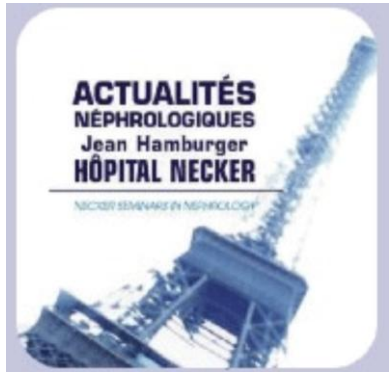
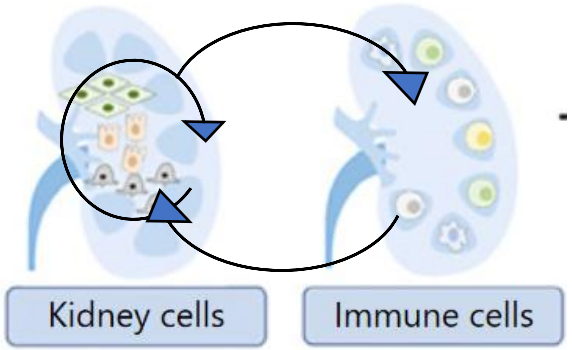


Single-cell RNA sequencing Implications en néphrologie

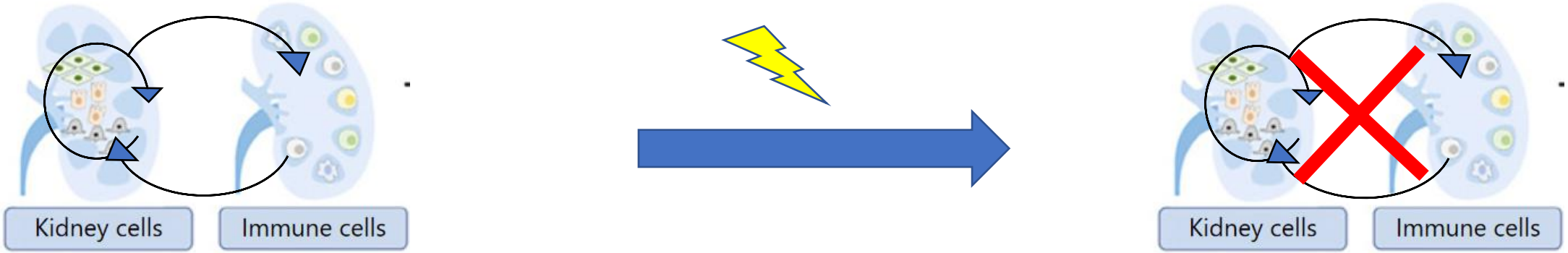
Guillaume DORVAL

Laboratoire des maladies rénales héréditaires
Institut Imagine
Service de médecine génomique des maladies rares
Necker Enfants Malades

Introduction



Introduction



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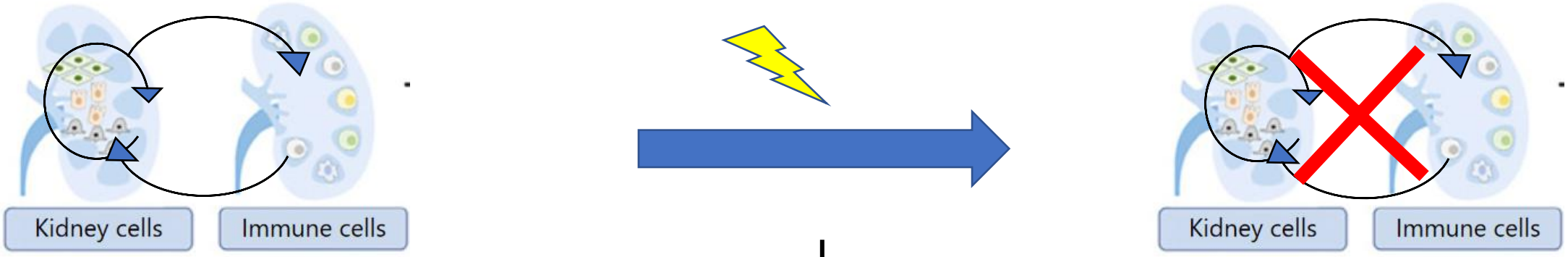

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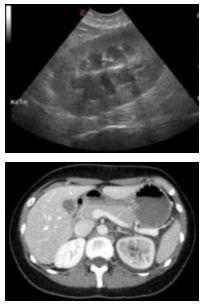
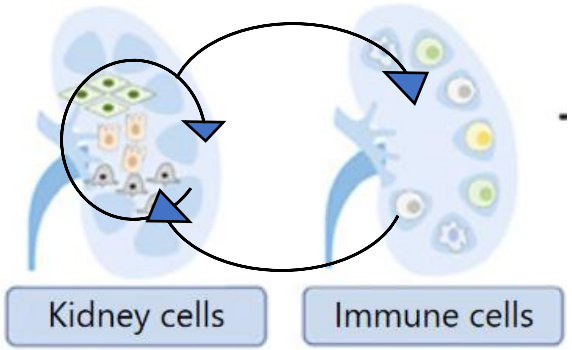
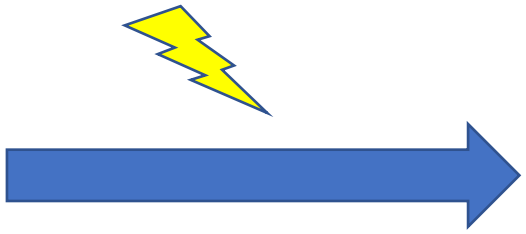


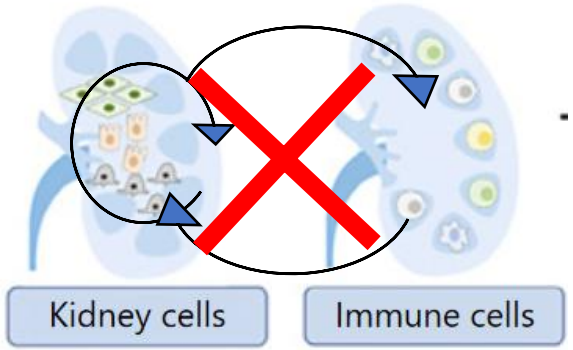
Anatomique macroscopique
Echographie
TDM
IRM

Moléculaires
Bulk RNA seq

Introduction







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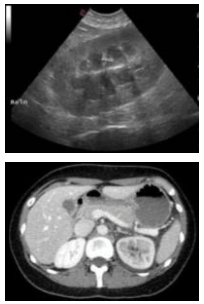

Anatomique macroscopique
Echographie
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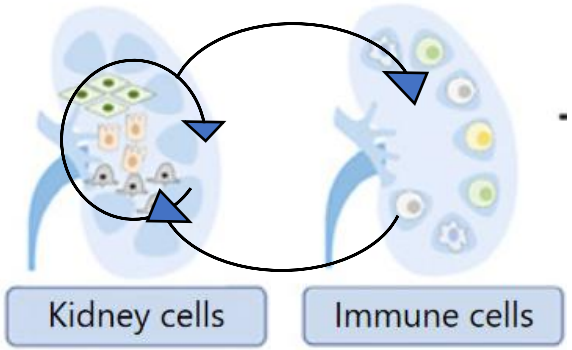
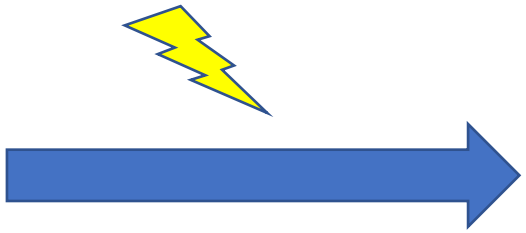
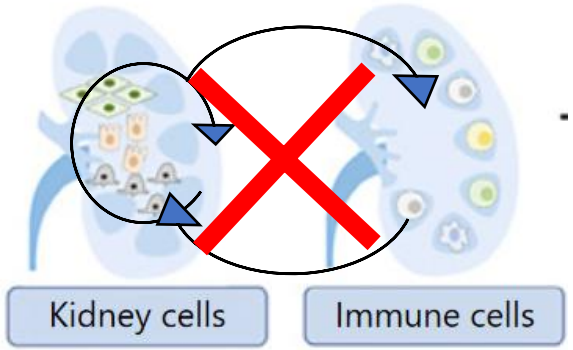
Moléculaires
Bulk RNA seq

Biomarqueurs Indirects
Créatinine
B2 microglob
Albuminurie
…

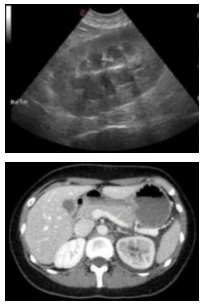
Introduction







Rein



Anatomique macroscopique
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Biomarqueurs Indirects

Créatinine
B2 microglob
Albuminurie
…

Cellule

Anatomique microscopique
Biopsie rénale

Single cell RNA seq

Médecine de Précision

KPMP

NIH National Institute of Diabetes and Digestive and Kidney Diseases

→ Passer à une **description moléculaire** des maladies

→ Les avancées dans la description de l'hétérogénéité différents constituants du rein sain sont indispensables pour la compréhension des états pathologiques

Reclasser les maladies rénales chroniques (MRC) en sous-groupes définis sur le plan moléculaire
qui reflètent les mécanismes sous-jacents de la maladie et pourraient
bénéficier d'interventions thérapeutiques spécifiques

