
- Abondant stroma fibreux



Ruben Verloy et al, 2020



Adénocarcinome canalaire du pancréas (HE x100)

ANNUAL REVIEWS

Annual Review of Pathology: Mechanisms of Disease

Tumor Microenvironment in Pancreatic Cancer Pathogenesis and Therapeutic Resistance

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*These authors contributed equally to this article.

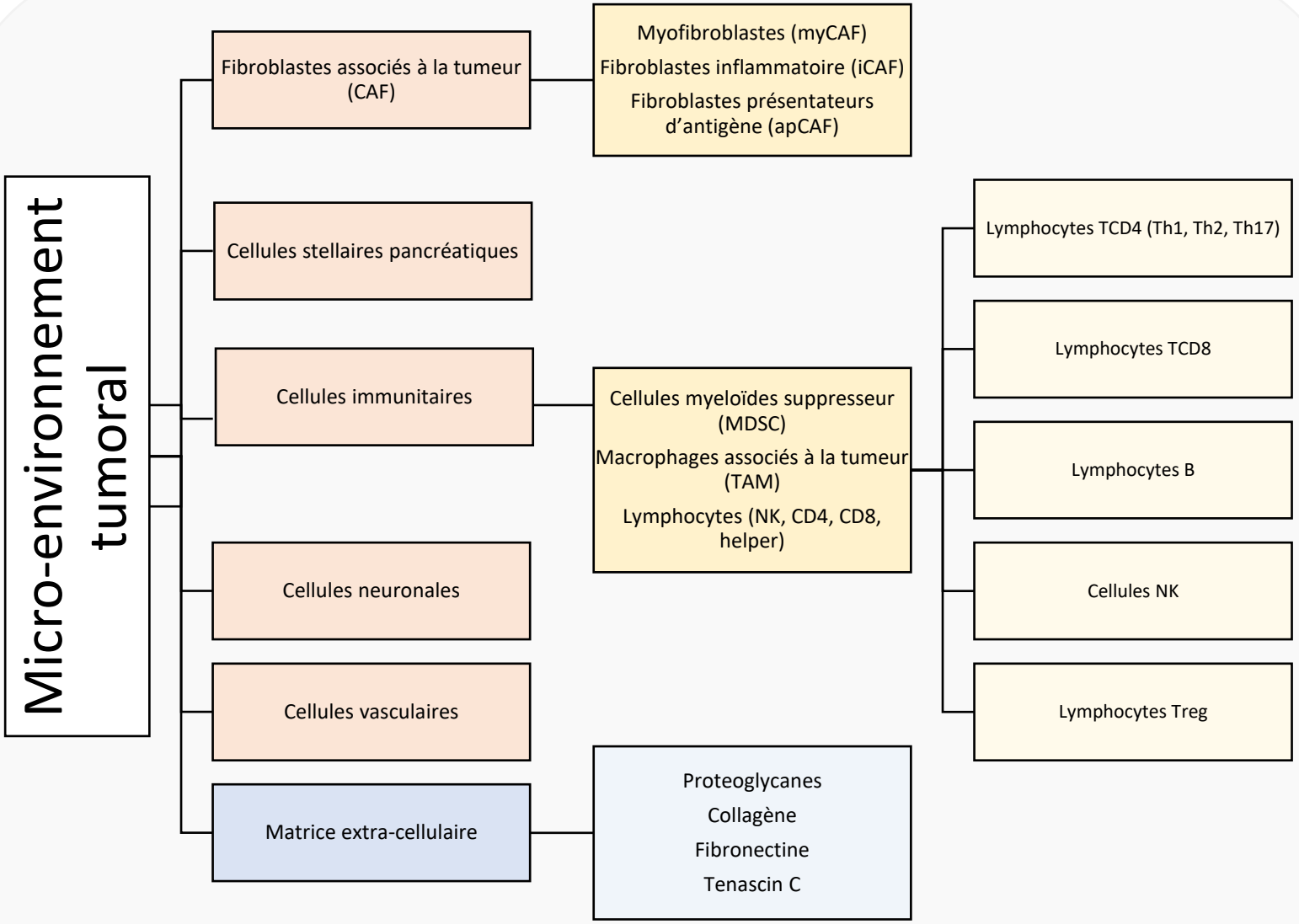
Keywords

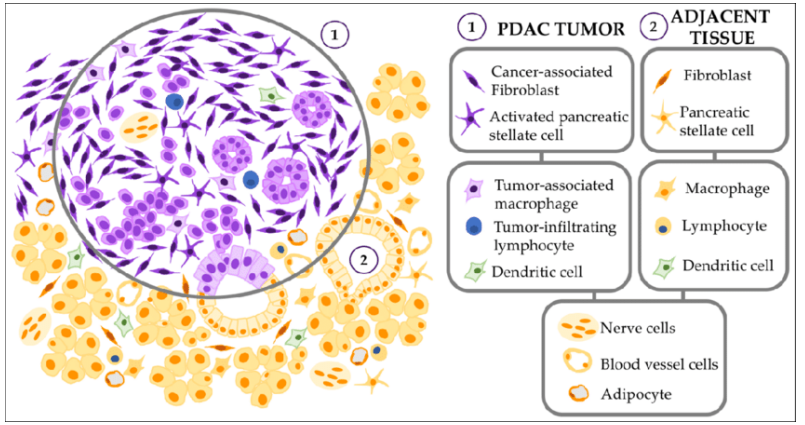
pancreatic cancer, tumor microenvironment, tumor immunology, stroma, cancer-associated fibroblast

Abstract

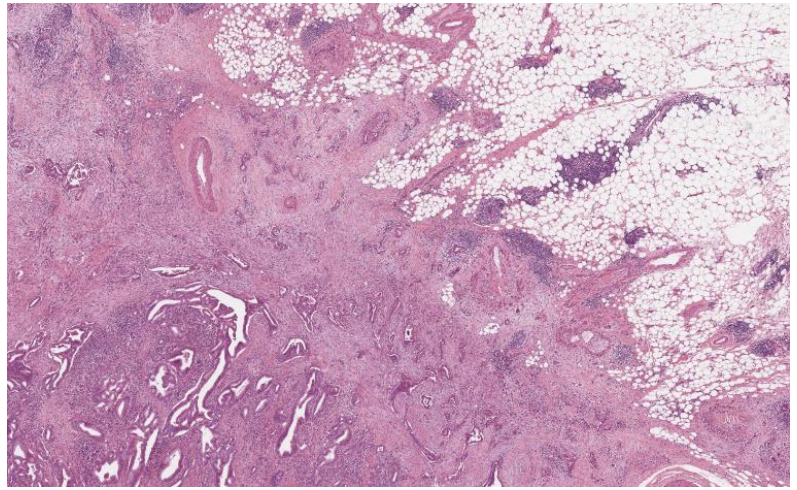
Pancreatic ductal adenocarcinoma (PDAC) features a prominent stromal microenvironment with remarkable cellular and spatial heterogeneity that meaningfully impacts disease biology and treatment resistance. Recent advances in tissue imaging capabilities, single-cell analytics, and disease modeling have shed light on organizing principles that shape the stromal complexity of PDAC tumors. These insights into the functional and spatial dependencies that coordinate cancer cell biology and the relationships that exist between cells and extracellular matrix components present in tumors are expected to unveil therapeutic vulnerabilities. We review recent advances in the field and discuss current understandings of mechanisms by which the tumor microenvironment shapes PDAC pathogenesis and therapy resistance.

Sherman et al, 2023

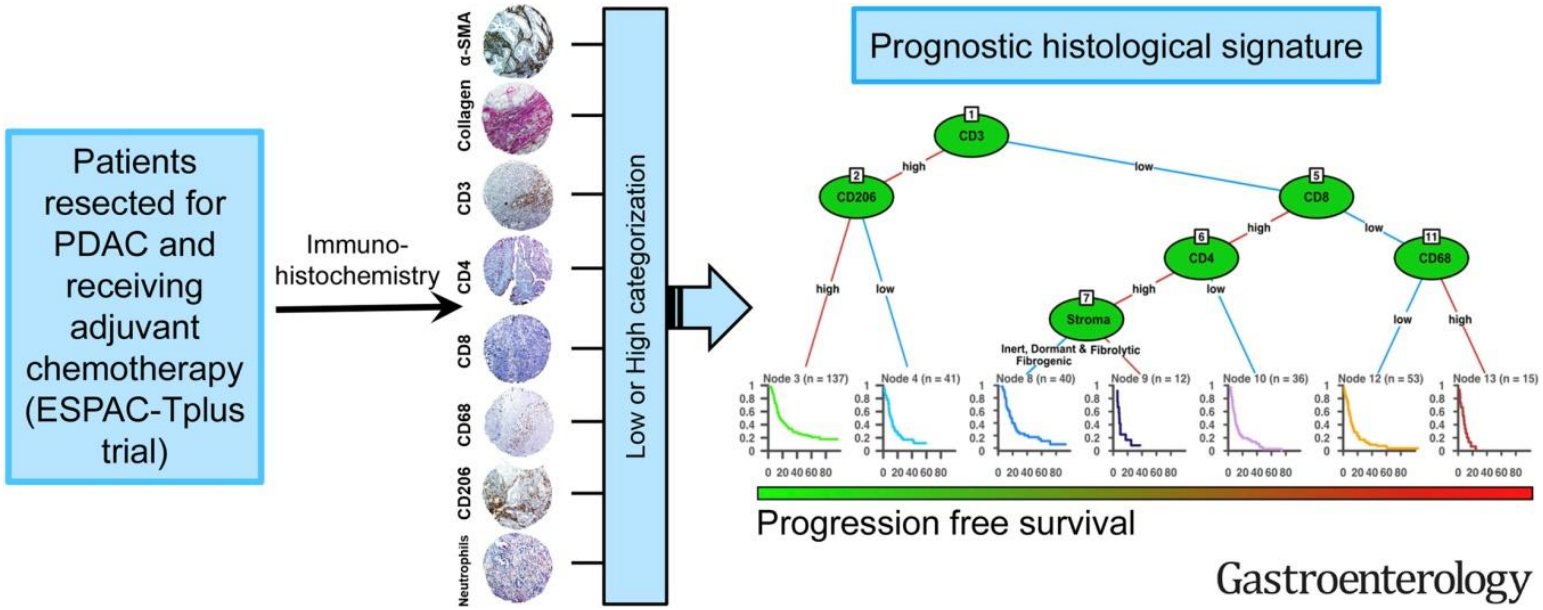




Pancreatic Adenocarcinoma Invasiveness and the Tumor Microenvironment: From Biology to Clinical Trials, Meija et al, 2020



Adénocacinome canalaire du pancréas (HE x50)



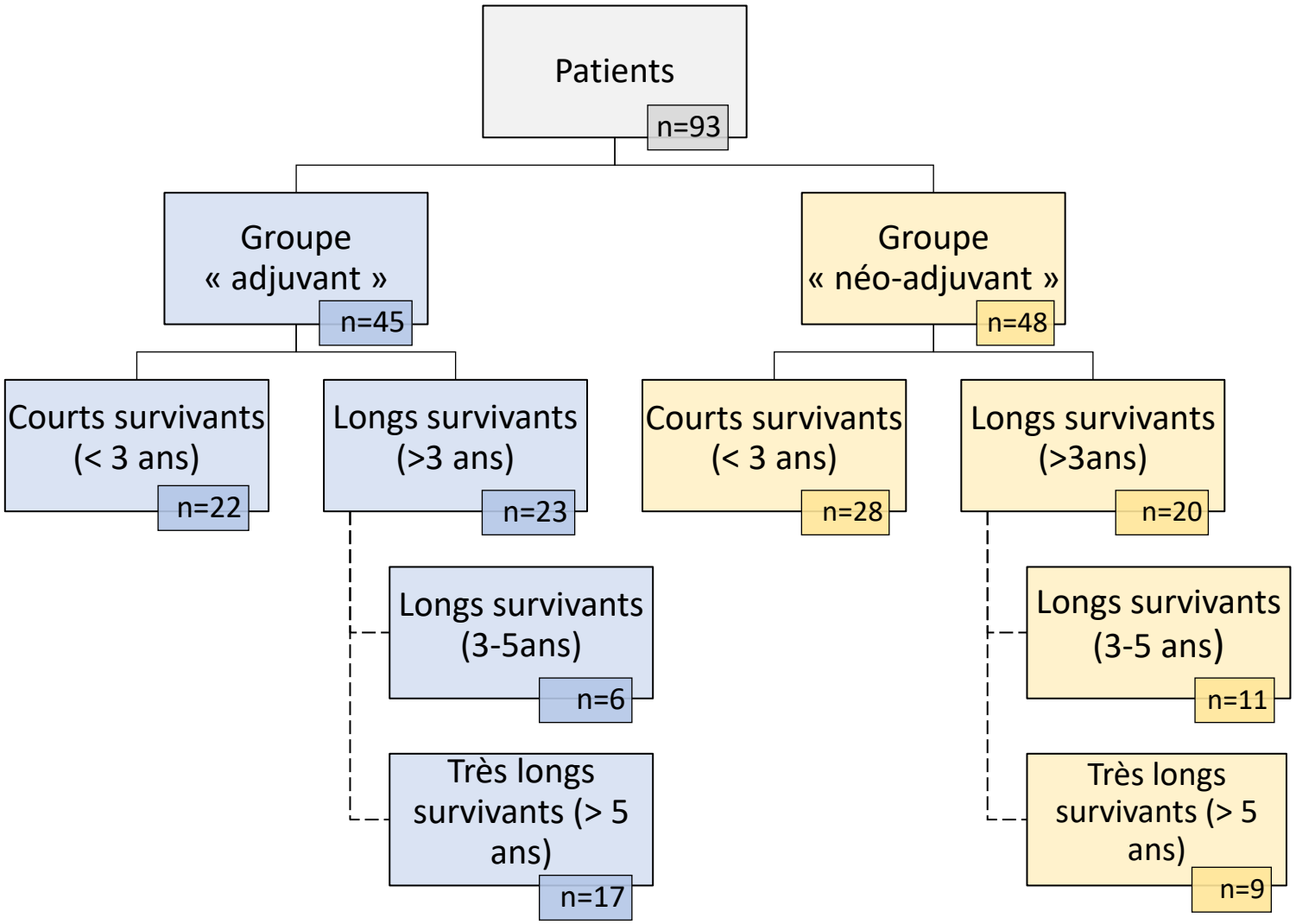
Mahajan et al, 2018

- Revue de la littérature : données semi quantitatives
- Reproductibilité inter-observateur médiocre

<u>Objectif principal</u> : caractériser le micro-environnement cellulaire de l'adénocarcinome pancréatique avec des méthodes de comptage fiables et reproductibles.

Autres objectifs :

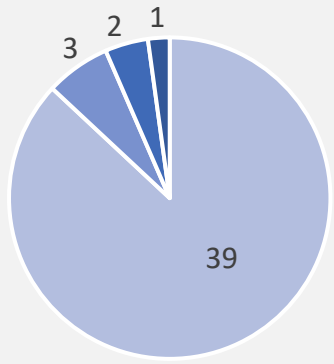
- évaluer l'impact de la chimiothérapie néo-adjuvante sur le micro-environnement tumoral
- évaluer l'impact du micro-environnement tumoral sur la survie



Diagnostic histologique "adjuvant"

Groupe« **adjuvant** »

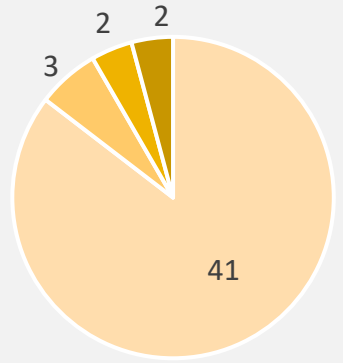
- 43-86 ans
- 2001 à 2011



Diagnostic histologique "néo-adjuvant"

Groupe « **néo-adjuvant**»

- 42-72 ans
- 2010 à 2018



Caractéristiques cliniques

Patients	Effectifs (n=93)	Groupe adjuvant (n=45)	Groupe néo-adjuvant(n=48)
Age médian	64,5	66	63
Sex Ratio (H/F)	53/40	26/19	28/21
Survie			
Survie <3ans)	50	21	28
Survie (3-5ans)	17	6	11
Survie (>5ans)	26	17	9
CA 19,9 (kU/l) médiane	55,45	143,3	35,6

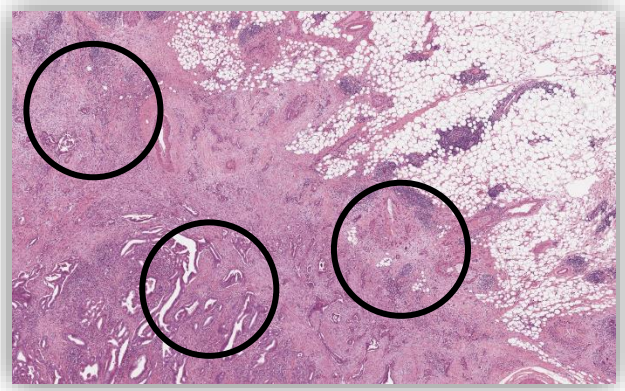
Caractéristiques chirurgicales et anatomopathologiques

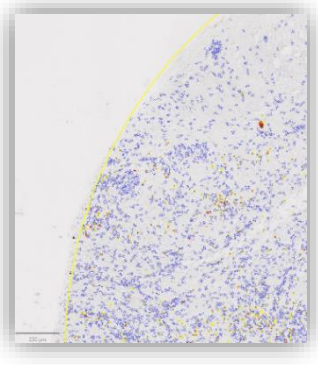
Patients	Effectifs (n=93)	Groupe adjuvant (n=45)	Groupe néo-adjuvant (n=48)
Type de résection			
DPC/DPT	75	43	32
SPG	18	2	16
Résection veineuse	66	23	43
Résection artérielle	33	0	33
Résection R0	46	21	25
Résection R1	44	21	23
TNM (8ème édition, 2017)			
T1	1	0	1
T2	26	19	7
T3	45	25	20
T4	21	1	20
Nombre moyen de N+	3,2	3,6	2,8
Grade			
Bien différencié	23	12	11
Moyennement différencié	37	22	15
Peu différencié	17	10	7
Grade inconnu/non spécifié	16	1	15