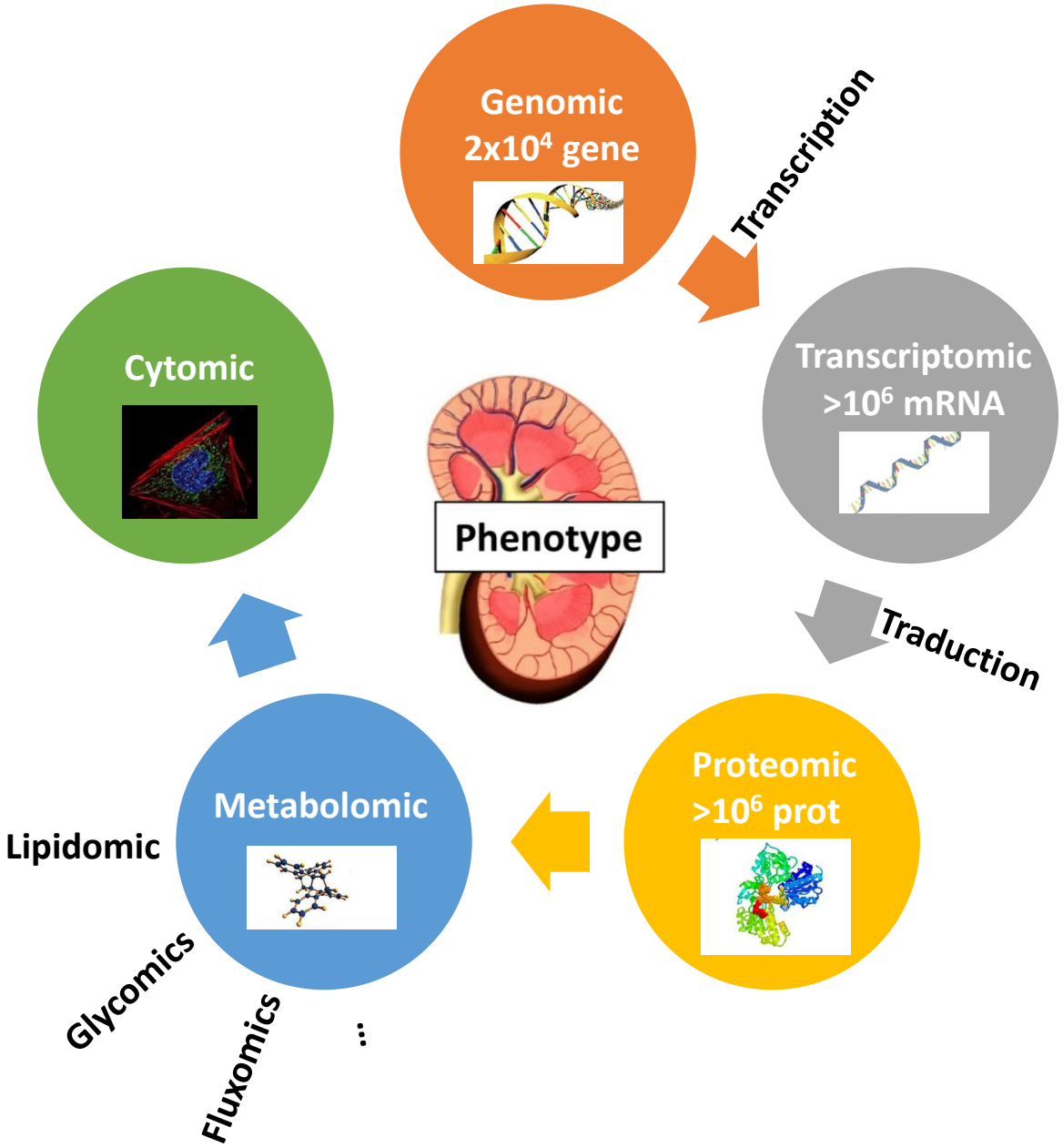
Création d'un atlas du rein

Définition des sous groupes de maladies

Identification des cellules pivots dans le développement des maladies

Identification de nouveaux pathways

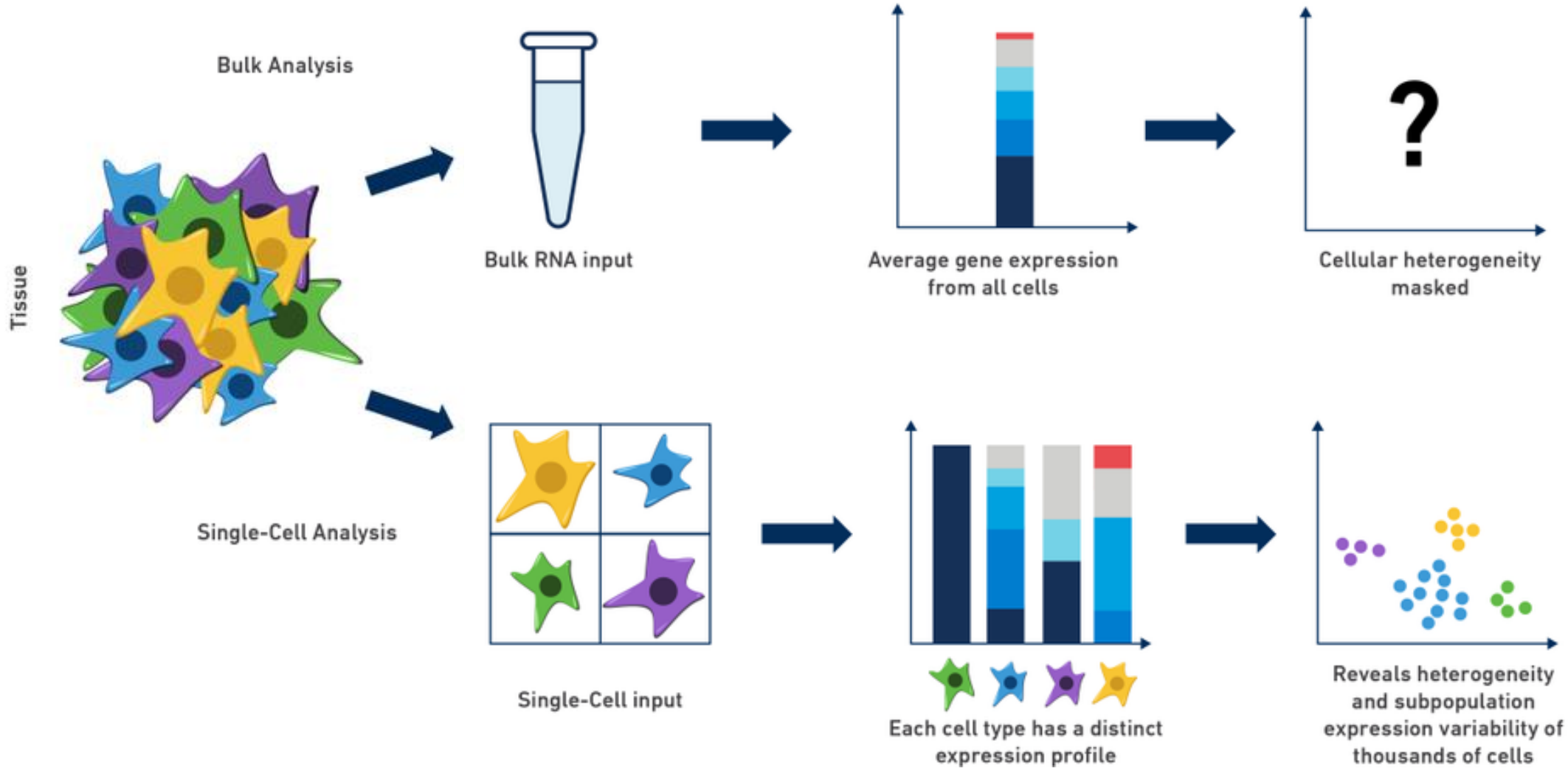
Identification de nouvelles cibles thérapeutiques



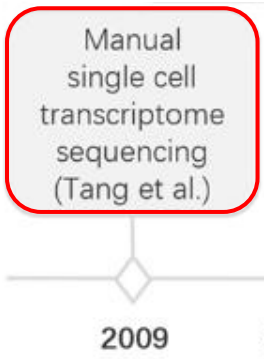
Single cell RNA sequencing
Bulk RNA sequencing

From Rhee et al. AJKD 2018

Bulk RNAseq ou Single cell RNAseq

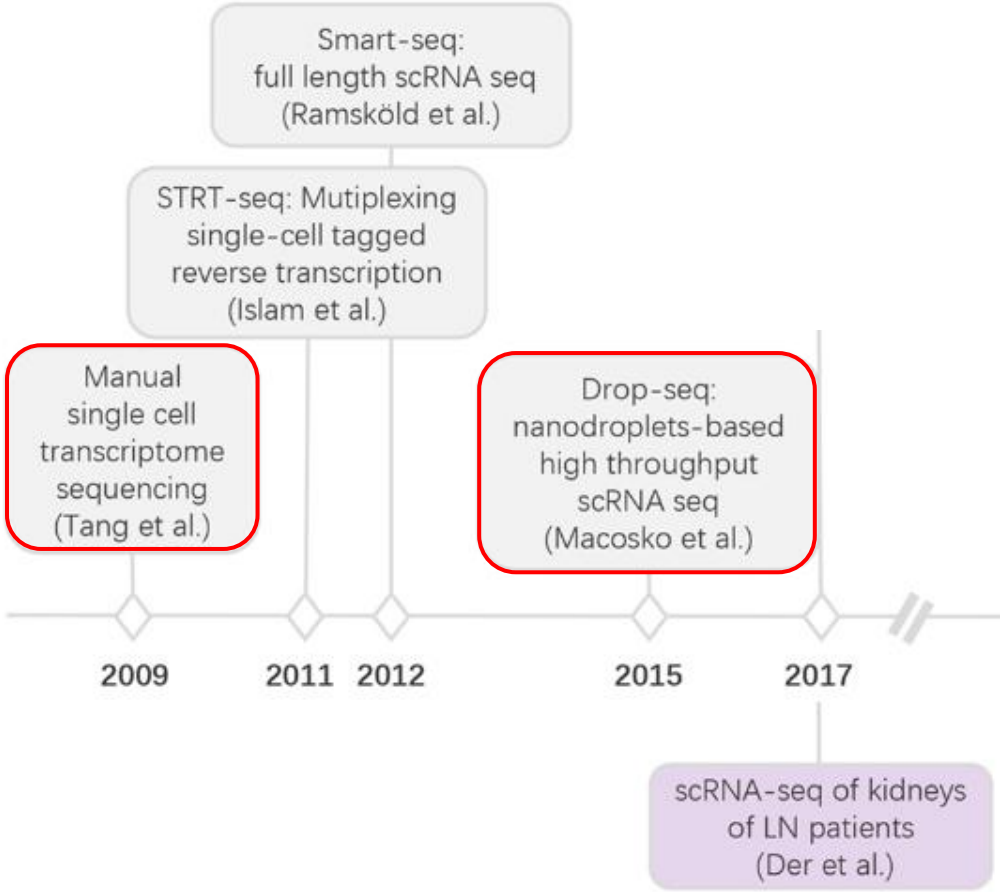


Historique du Single cell RNAseq



1 cellule

Adapted from Svensson et al. Zheng et al.



1 cellule

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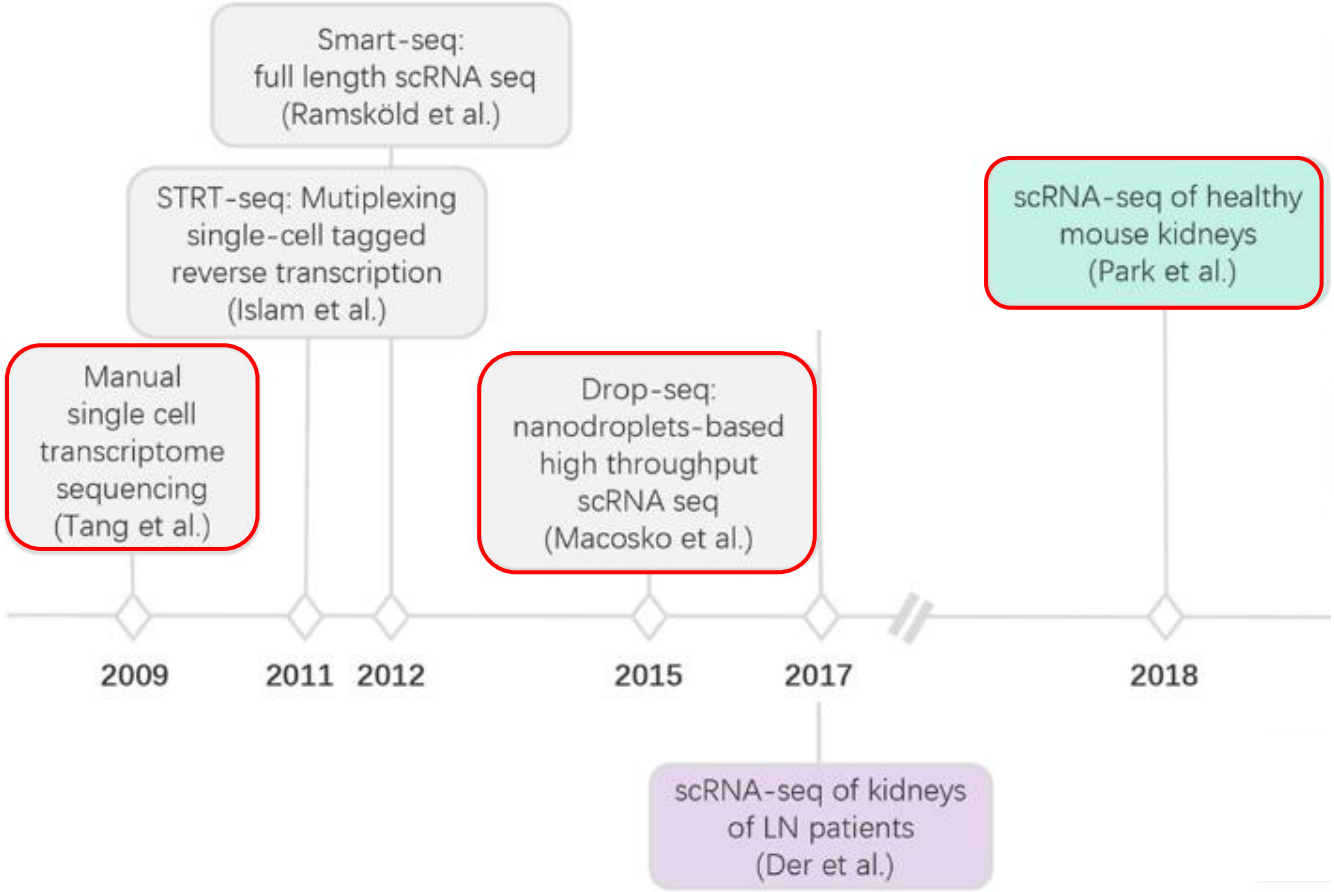