- Désarchivage lames et blocs
- Sélection de région d'intérêt (3 zones par tumeur)
- Réalisation de 3 punch (6 mm) par tumeur, et intégration dans un TMA
- 12 TMA par cohorte
- Réalisation de lames H&E ainsi que des immunomarquages : *CD1a, CD3, CD4, CD8, CD163, CD68, FOXP3*
- Numérisation et quantification cellulaire de l'ensemble des punchs

Immunomarquages utilisés

Anticorps	Cible
CD1a	Cellules dendritiques
CD3	Lymphocytes T
CD4	Lymphocytes T CD4
CD8	Lymphocytes T cytotoxiques
CD163	Macrophages M2
CD68	Macrophages
FoxP3	Lymphocytes T Régulateurs

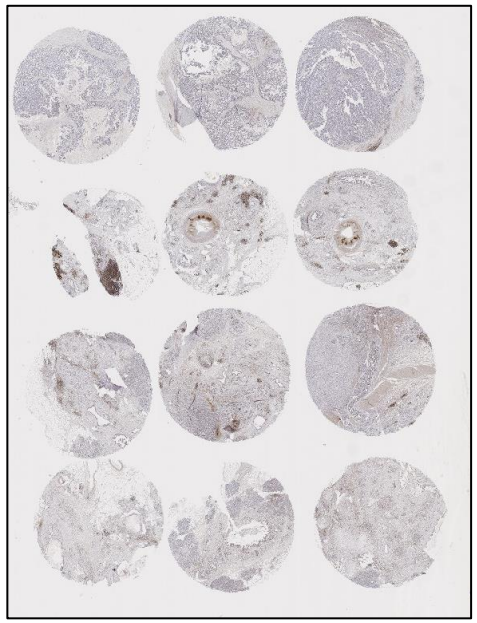




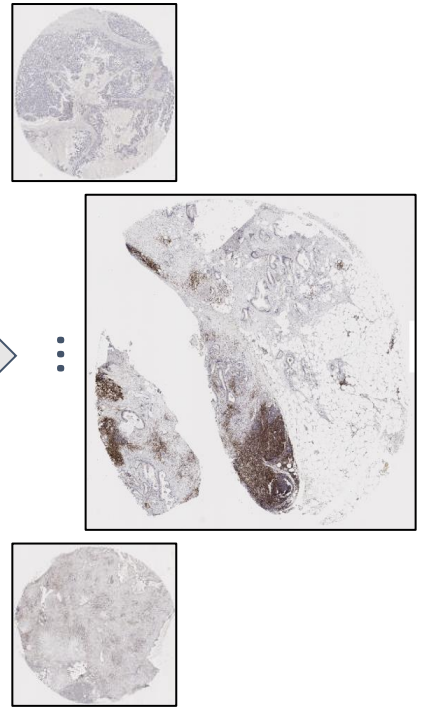
Désarchivage lames + blocs → Ciblage des régions d'intérêt → Réalisation des TMA → Immunohistochimie → Numérisation → Quantification par QuPath et par QMS

Processus de traitement

Tissue MicroArray

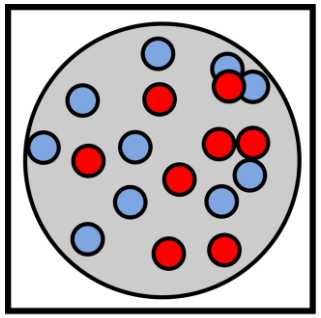


Punchs



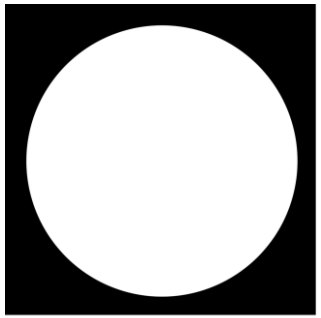
Extraction en punchs
avec QuPath [1] et Fiji [2]

Quantification cellulaire
(2 méthodes)



$n_{positif}$

Estimation surfacique
(tissu)



A_{tissu}

Métrique : nombre de cellules positives par mm²

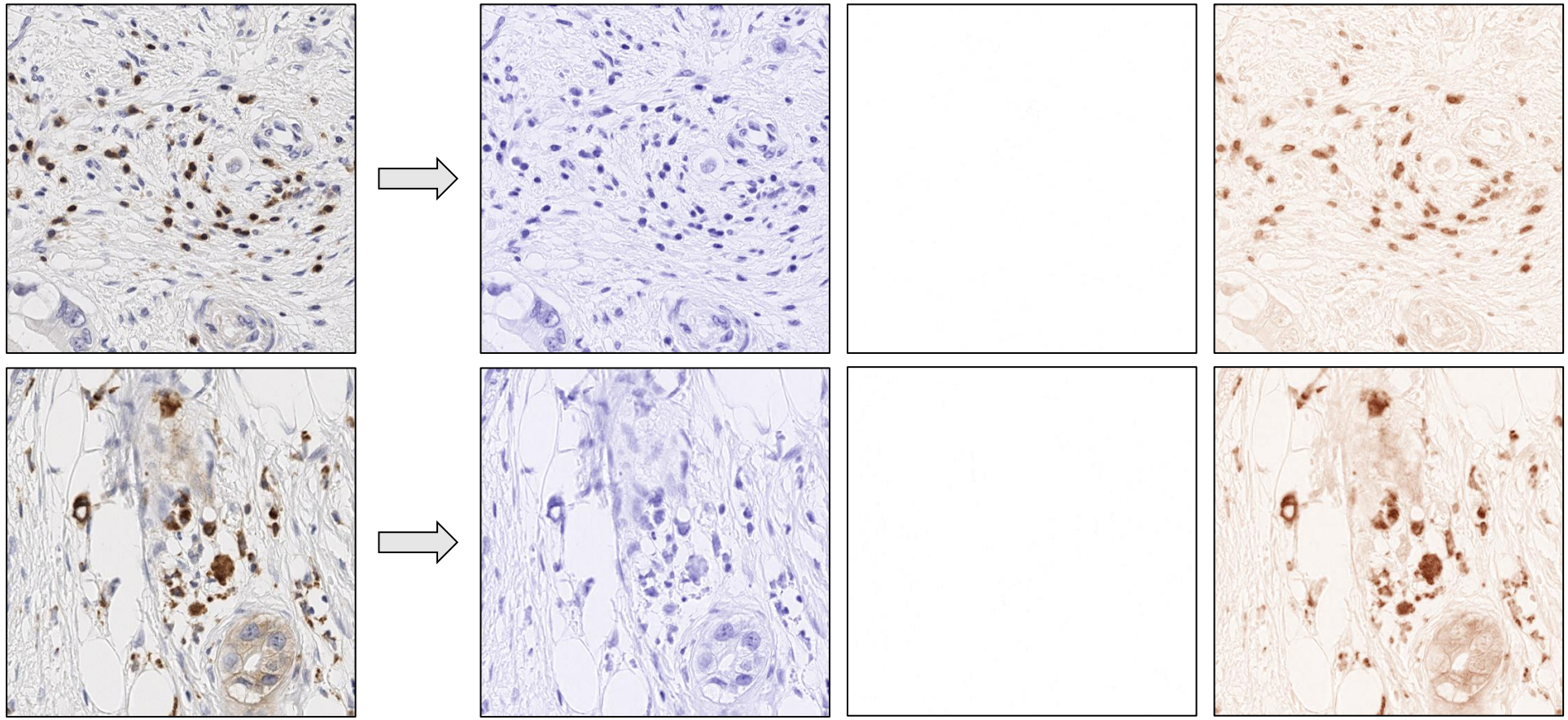
$$\frac{n_{positif}}{10^6 \times A_{tissu}}$$

[1] Bankhead, P. et al. QuPath: Open source software for digital pathology image analysis. *Scientific Reports* (2017).
[2] Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. *Nature Methods*, *9*(7), 676–682 (2012).

Quantification des cellules positives (Méthode QMS)

QMS : Quantification Morphologique et ratios de Surfaces

Séparation des canaux {R, V, B} en {Hématoxyline, Éosine, DAB} [3]

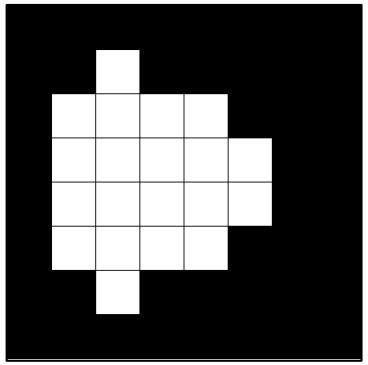
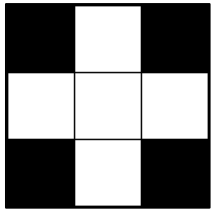
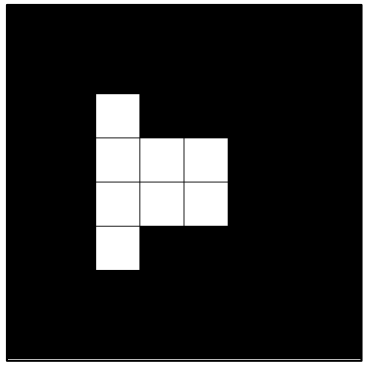


[3] Ruifrok A.C. and Johnston D.A. Quantification of histochemical staining by color deconvolution. Anal Quant Cytol Histol. 2001 Aug;23(4):291-9.