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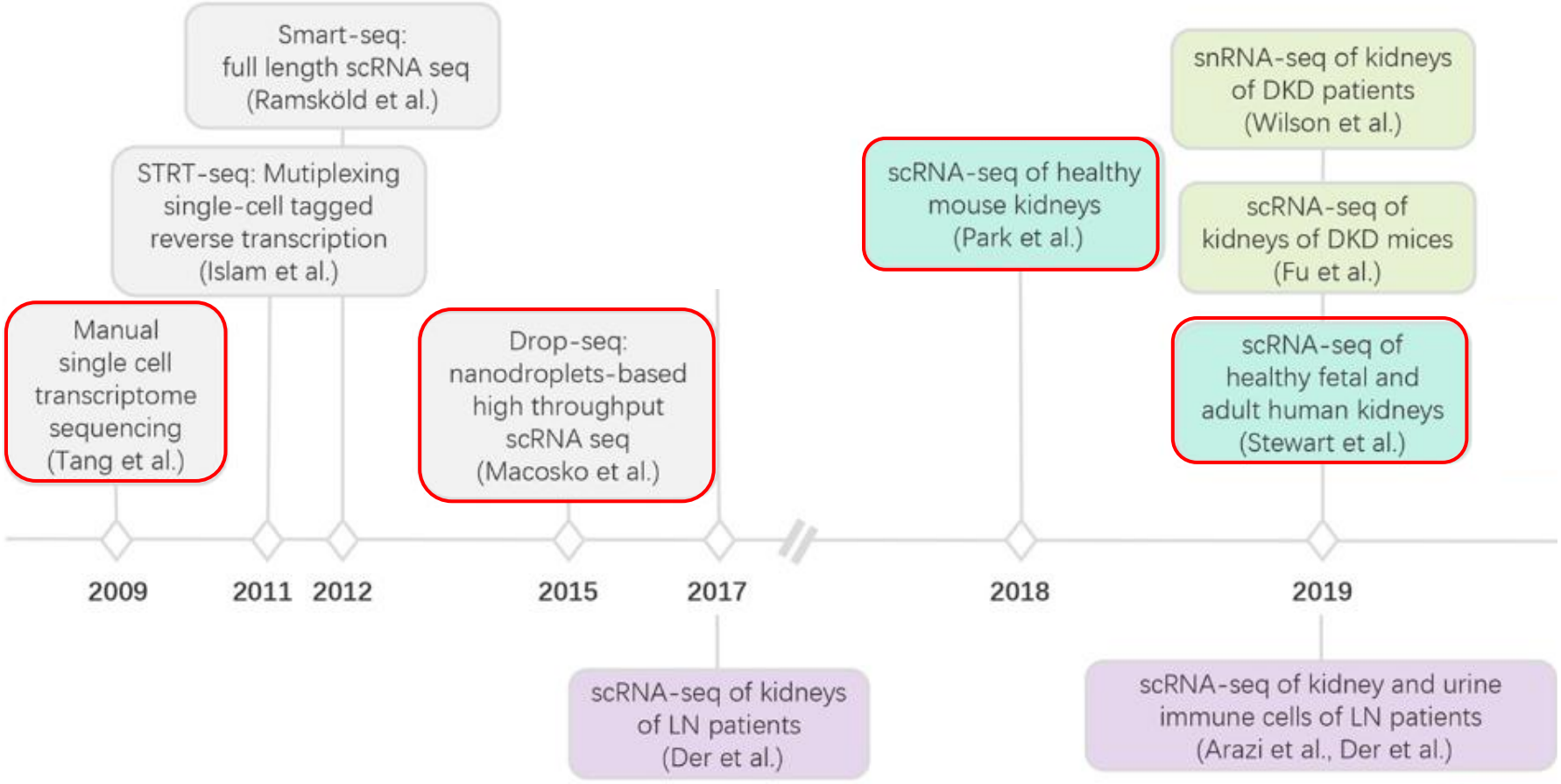
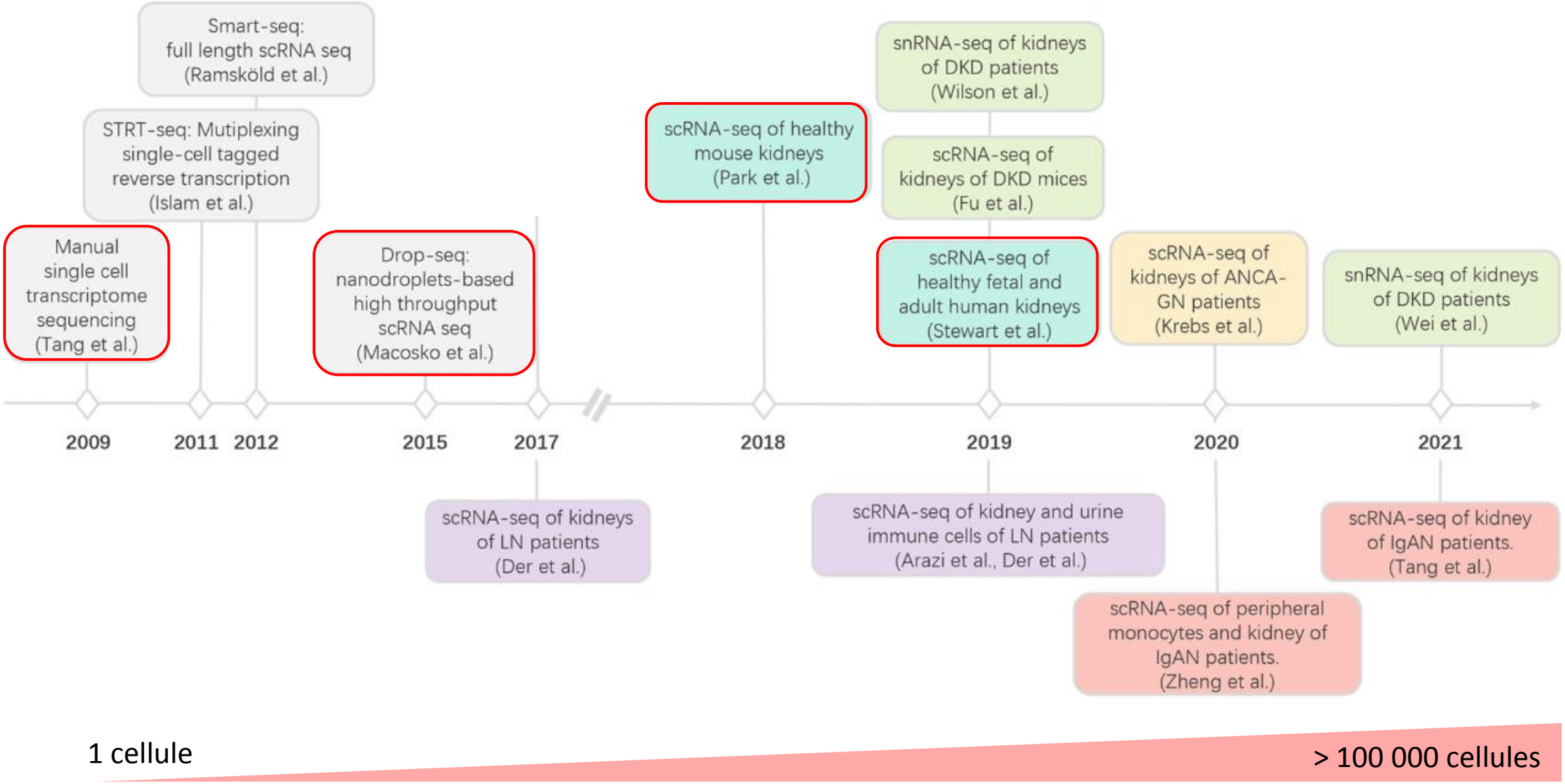


1 cellule

Adapted from Svensson et al. Zheng et al.

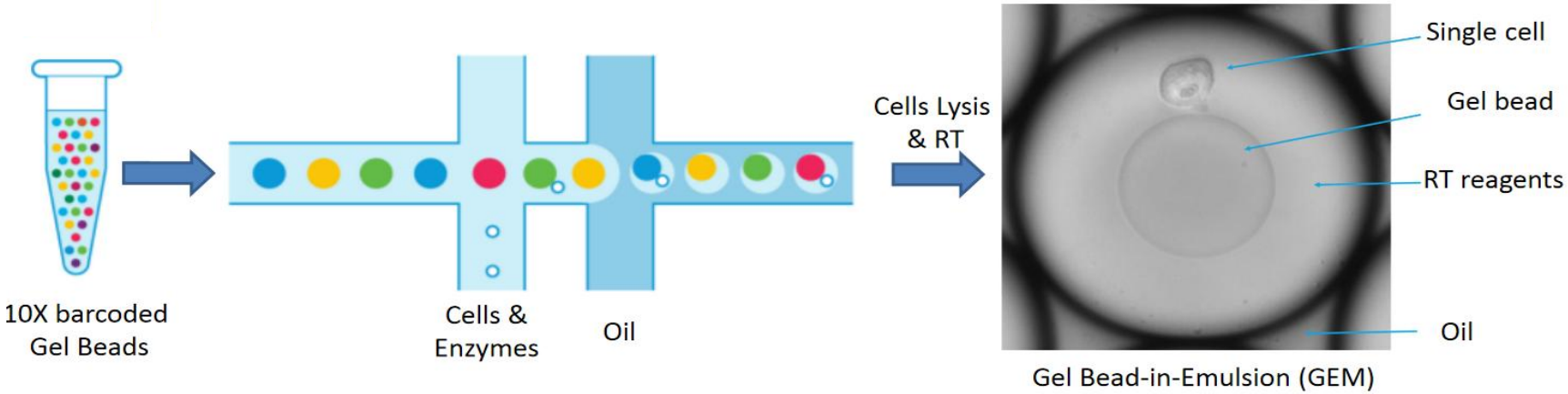


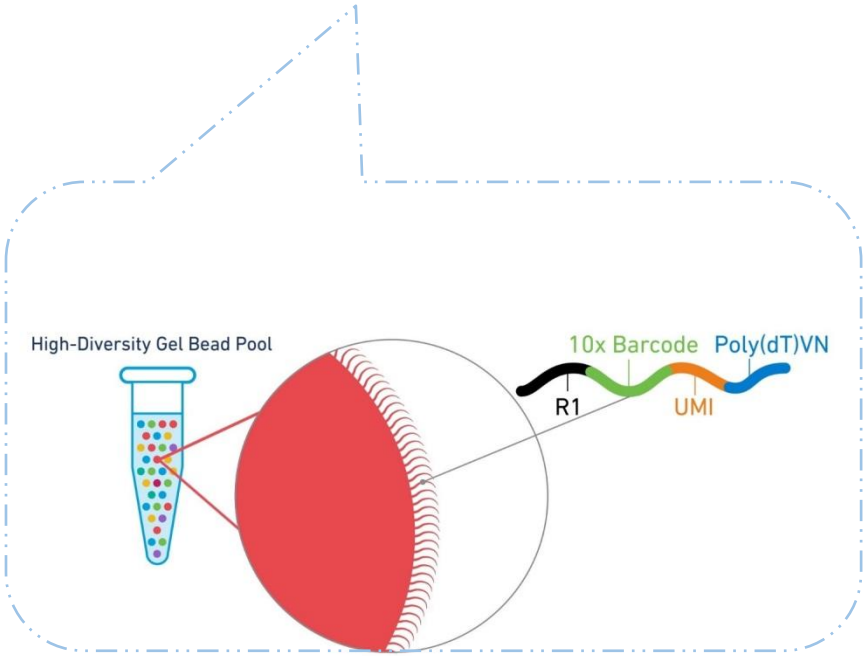
1 cellule

> 100 000 cellules

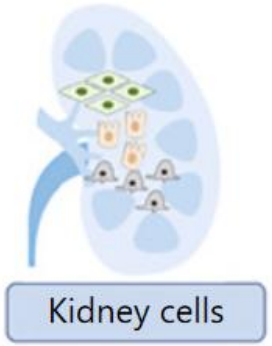
Adapted from Svensson et al. Zheng et al.

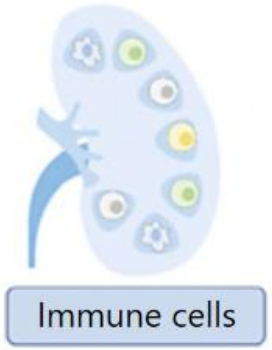
Principes du single cell RNA sequencing (10X genomics)



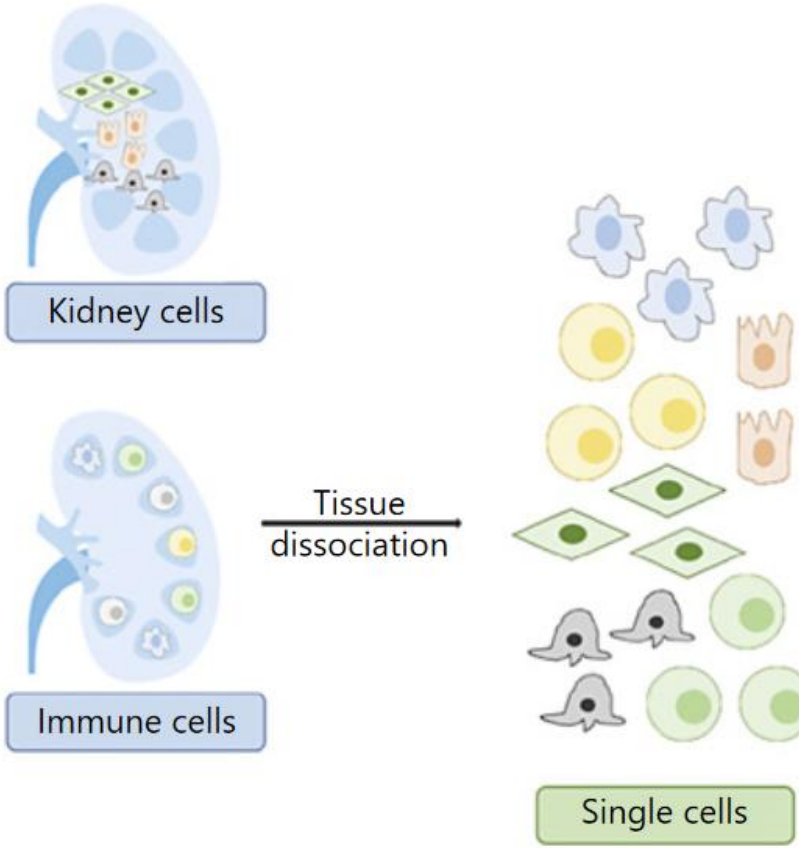


Objectifs du single cell RNA sequencing





Objectifs du single cell RNA sequencing



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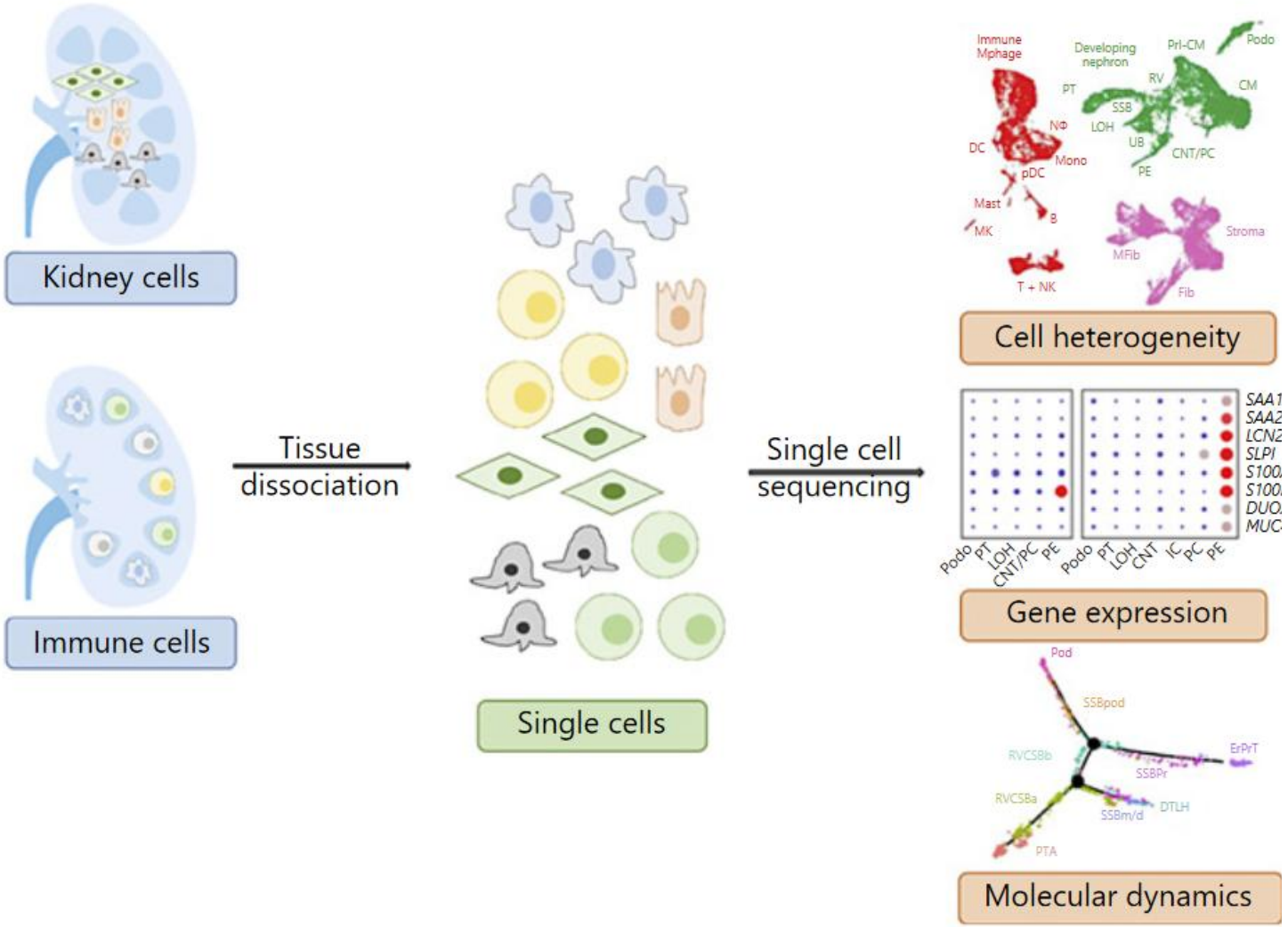
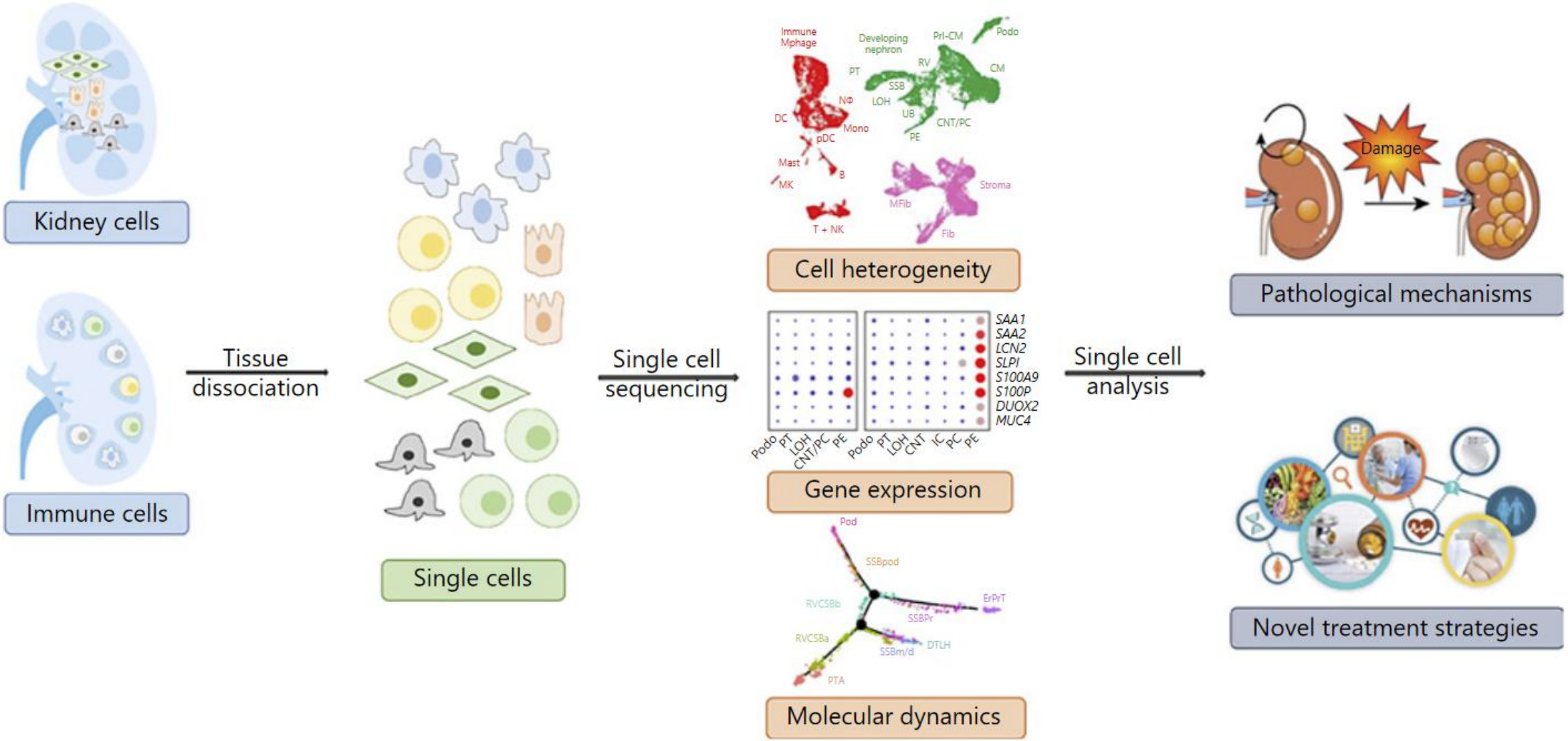


Objectifs du single cell RNA sequencing

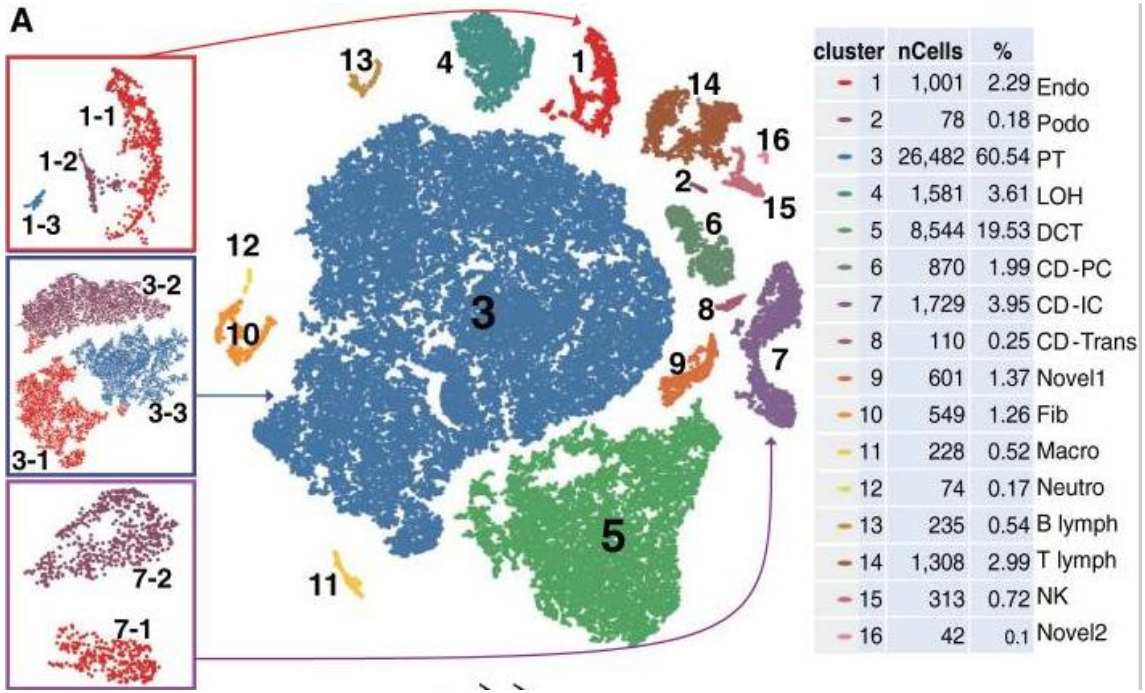


Single-cell transcriptomics of the mouse kidney reveals potential cellular targets of kidney disease

JIHWAN PARK, ROJESH SHRESTHA, CHENGXIANG QIU, AYANO KONDO, SHIZHENG HUANG, MAX WERTH, MINGYAO LI, JONATHAN BARASCH, AND KATALIN SUSZTÁK Authors Info & Affiliations

Science AAAS

Atlas des cellules rénales pour essayer de caractériser le comportement moléculaire de chaque sous-type cellulaires



cluster	nCells	%	
1	1,001	2.29	Endo
2	78	0.18	Podo
3	26,482	60.54	PT
4	1,581	3.61	LOH
5	8,544	19.53	DCT
6	870	1.99	CD-PC
7	1,729	3.95	CD-IC
8	110	0.25	CD-Trans
9	601	1.37	Novel1
10	549	1.26	Fib
11	228	0.52	Macro
12	74	0.17	Neutro
13	235	0.54	B lymph
14	1,308	2.99	T lymph
15	313	0.72	NK
16	42	0.1	Novel2

57 979 cellules rénales

7 souris mâles

Identification de 16 clusters cellulaires

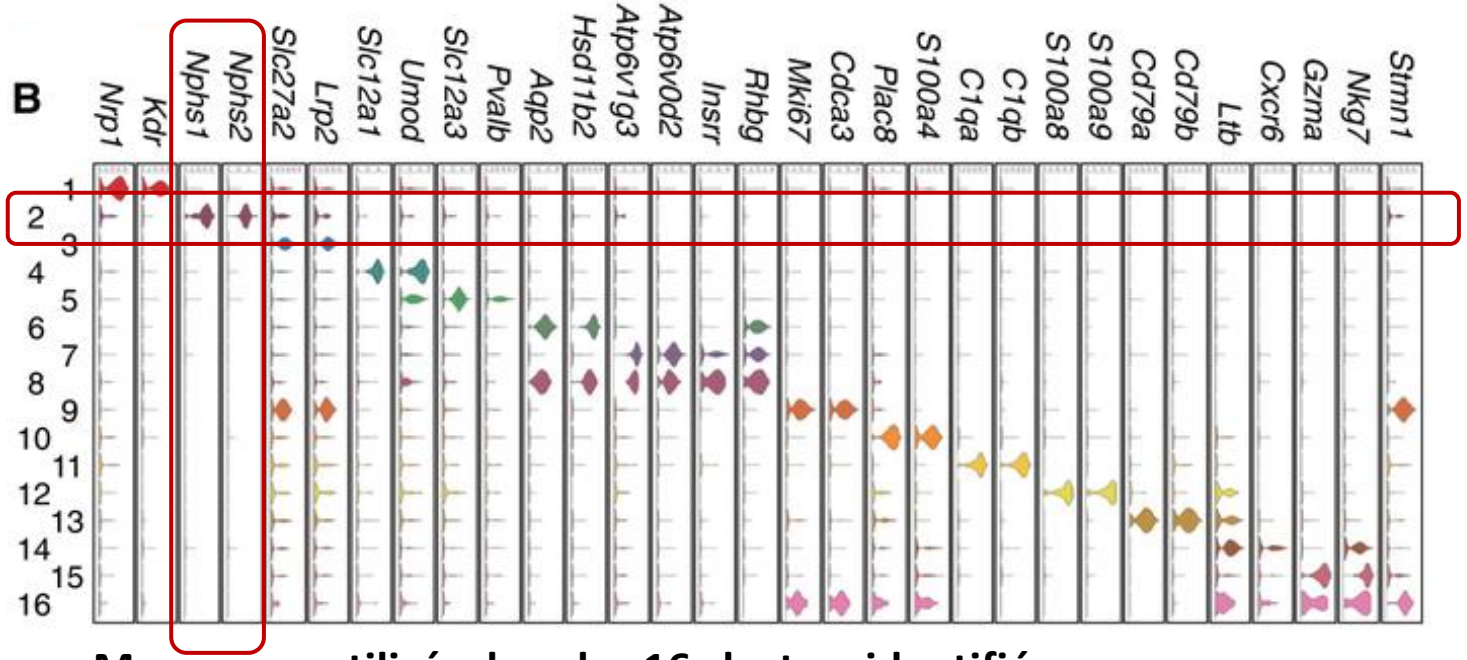
2 nouveaux cluster

Max > 26 000 cellules : tubule proximal

Min 42 cellules (Cellules non identifiées)

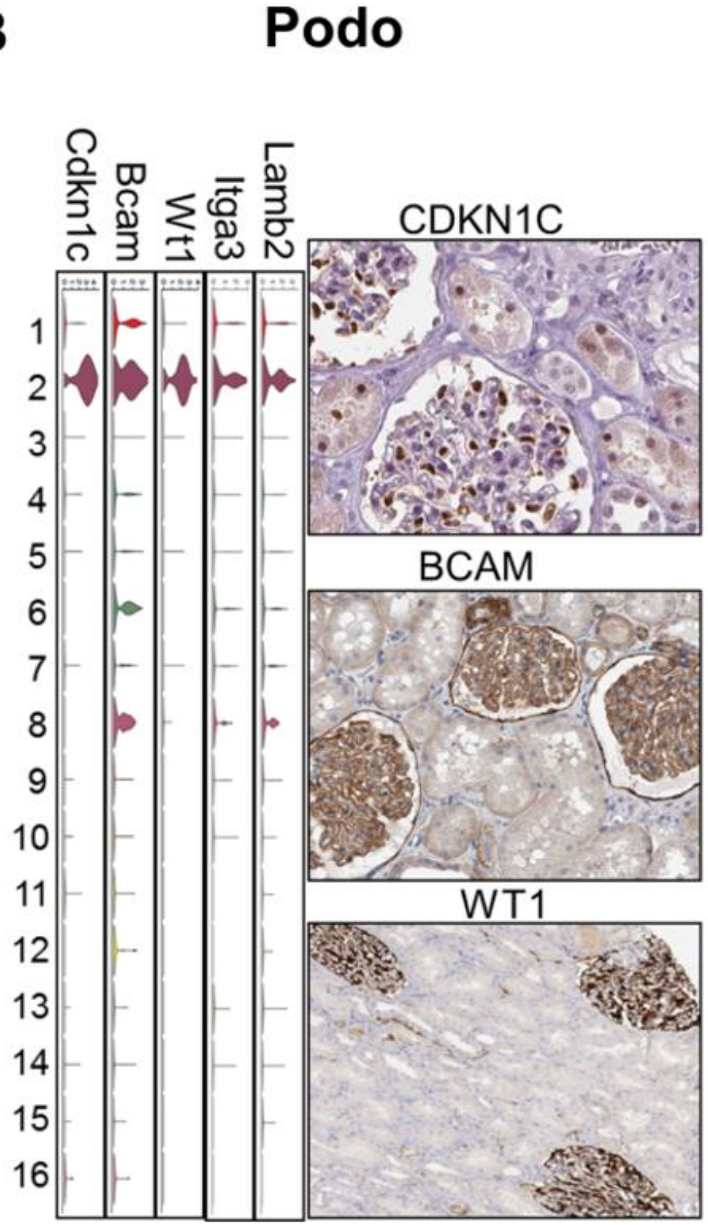
2018 : Park et al Science

Mise en évidence de nouveaux marqueurs cellulaires : exemple du podocyte



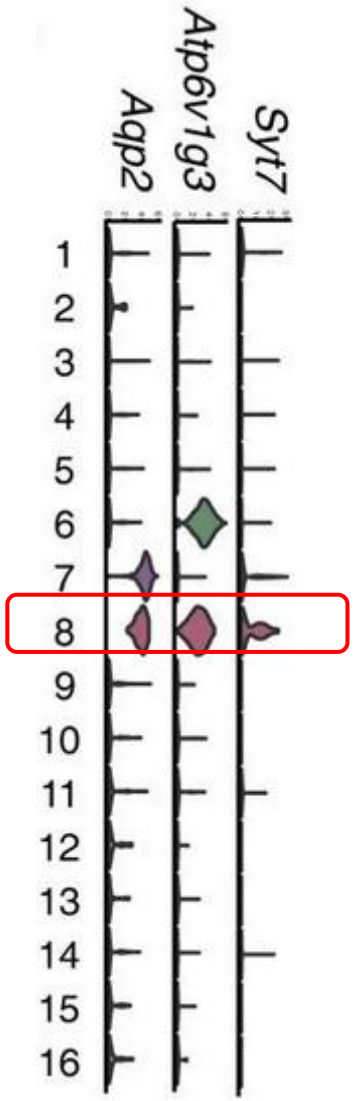
Marqueurs utilisés dans les 16 clusters identifiés