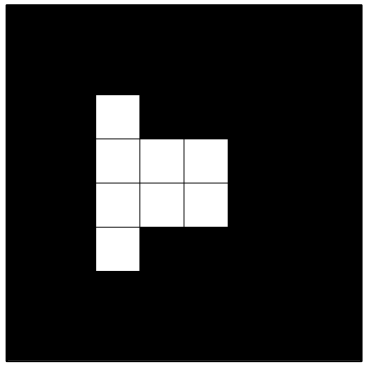
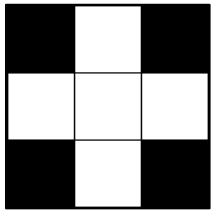
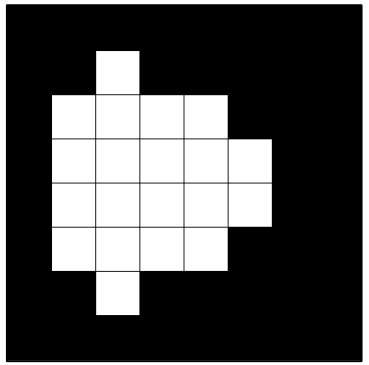
Quantification des cellules positives (Méthode QMS)

Opérations de base : erosion et dilatation morphologiques

$$\epsilon_B(X) = X \ominus B = \{x \,|\, B_x \subset X\}$$

X :  B :  $\epsilon_B(X)$: 

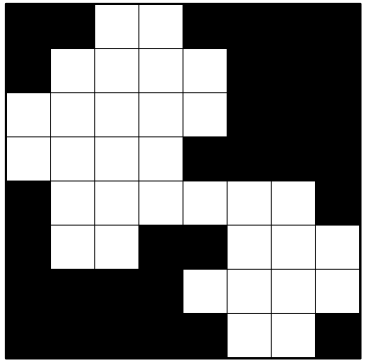
$$\delta_B(X) = X \oplus B = \{x + b \,|\, b \in B, x \in X\}$$

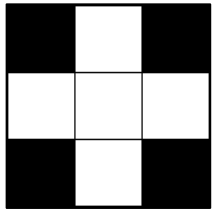
X :  B :  $\delta_B(X)$: 

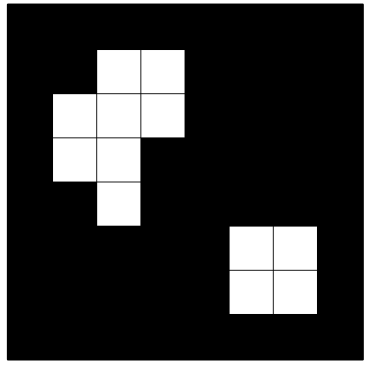
Quantification des cellules positives (Méthode QMS)

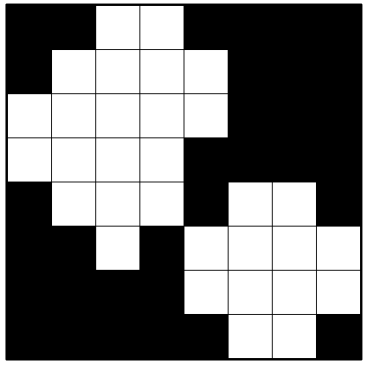
Opération complexes : ouverture et fermeture morphologiques

$$\gamma_B(X) = X \circ B = \delta_B(\epsilon_B(X))$$

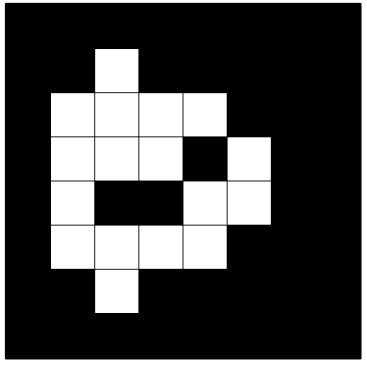
X : 

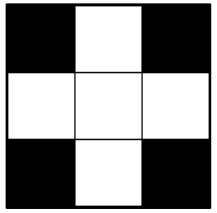
B : 

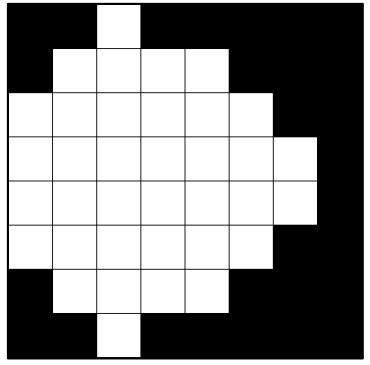
$\epsilon_B(X)$: 

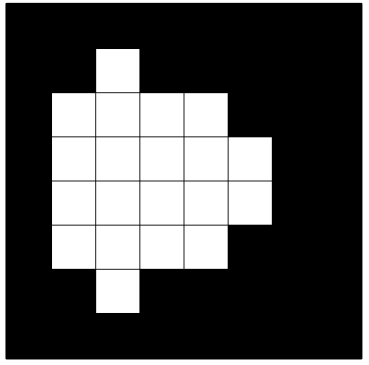
$\delta_B(\epsilon_B(X))$: 

$$\phi_B(X) = X \bullet B = \epsilon_B(\delta_B(X))$$

X : 

B : 

$\delta_B(X)$: 

$\epsilon_B(\delta_B(X))$: 

Quantification des cellules positives (Méthode QMS)

Seuillage du signal DAB et filtrage morphologique

$$f_B(X) = \phi_B(\gamma_B(X))$$

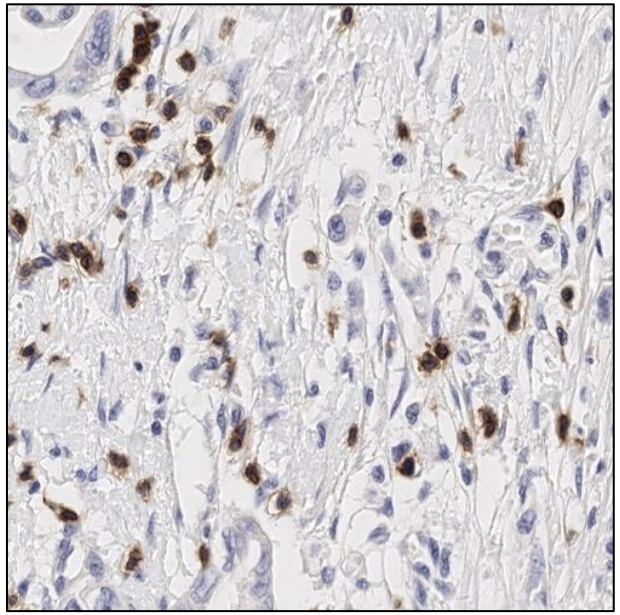
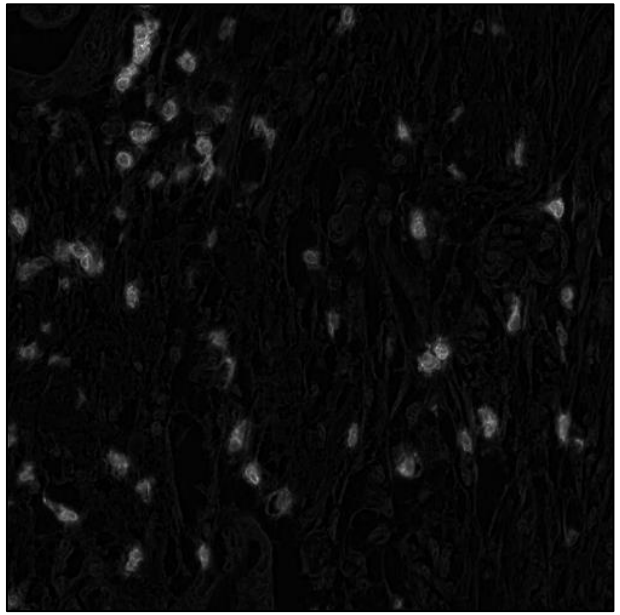
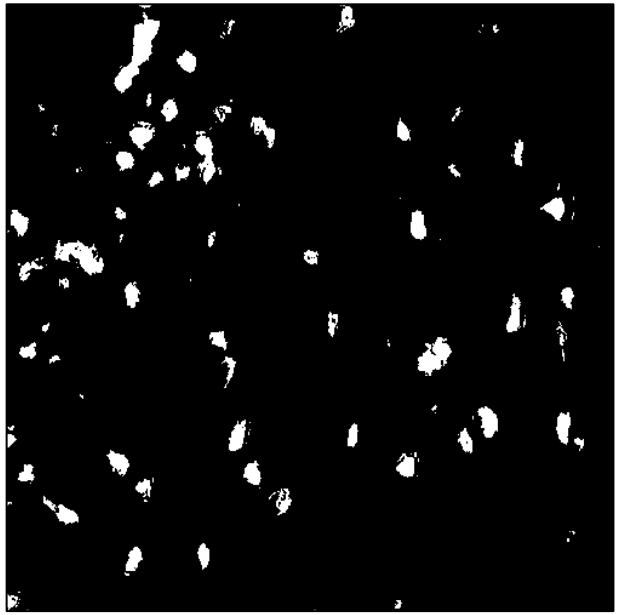