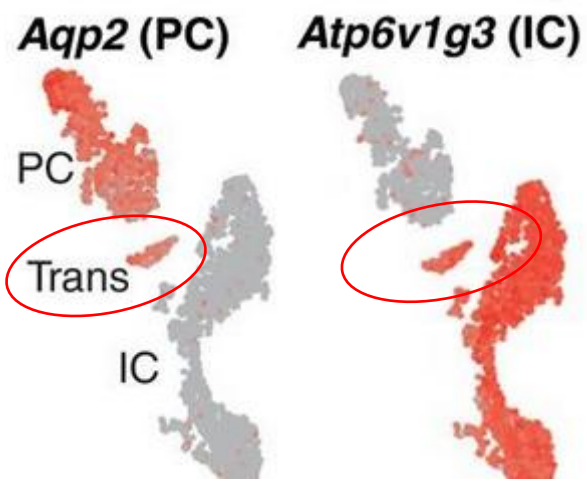
Identification de nouveaux marqueurs spécifiques : *Bcam* et *Cdkn1c* dans les podocytes

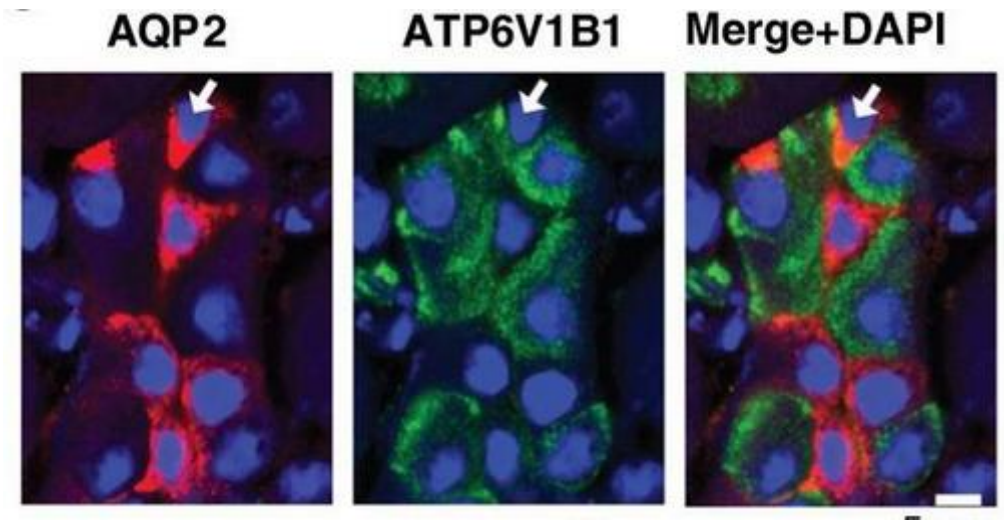


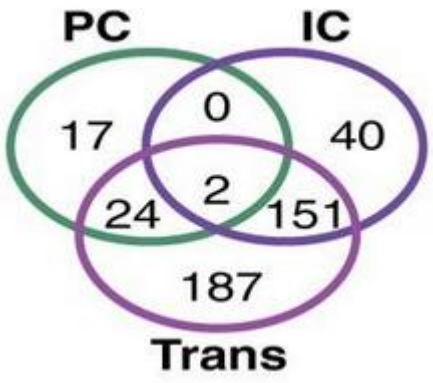
2018 : Park et al Science

Mise en évidence de nouveaux types cellulaires : exemple des cellules du tube collecteur









Cellules tansitionnelles exprimant les marqueurs des PC et des cellules intercallaires

Transcriptome croisé entre CP et CI

2018 : Park et al Science

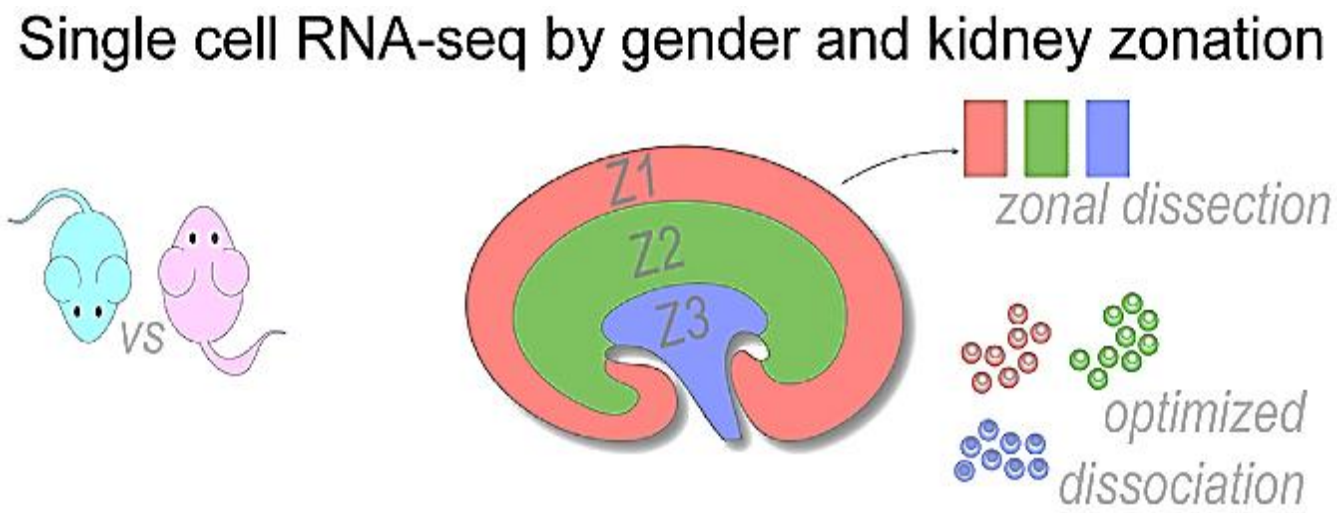
Single-Cell Profiling Reveals Sex, Lineage, and Regional Diversity in the Mouse Kidney

Developmental Cell
Resource

Microdissection rein souris males et femelles

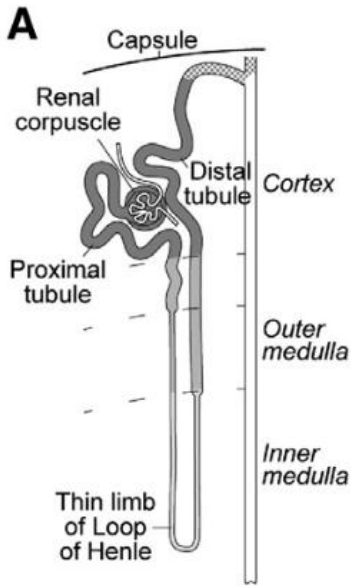
Single-cell « anatomique »

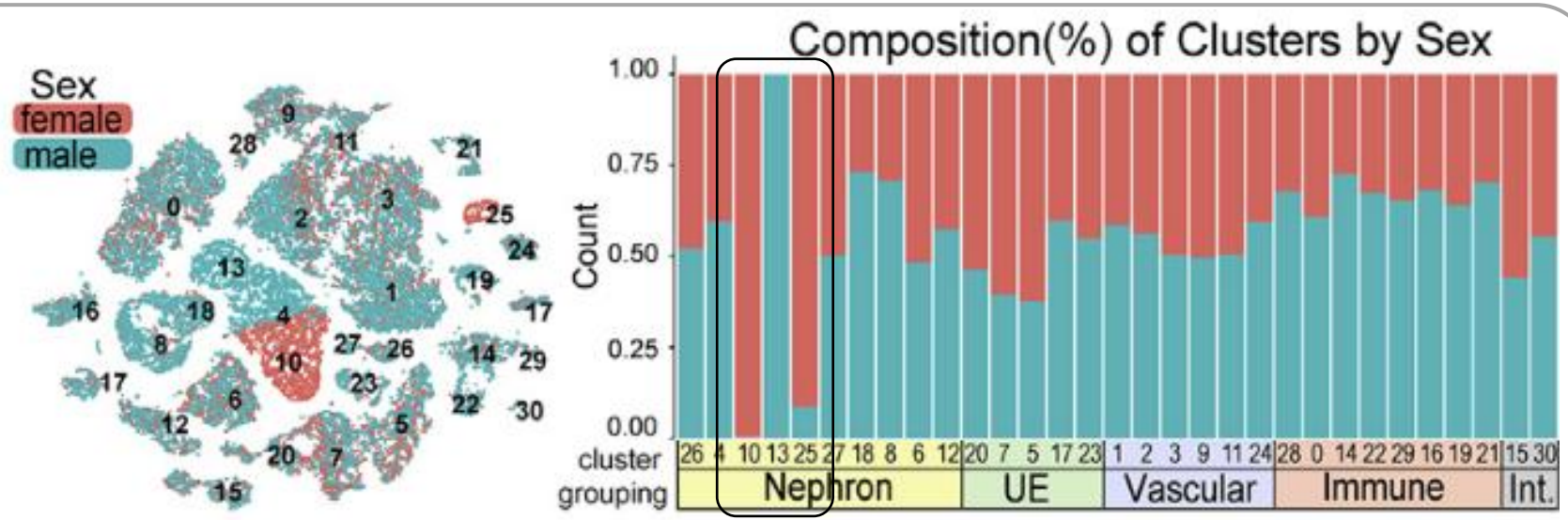
31 265 cellules



Ranswick et al. Dev Cell. 2019

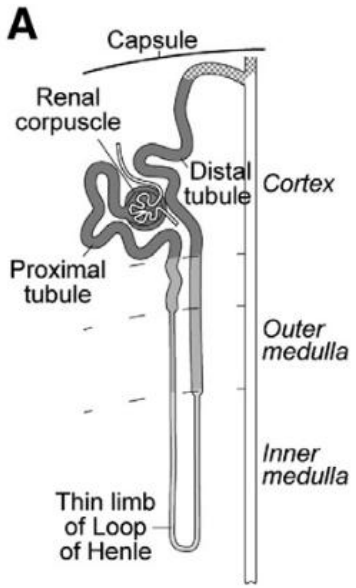
Types cellulaires spécifiques de zones … et de sexe chez la souris

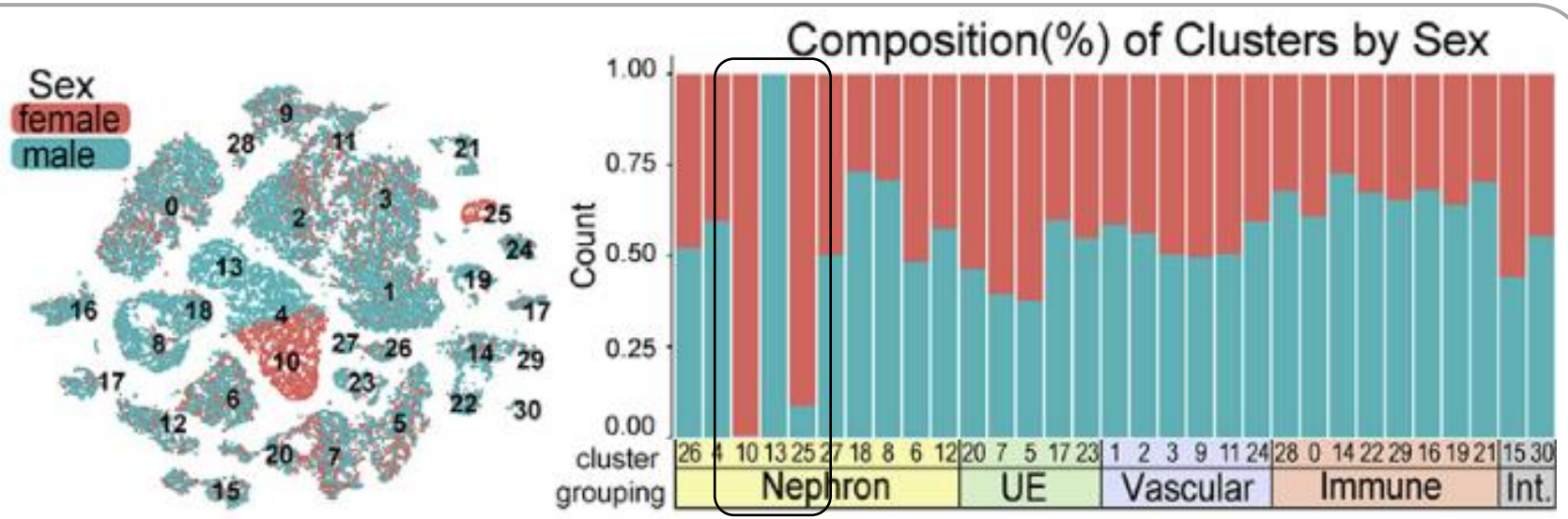




10 et 13 – cellules du tube proximale

Types cellulaires spécifiques de zones … et de sexe chez la souris

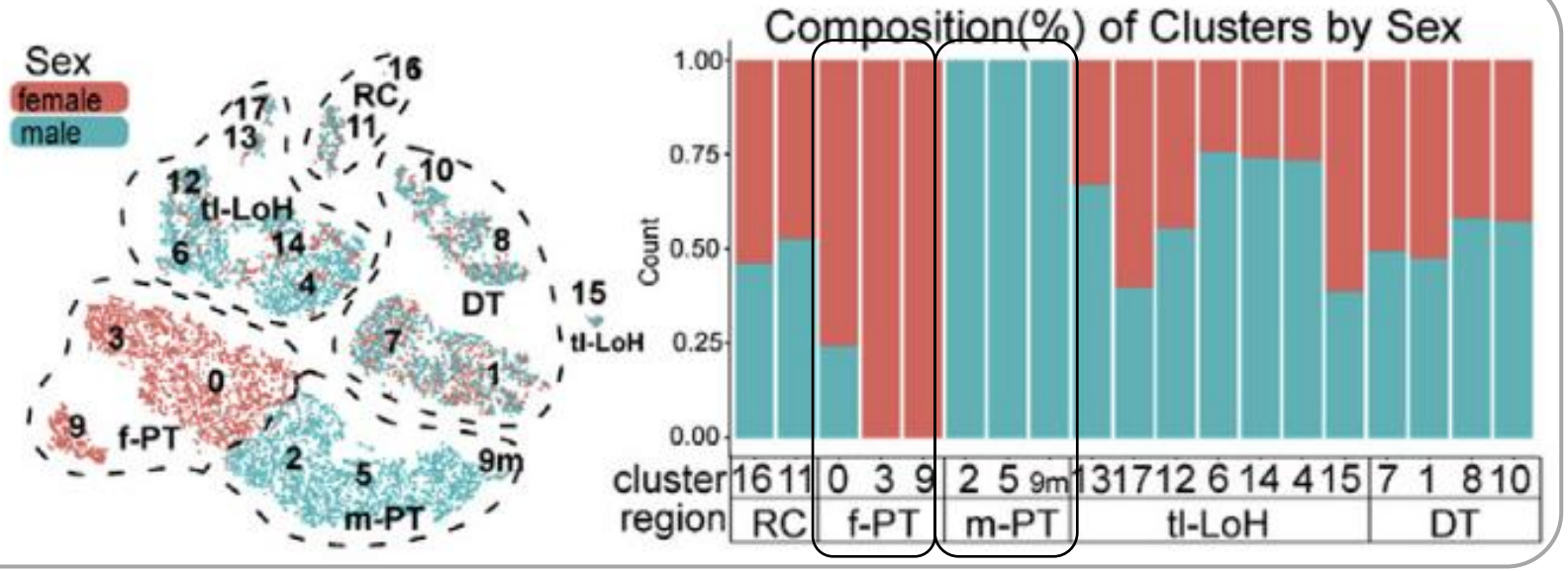




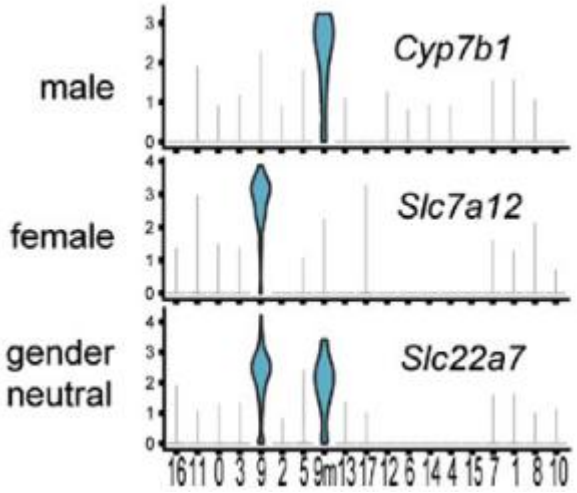
10 et 13 – cellules du tube proximale

Analyse ciblée sur le néphron

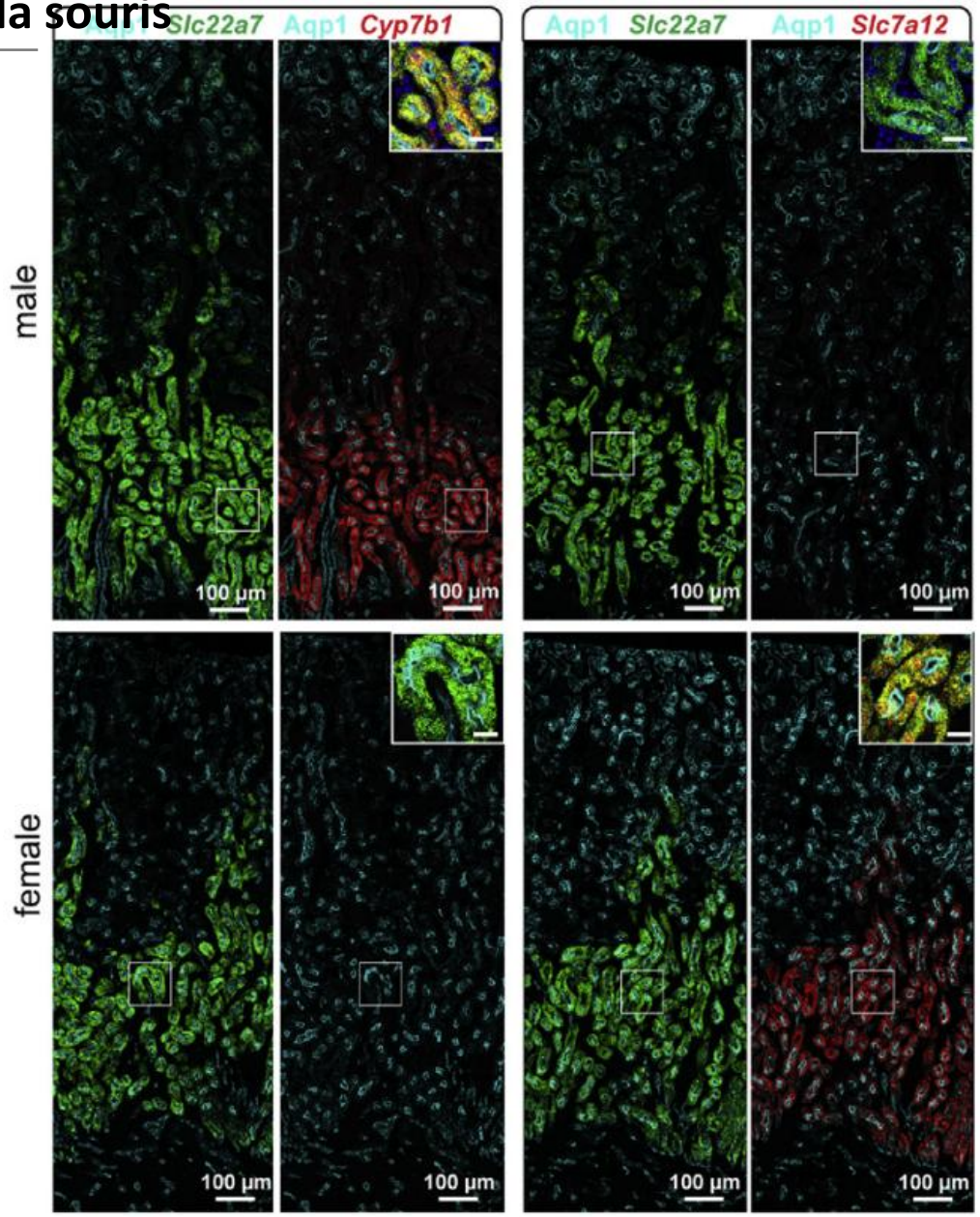
→ cellules tubulaires proximales mâles et femelles



Types cellulaires spécifiques de zones … et de sexe chez la souris



Slc22a7 = segment S3 du TP



Données non nouvelles : études sur rein entier ++
(Rinn et al., 2004, Si et al., 2009, Sabolić et al., 2007)

→ Augmentation du repertoire de genes differentiellement exprimés et du type celulaire

Région S3 particulièrement vulnérable aux lésions d'ischémie chez les males ++ (Park et al. 2004, Neugarten et al. 2018)

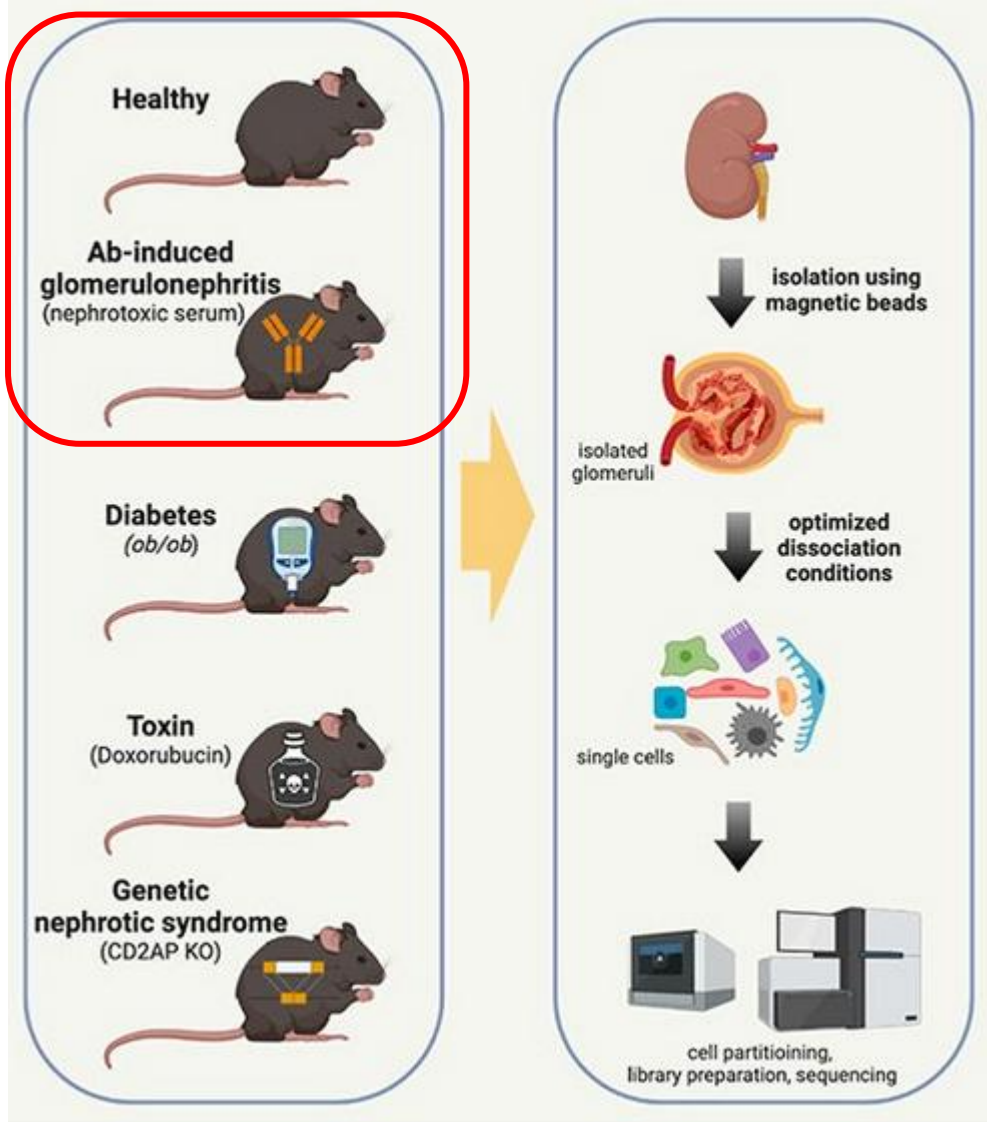
→ Explication par un contingent cellulaire différent ?