Image RVB



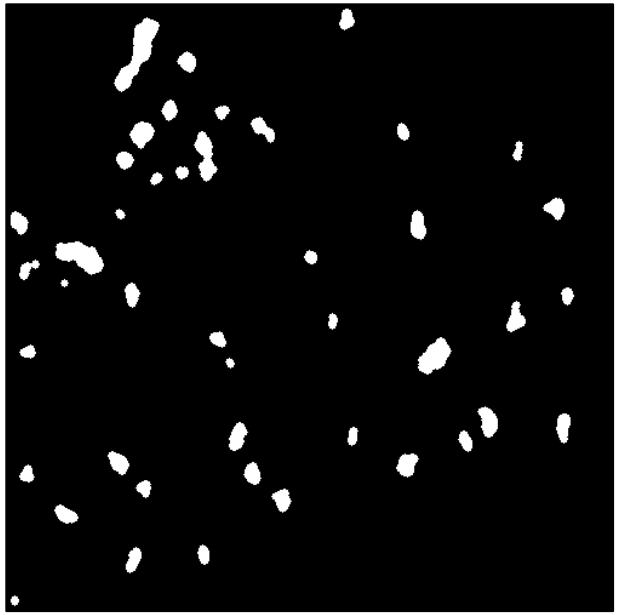
Signal DAB

X



Signal DAB
seuillage + binarisation

$f_B(X)$



Signal DAB
morphologie

Quantification des cellules positives (Méthode QMS)

Détection sur le signal DAB binarisé et nettoyé par filtrage morphologique

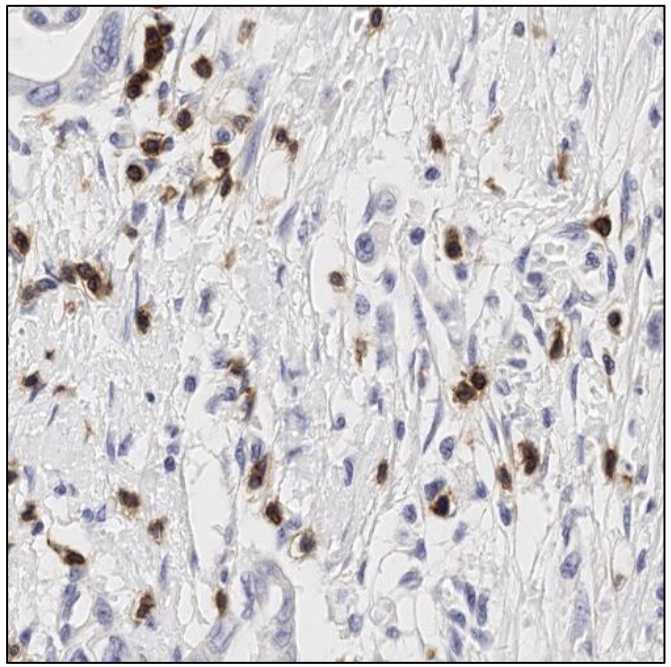
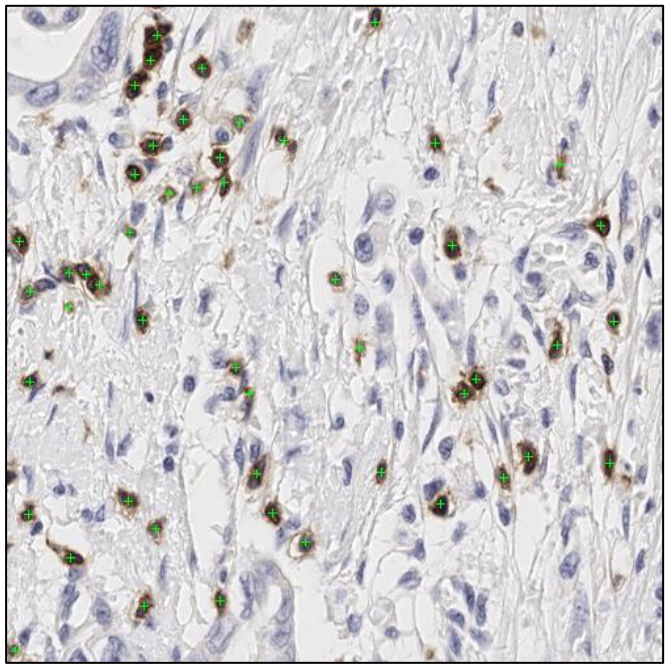


Image RVB

Signal DAB binarisé et nettoyé par filtrage morphologique



Détection et quantification des cellules positives

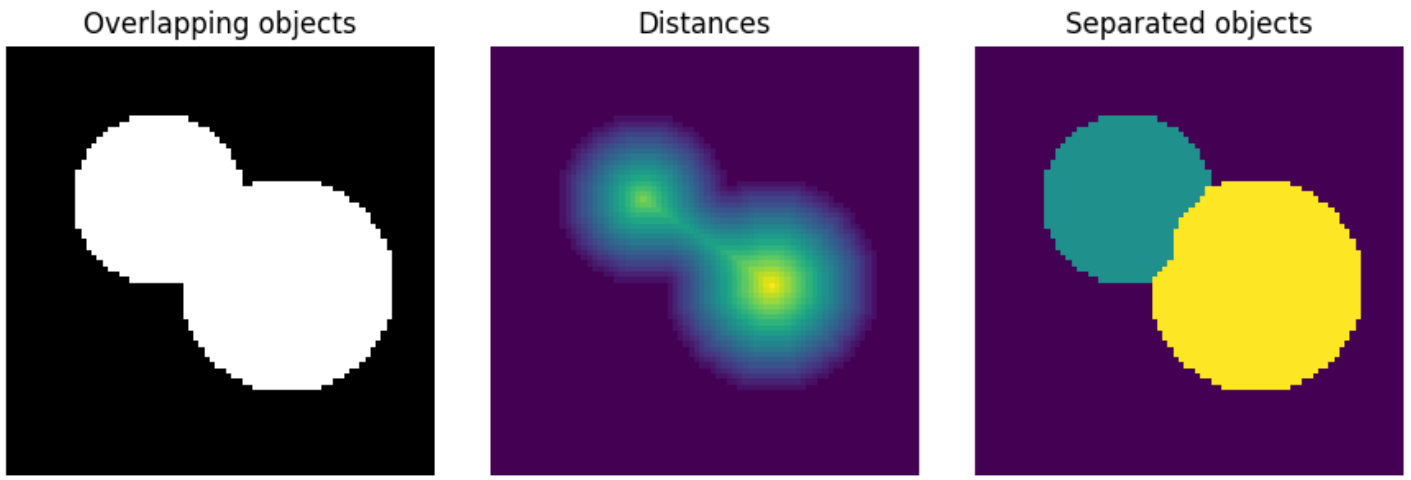
Détection des cellules positives (Méthode QuPath)

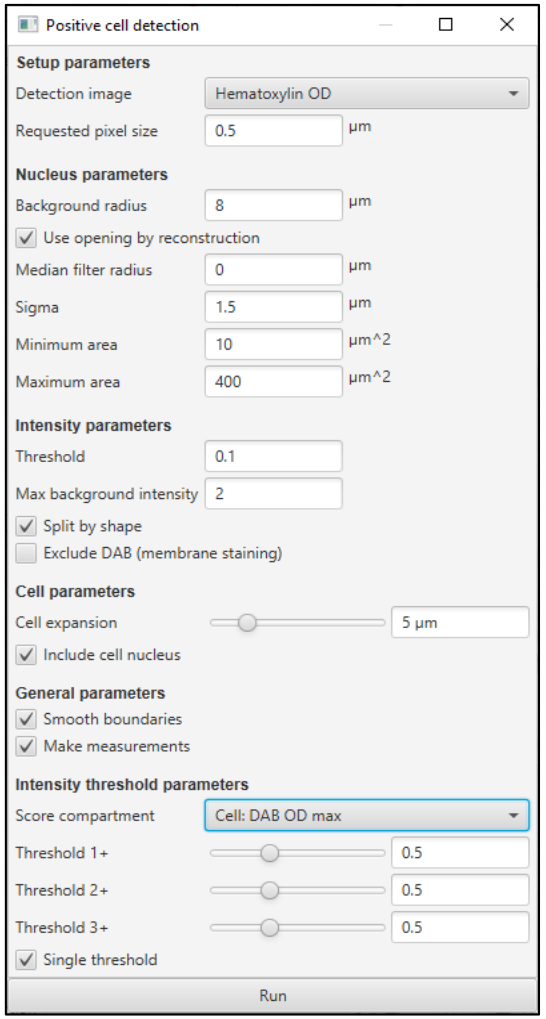
Module *Positive cell detection* de QuPath [1]