Single-Cell Transcriptome Profiling of the Kidney Glomerulus Identifies Key Cell Types and Reactions to Injury

Jun-Jae Chung, Leonard Goldstein, Ying-Jiun J. Chen, Jiyeon Lee, Joshua D. Webster, Merone Roose-Girma, Sharad C. Paudyal, Zora Modrusan, Anwesha Dey and Andrey S. Shaw

JASN October 2020, 31 (10) 2341-2354; DOI: https://doi.org/10.1681/ASN.2020020220

JASN
JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY



Exemple de la GN à Ac anti-MBG

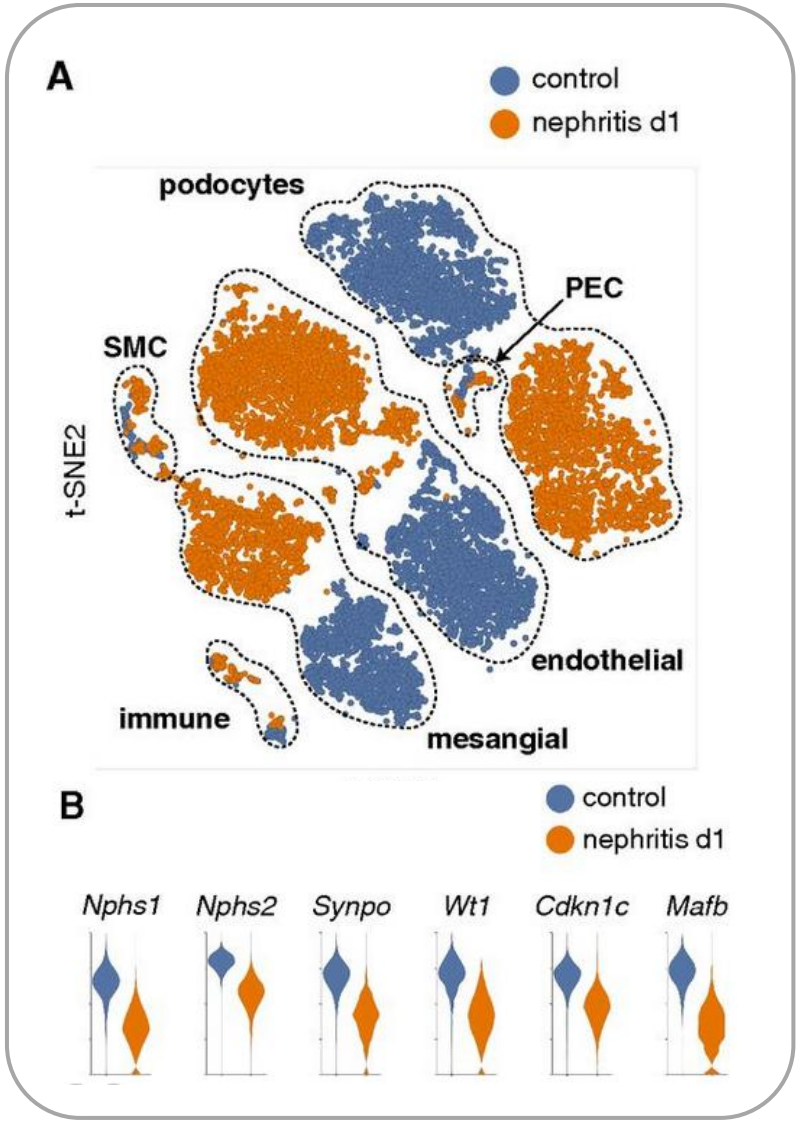
Injection de sérum contenant des Ac anti MBG

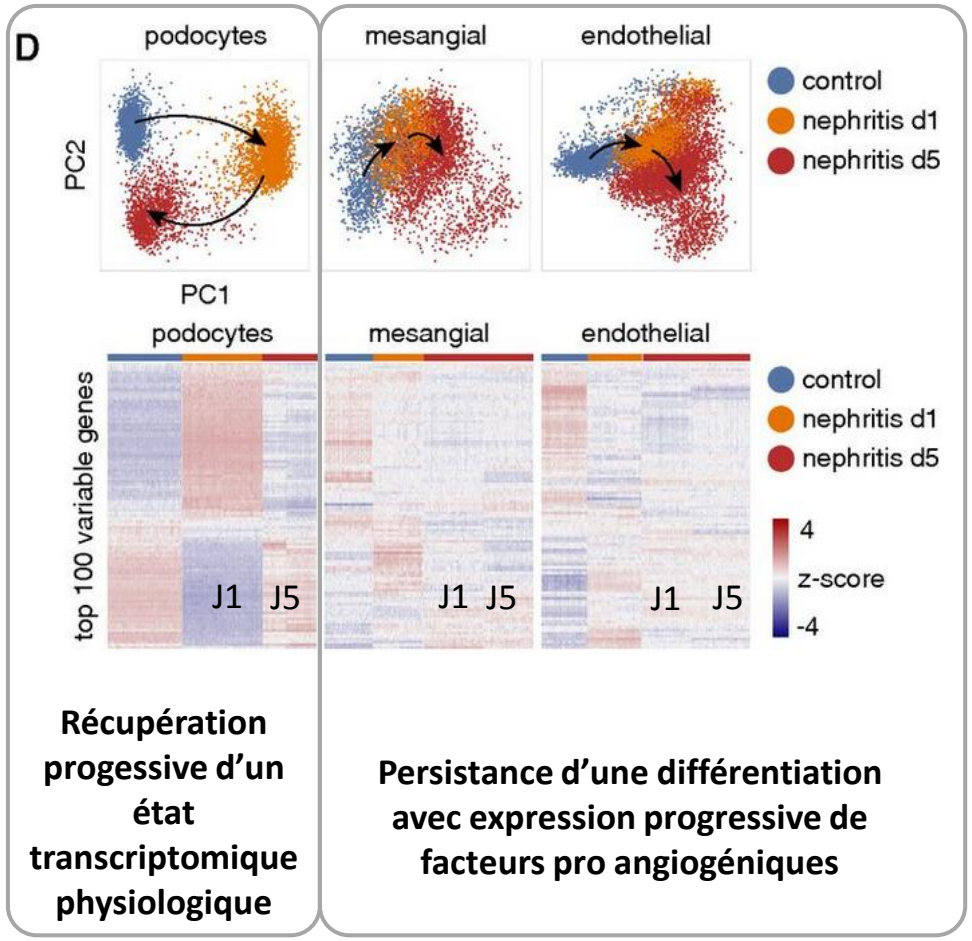
Développement d'une **protéinurie à J1**

Disparition de la protéinurie à J6

Développement progressif d'une **fibrose rénale**

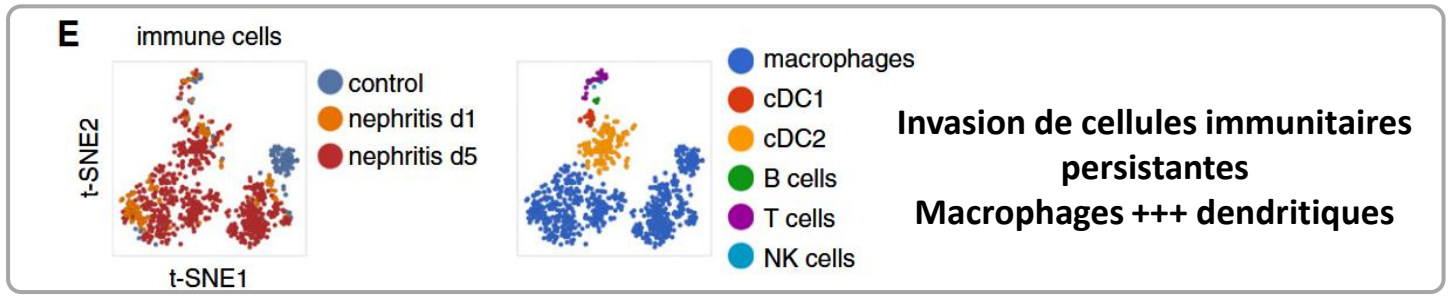
Modèle Ac anti-MBG : dérégulation des cellules glomérulaires après agression immunologique





Récupération progessive d'un état transcriptomique physiologique

Persistance d'une différentiation avec expression progressive de facteurs pro angiogéniques



Invasion de cellules immunitaires persistantes
Macrophages +++ dendritiques

The immune cell landscape in kidneys of patients with lupus nephritis

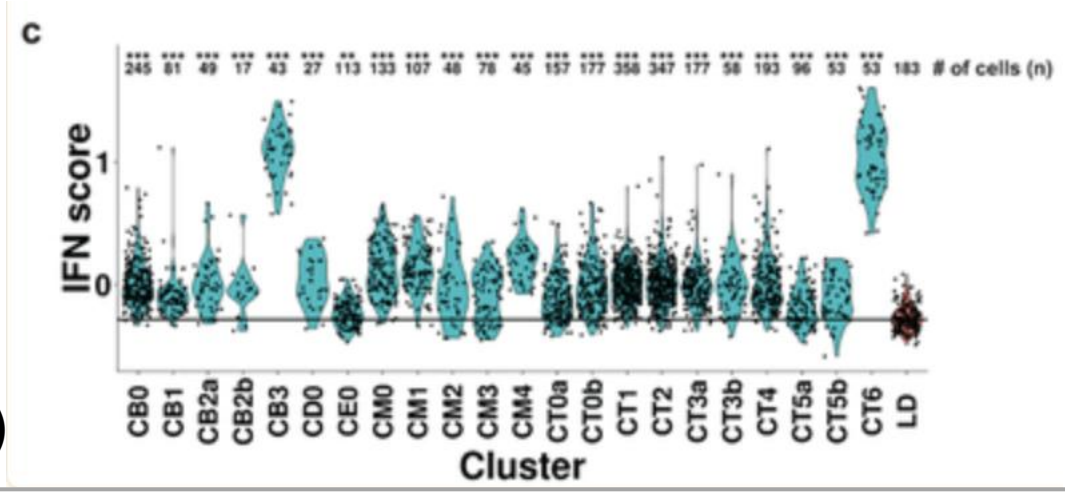
Arnon Arazi, Deepak A. Rao, ... the Accelerating Medicines Partnership in SLE network

+ Show authors

Nature Immunology **20**, 902–914 (2019) | Cite this article

21 types de cellules immunitaires différents chez les patients avec néphropathie lupique (sc-RNASeq du rein et des urines)

Signature interféron dans les cellules immunitaires rénales (taux ISG)



Single cell RNA sequencing to dissect the molecular heterogeneity in lupus nephritis

Evan Der,[1] Saritha Ranabothu,[2] Hemant Suryawanshi,[3] Kemal M. Akat,[3] Robert

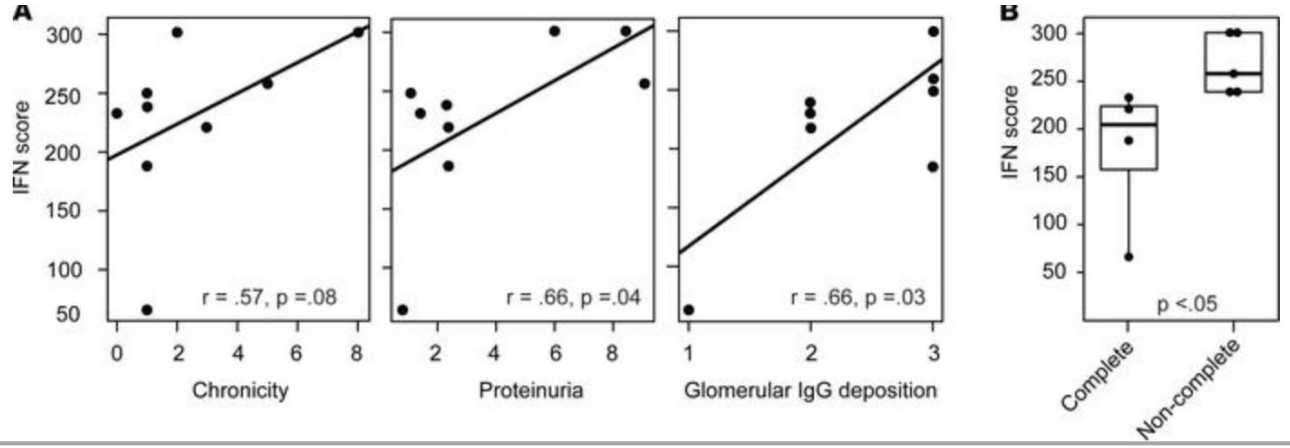
Cellules de biopsie rénale ou cutanée

Signature interferon dans les cellules epithéliales de patients avec néphropathie lupique

Corrélation avec la progression de la maladie et la réponse au traitement

Tubular cell and keratinocyte single-cell transcriptomics applied to lupus nephritis reveal type I IFN and fibrosis relevant pathways

Evan Der, Hemant Suryawanshi, ... the Accelerating Medicines Partnership Rheumatoid Arthritis and



Single-cell RNA sequencing et FSGS

> JCI Insight. 2020 Mar 26;5(6):e133267. doi: 10.1172/jci.insight.133267.

Single cell transcriptomics identifies focal segmental glomerulosclerosis remission endothelial biomarker

Rajasree Menon [1], Edgar A Otto [1], Paul Hoover [2], Sean Eddy [1], Laura Mariani [1],

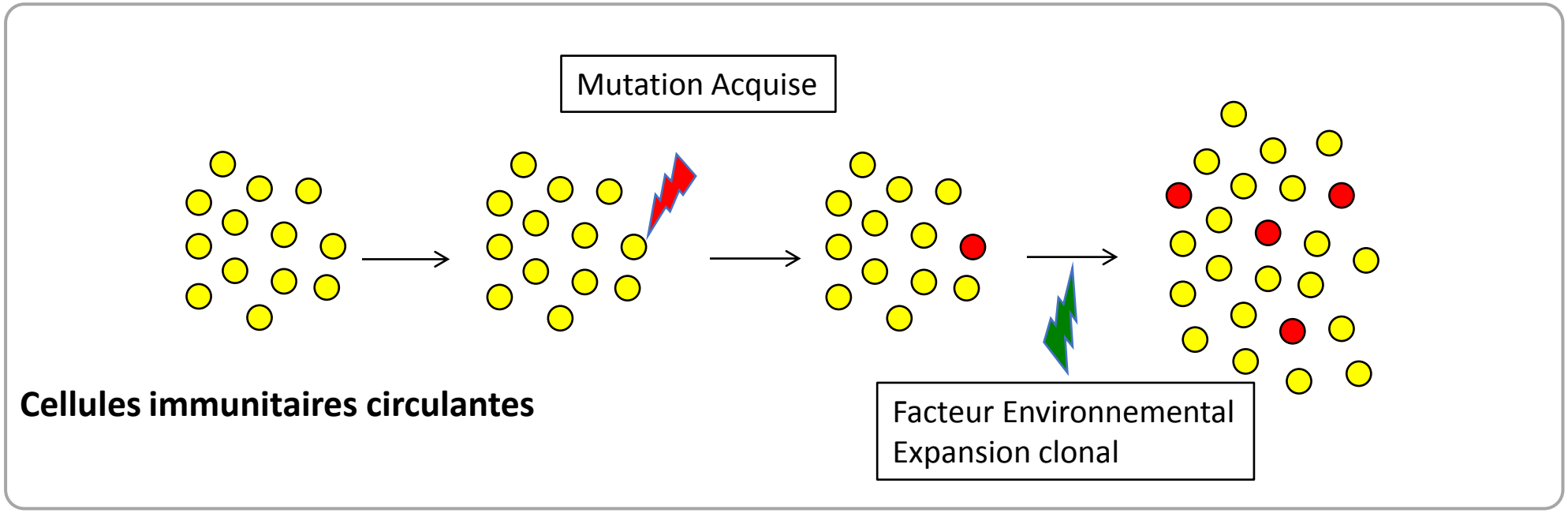
→ a-2 macroglobuline est un facteur prédictif de mauvaise progression de la FSGS