Séparation {R,V,B} → {Hématoxyline, Éosine, DAB}

Traitement des canaux Hématoxyline et DAB

Segmentation avec algorithme de ligne de partage des eaux



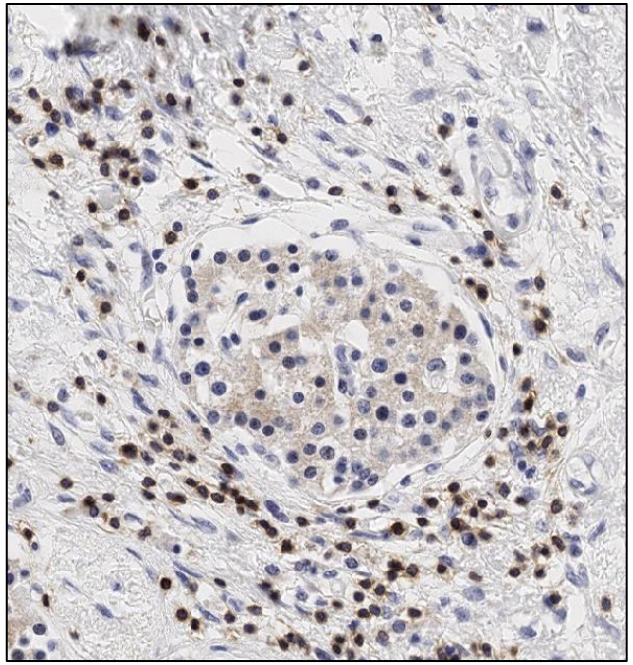


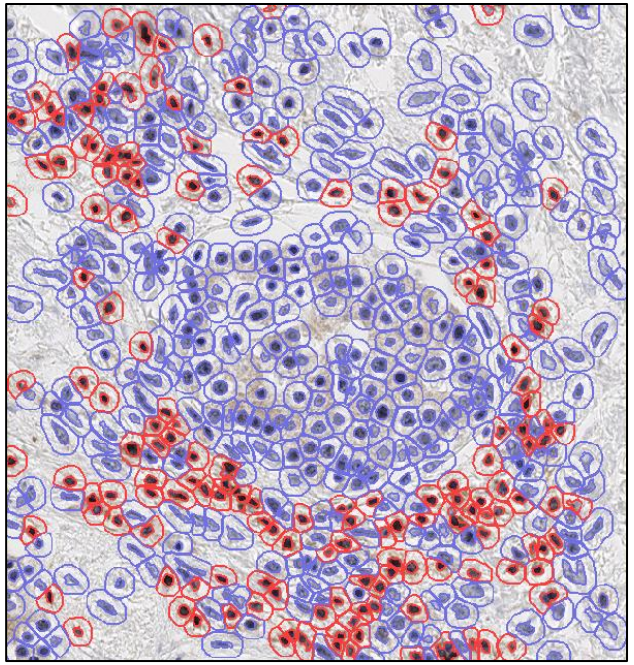
[1] Bankhead, P. et al. QuPath: Open source software for digital pathology image analysis. *Scientific Reports* (2017).

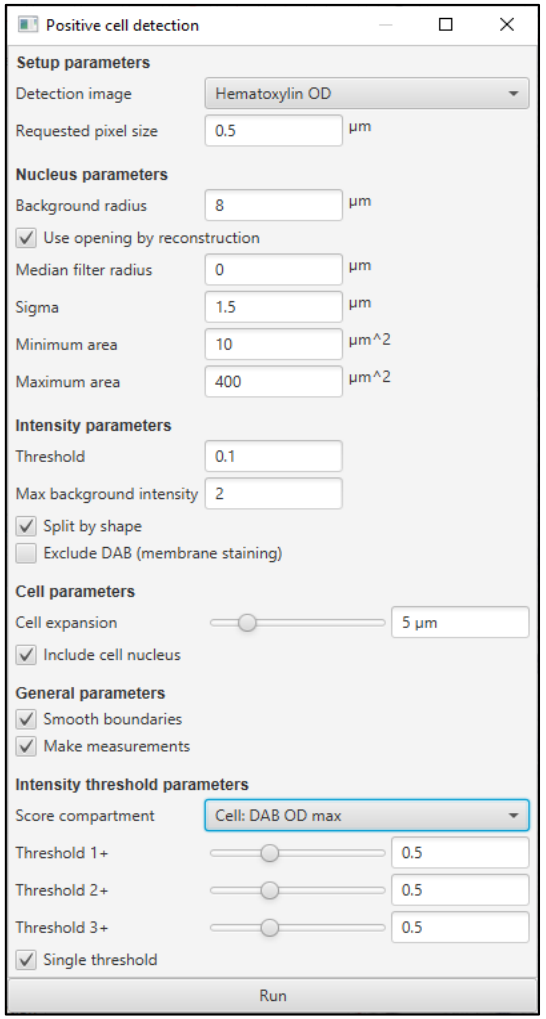
Détection des cellules positives (Méthode QuPath)

Module *Positive cell detection* de QuPath [1]

Quantification : # noyaux positifs







[1] Bankhead, P. et al. QuPath: Open source software for digital pathology image analysis. *Scientific Reports* (2017).

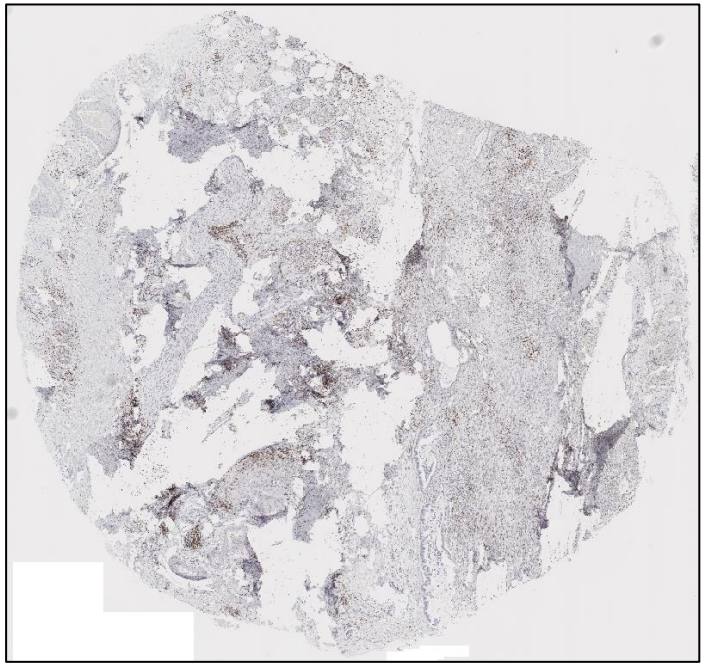
Estimation surfacique

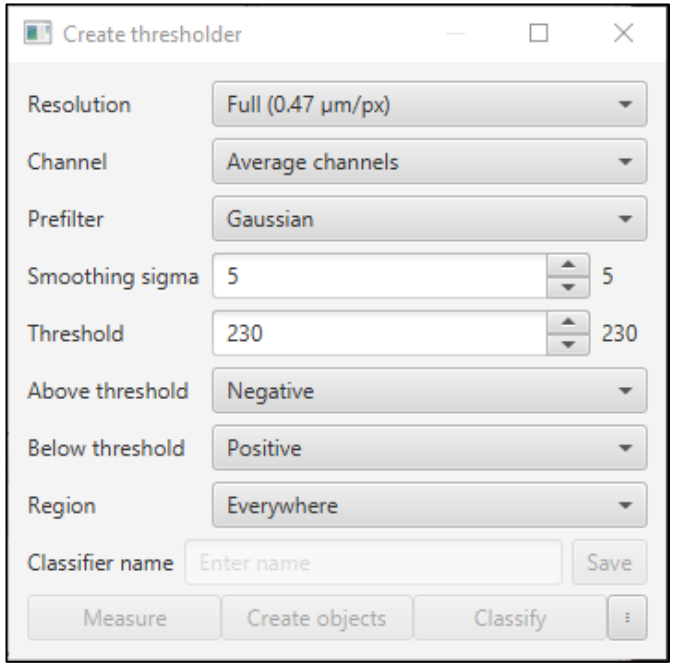
QuPath : classificateur binaire (pixel thresholder) [1]

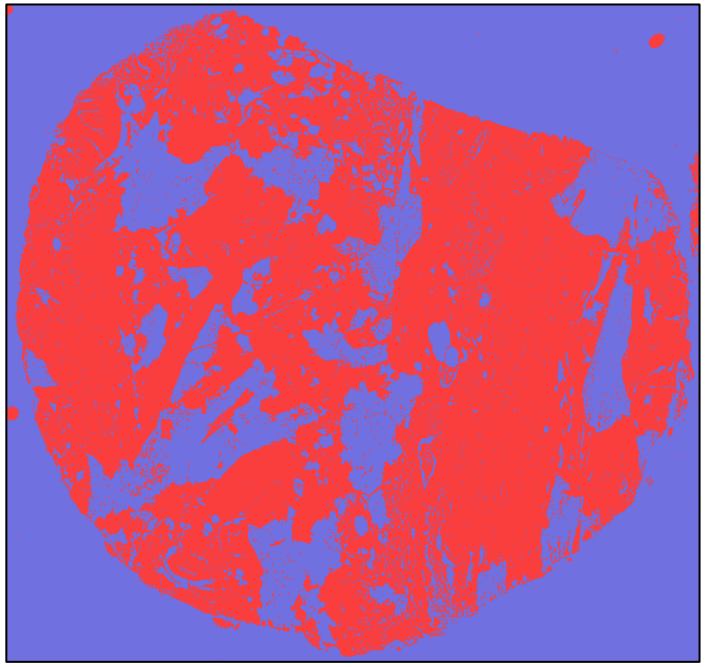
Classify → Pixel thresholder → Create thresholder

Résultat : aire en μm^2

Key	Value
Image	TMA_1_patient_1_punch_2.tif
Name	Image
(Live) Positive %	49.9769
(Live) Positive area µm^2	19690398
(Live) Negative %	50.0231
(Live) Negative area µm^2	19708588







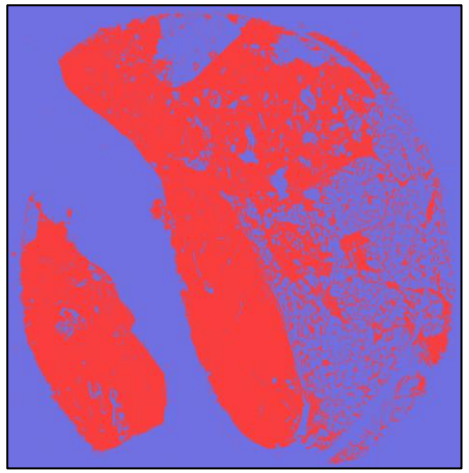
[1] Bankhead, P. et al. QuPath: Open source software for digital pathology image analysis. *Scientific Reports* (2017).

Métrique finale

Pour chaque cohorte c, patient p, punch i, marqueur m :

$$n_{positif}(c,p,i,m)$$

$$i.e.\ 24752$$



$$\mathcal{A}(c,p,i,m)$$

$$i.e.\ 20912508\,\mu m^2$$

$$Métrique(c,p,i,m) = \frac{n_{positif}(c,p,i,m)}{10^6 \times \mathcal{A}(c,p,i,m)}$$

$$i.e.\ 118.4\,m^+/mm^2$$

Moyennes : cellules/mm², chiffres bruts

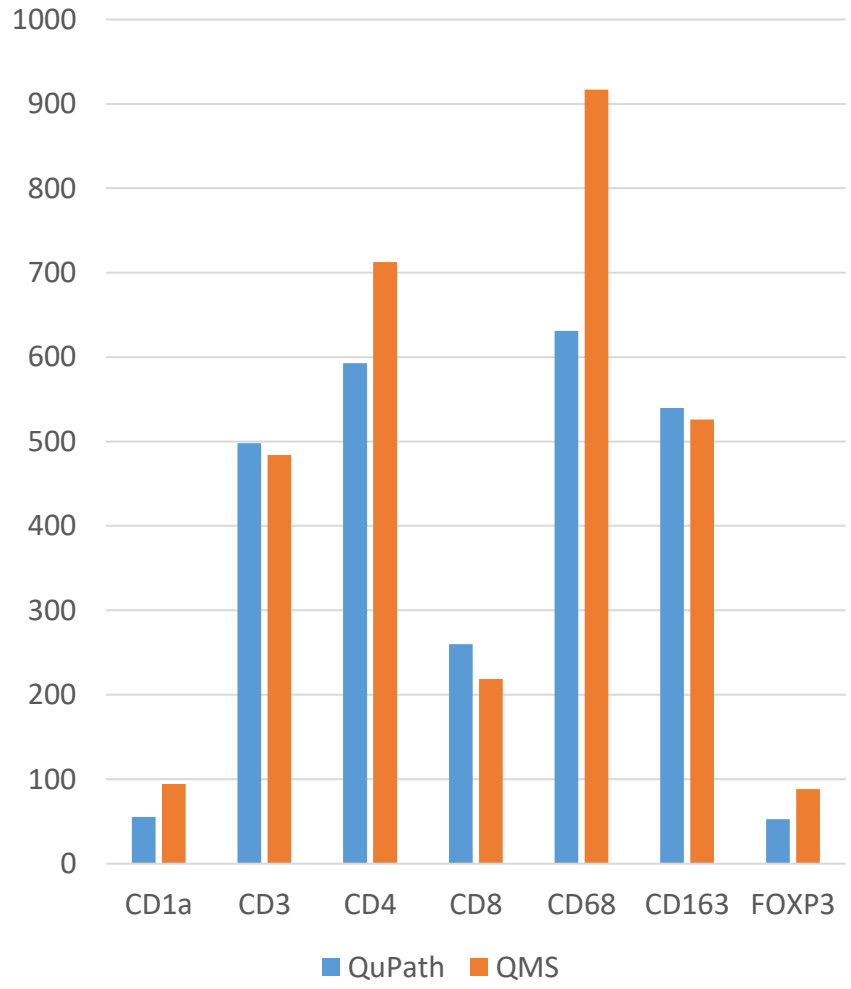
QuPath

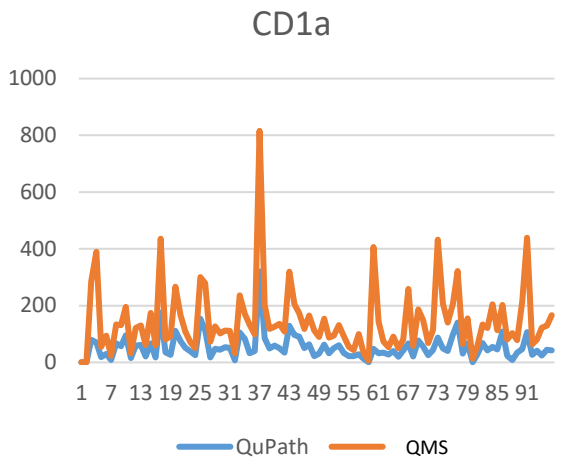
Marqueur	Moyenne	Médiane	Ecart Type
CD1a	55,1	45,6	43,6
CD3	498,12	460,9	318,6
CD4	592,6	540,6	353,8
CD8	260,0	238,3	189,9
CD68	631,1	556,8	364,5
CD163	539,6	511,9	302,3
FOXP3	52,7	39,7	60,3
CD8/CD3	0,57	0,49	0,48
CD8/CD4	0,48	0,41	0,37

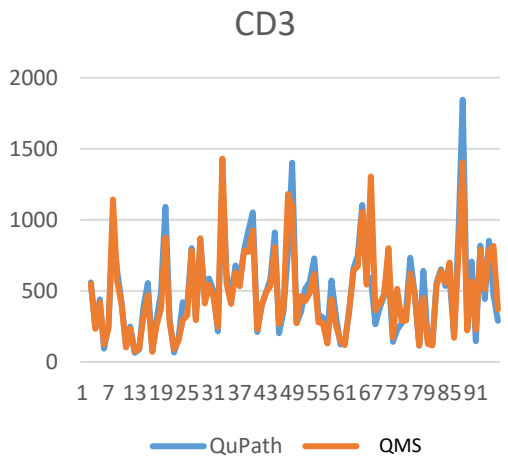
QMS

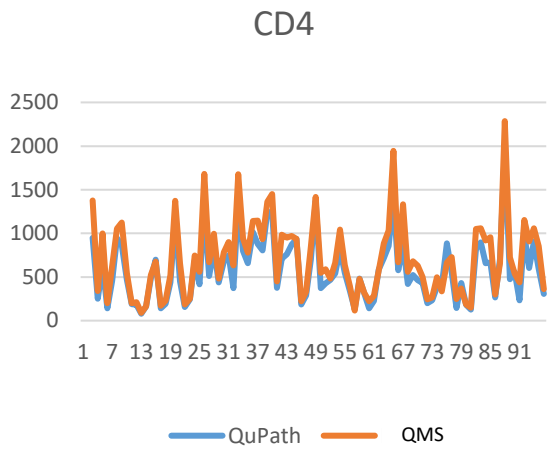
Marqueur	Moyenne	Médiane	Ecart Type
CD1a	94,2	69,9	82,2
CD3	484,1	436,2	305,9
CD4	712,7	657,7	431,4
CD8	218,4	208,9	146,3
CD68	916,8	797,9	551,9
CD163	526,1	477,8	290,3
FOXP3	88,1	39,4	218,6
CD8/CD3	0,50	0,46	0,38
CD8/CD4	0,35	0,29	0,25