Urine single cell RNA-sequencing in focal segmental glomerulosclerosis reveals inflammatory signatures in immune cells and podocytes

Khun Zaw Latt, Jurgen Heymann, Joseph H. Jessee, Avi Z. Rosenberg, Celine C. Berthier, Sean Eddy,
Teruhiko Yoshida, Yongmei Zhao, Vicky Chen, George W. Nelson, Margaret Cam, Parimal Kumar,
Monika Mehta, Michael C. Kelly, Matthias Kretzler, The Nephrotic Syndrome Study Network (NEPTUNE),
The Accelerating Medicines Partnership in Rheumatoid Arthritis and Systemic Lupus Erythematosus (AMP RA/SLE)
A. Winkler, Jeffrey B. Kopp

→ Transition epithélio-mésenchymateuse dans les biopsies FSGS > MCD

Single-cell RNA sequencing et SNI : hypothèse du double hit



Isolation et caractériser une population clonale +++++

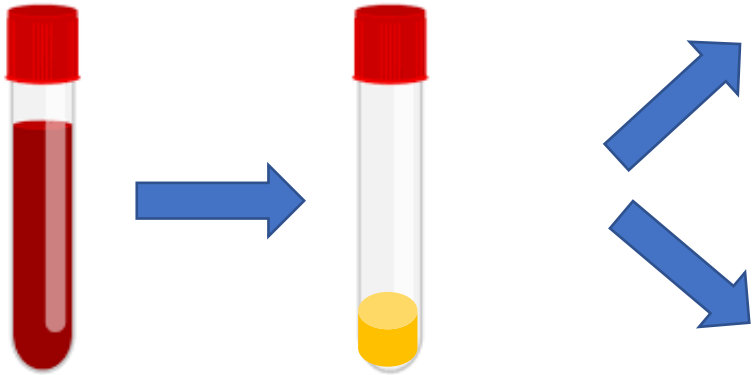
Auto-immune lymphoproliferative syndrome (ALPS)

Hauck et al. *Clin Immuno* 2013

Single-cell RNA sequencing et syndrome néphrotique

5 patients pédiatriques – SNI avant traitement
3 contrôles pédiatriques – prélevés au même moment – pas de traitement IS

} Single cell 10X genomics



PBMC

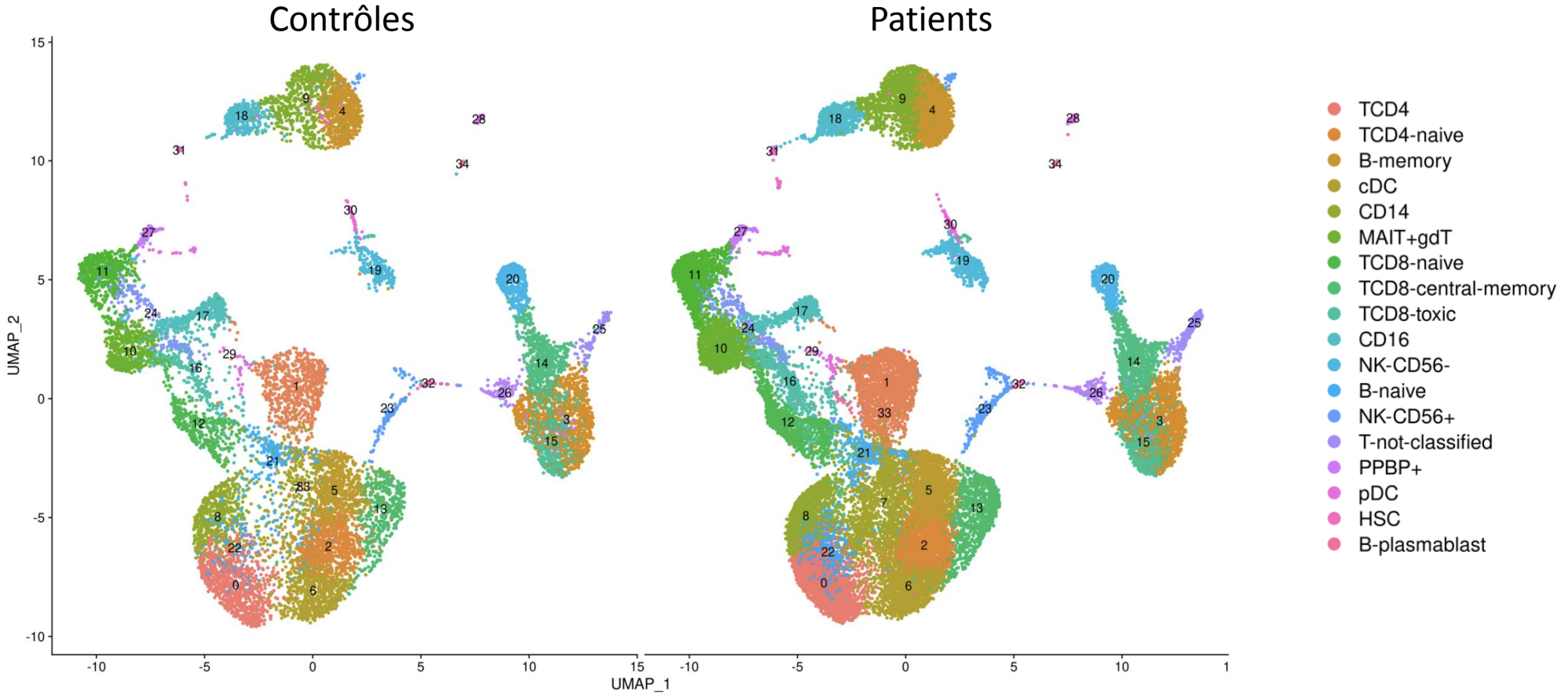
Single-cell transcriptomics

Flow Cytometry 48 couleurs
3 panels (T – Treg – B cells)

Single-cell RNA sequencing et syndrome néphrotique

5 patients pédiatriques – SNI avant traitement
3 contrôles pédiatriques – prélevé au même moment – pas de traitement IS

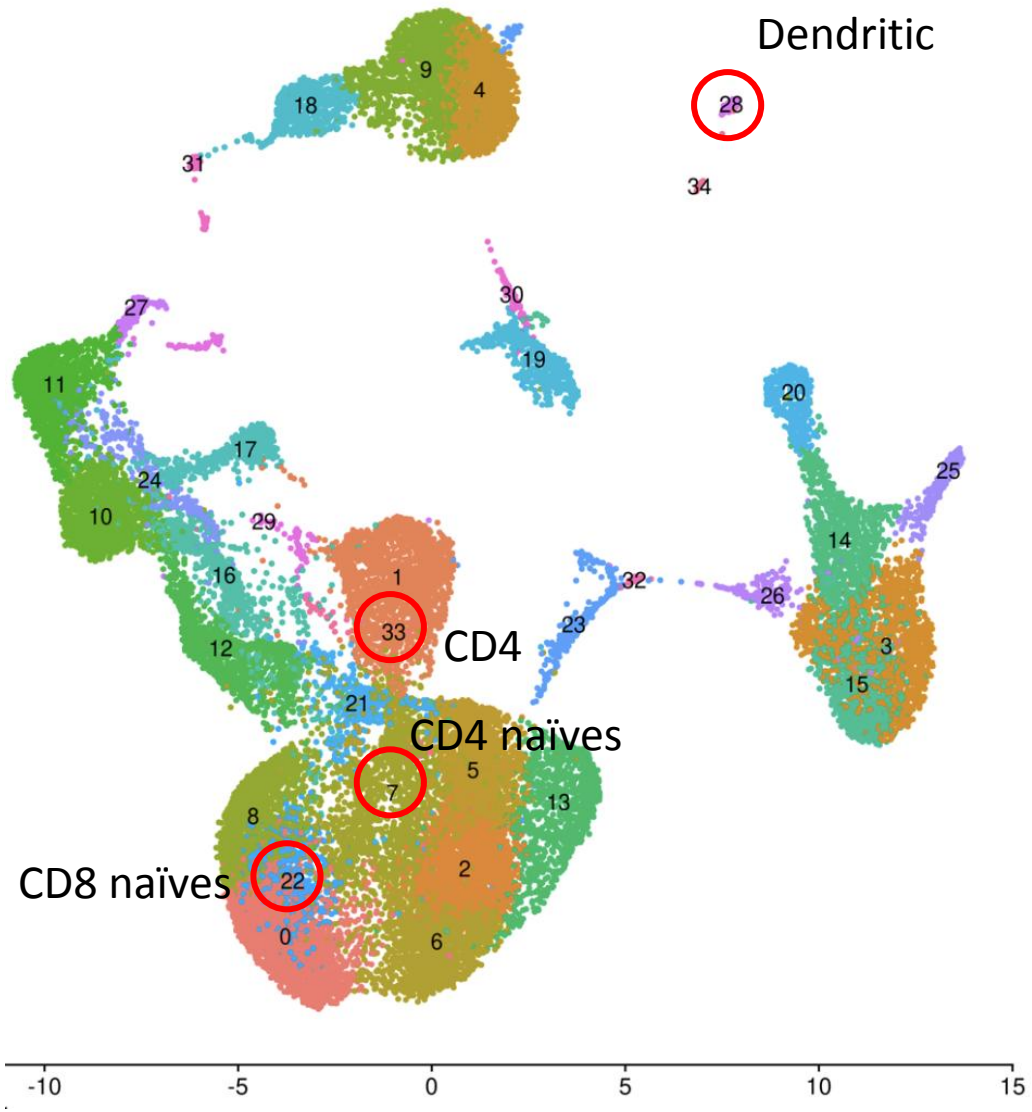
} Single cell 10X genomics

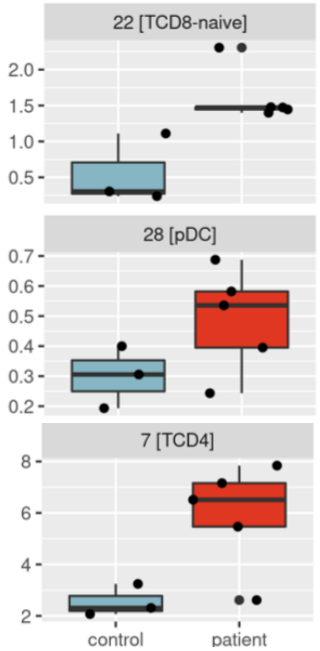


UMAP superposables – Pas de population cellulaire différente

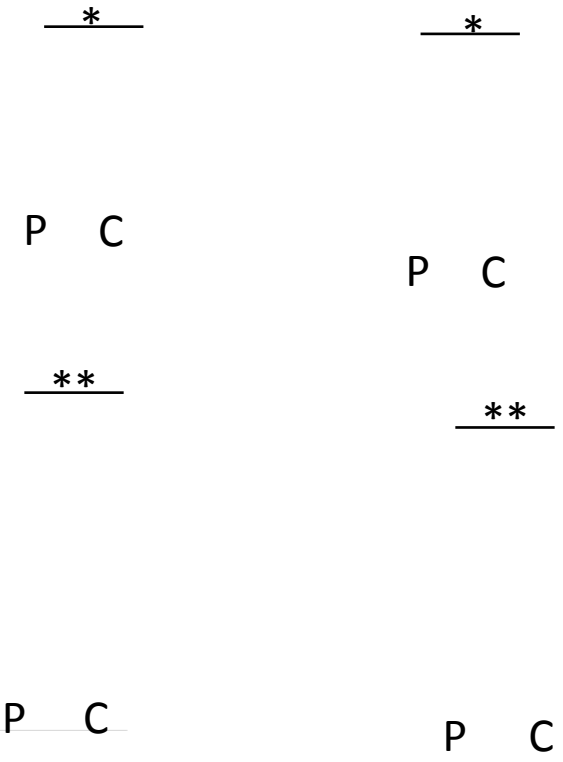
Single-cell RNA sequencing et syndrome néphrotique

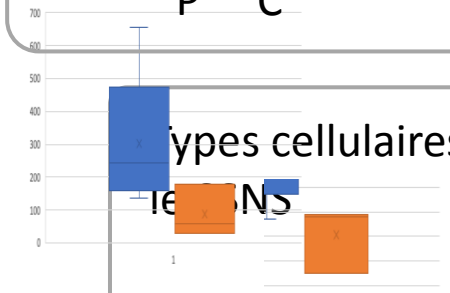
Single-cell UMAP





FACS





ypes cellulaires déjà incriminés dans
le SNS

Caractérisation des gènes différentiellement exprimés +++

Signature unicellulaire ?

Conclusions – Single cell RNA sequencing dans les maladies rénales

Nouvelle technologie qui offre des perspectives majeures

→ de compréhension de la physio(patho)logie
→ de détection de biomarqueurs des maladies rénales
→ de détection de cibles thérapeutiques

Attention

→ Interprétation délicate des résultats
→Risque de « biais » important +++
→ interprétation faussée des résultats

Remerciements

Laboratoire des maladies rénales héréditaires

Corinne Antignac
Sophie Saunier
Alexandre Benmerah
Géraldine Mollet
Marceau Quatredenniers
…

Plateforme de single cell analysis

Mickael Ménager

Plateforme de génomique

Christine Bole

MERCI POUR VOTRE ATTENTION