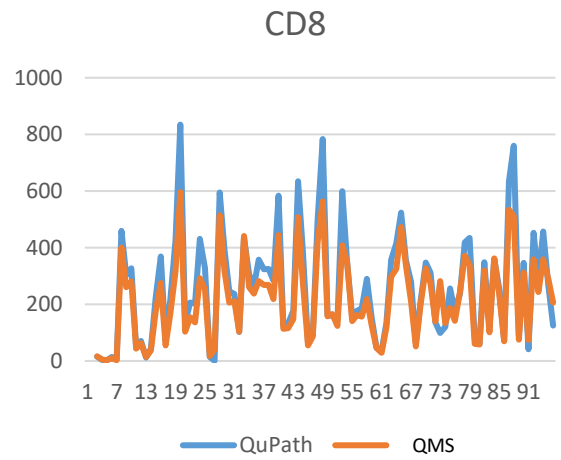
Comparaison de moyennes

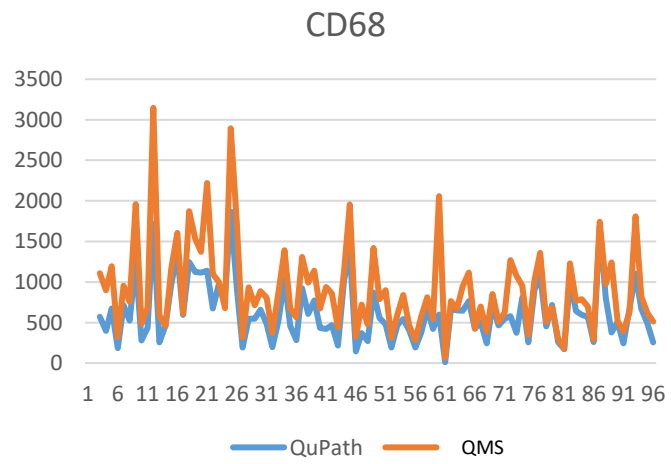


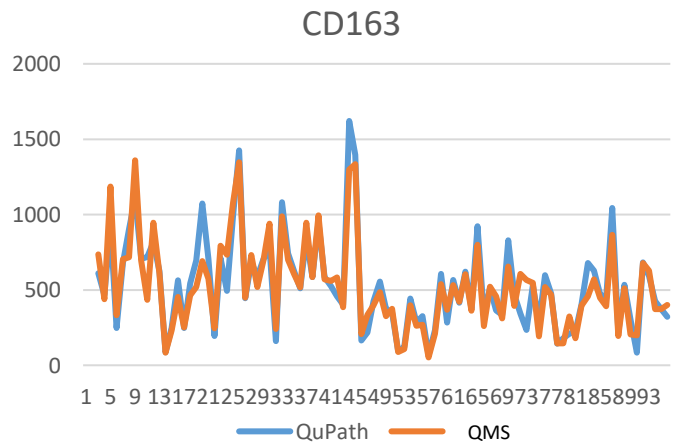

CD1a
1000
800
600
400
200
0
1 7 13 19 25 31 37 43 49 55 61 67 73 79 85 91
QuPath
QMS

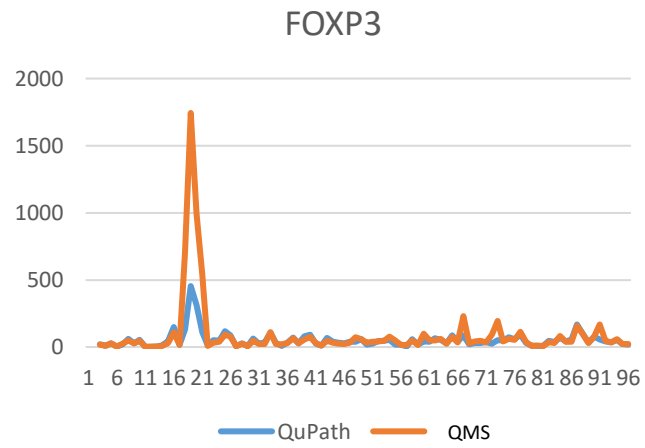

CD3
2000
1500
1000
500
0
1 7 13 19 25 31 37 43 49 55 61 67 73 79 85 91
QuPath
QMS


CD4
2500
2000
1500
1000
500
0
1 7 13 19 25 31 37 43 49 55 61 67 73 79 85 91
QuPath
QMS


CD8
1000
800
600
400
200
0
1 7 13 19 25 31 37 43 49 55 61 67 73 79 85 91
QuPath
QMS

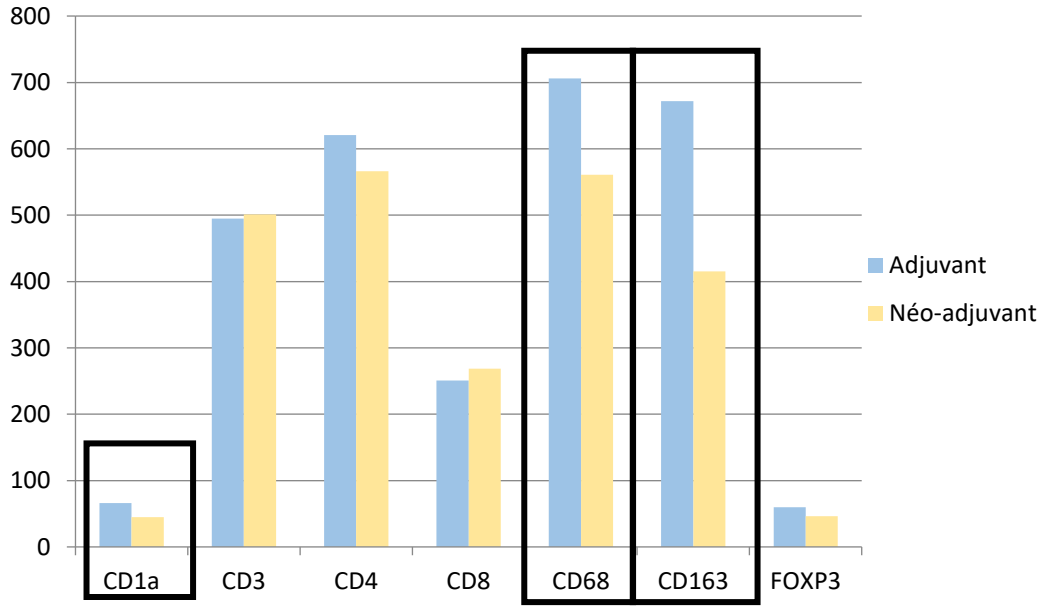

CD68
3500
3000
2500
2000
1500
1000
500
0
1 6 11 16 21 26 31 36 41 46 51 56 61 66 71 76 81 86 91 96
QuPath
QMS


CD163
2000
1500
1000
500
0
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61 65 69 73 77 81 85 89 93
QuPath
QMS


FOXP3
2000
1500
1000
500
0
1 6 11 16 21 26 31 36 41 46 51 56 61 66 71 76 81 86 91 96
QuPath
QMS

Comparaison groupe adjuvant I néo-adjuvant

- CD1a : 66/mm² I 45/mm² (p=0,01)
- CD68 : 706/mm² I 560/mm² (p=0,05)
- CD163 : 672/mm² I 415/mm² (p<0,0001)



Rappel :
- CD1a : cellules dendritiques
- CD68 : pan macrophagique
- CD163 : macrophages M2

Comparaison survivant > 3 ans I < 3ans

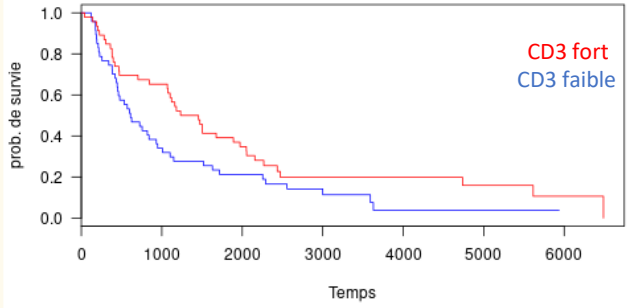
CD3 : 628/mm² I 447/mm² (p=0,01)

CD4 : 762/mm² I 526/mm² (p=0,003)

Comparaison survivant > 5 ans I < 5ans

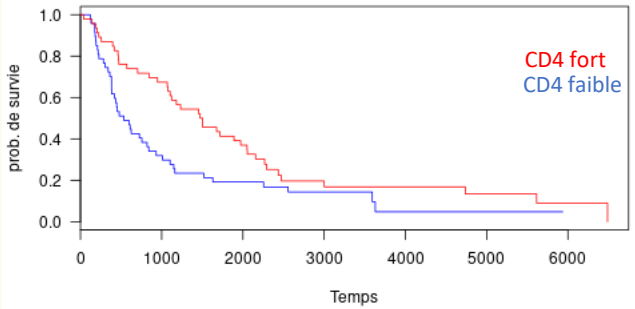
CD3 : 576/mm² I 431/mm² (p=0,02)

CD4 : 692/mm² I 506/mm² (p=0,01)



Test du log-rank

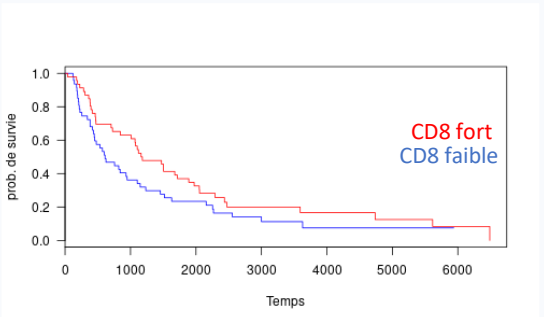
- Statistique du test (x^2) : 3.9564602115058
- p-value: 0.046691791897494



Test du log-rank

- Statistique du test (x^2) : 5.4982322053779
- p-value: 0.019035708024361

Courbe de Survie



Test du log-rank

- Statistique du test (x^2) : 2.5088632706238
- p-value: 0.11320756424166

Comparaison survivant > 3 ans I < 3ans

CD8 : 289/mm² I 235/mm² (p=0,17)

Comparaison survivant > 5 ans I < 5ans

CD8 : 304/mm² I 242/mm² (p=0,16)

Messages clés

- Nouvelle méthode de **quantification cellulaire sur grande surface tumorale** : morphologique, reproductible et personnalisable.

- **Amélioration de survie significative des patients avec important infiltrat lymphocytaire (CD3, CD4)** dans un effectif avec une prédominance de cancers localement avancés.

- Démonstration d'une **baisse d'expression significative de CD163, CD68 et CD1a après chimiothérapie néo-adjuvante**.

- Perspectives : exploitation des données complètes, recherche de paramètres optimaux et introduction d'un modèle d'apprentissage semi-supervisé.

Merci pour votre attention