How Digital pathology can help to speed-up Biomarkers detection in Pharma Research ?

10th Digital Pathology & AI congress : Europe

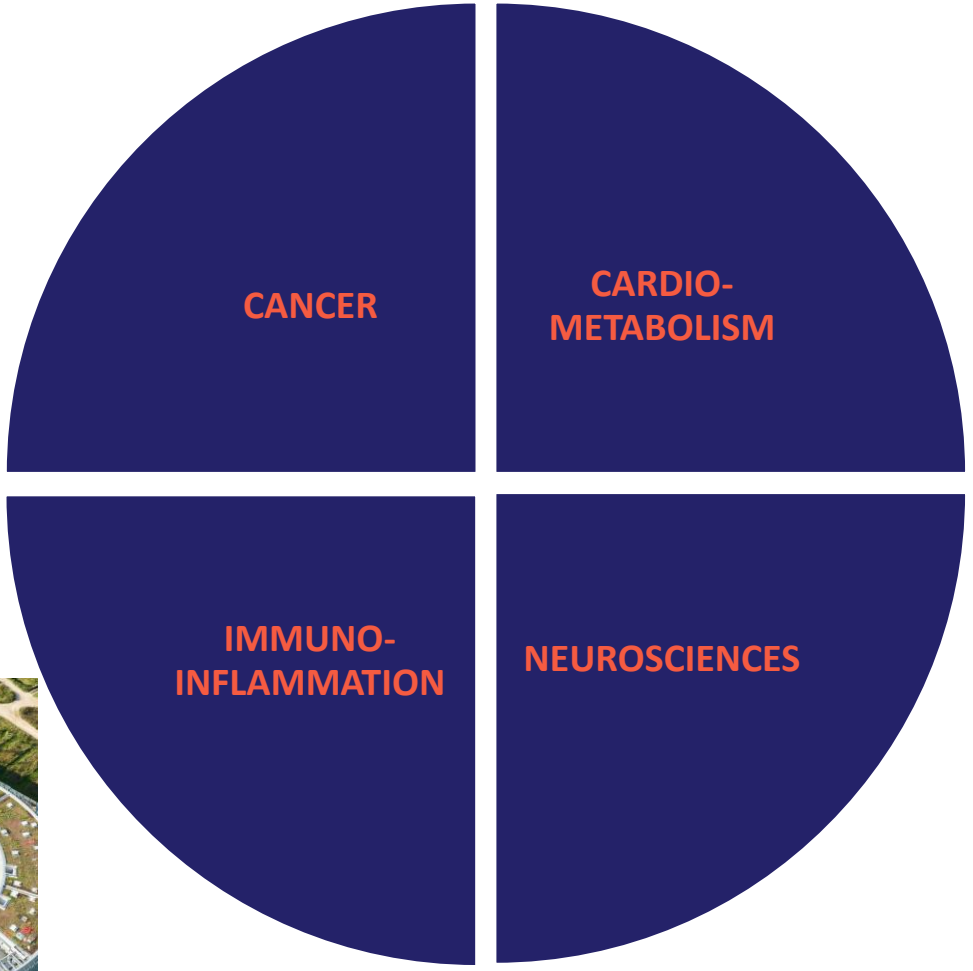
Carole CHOTARD, PhD
Head of the Histopathology Pole
NonClinical Safety Unit – Translational Medicine

08/12/2023

SERVIER
moved by you

Servier Pharmaceutical Company

- Foundation
- **21400** employees within the Group
- More than **20%** of our revenue in Research and Development
- **30** medicines available to patients in over 150 countries
- **New R&D Center** opened in Feb 2023 (Paris/Saclay Campus) with **the Research**, **Translational Medicine** & **Clinical** departments

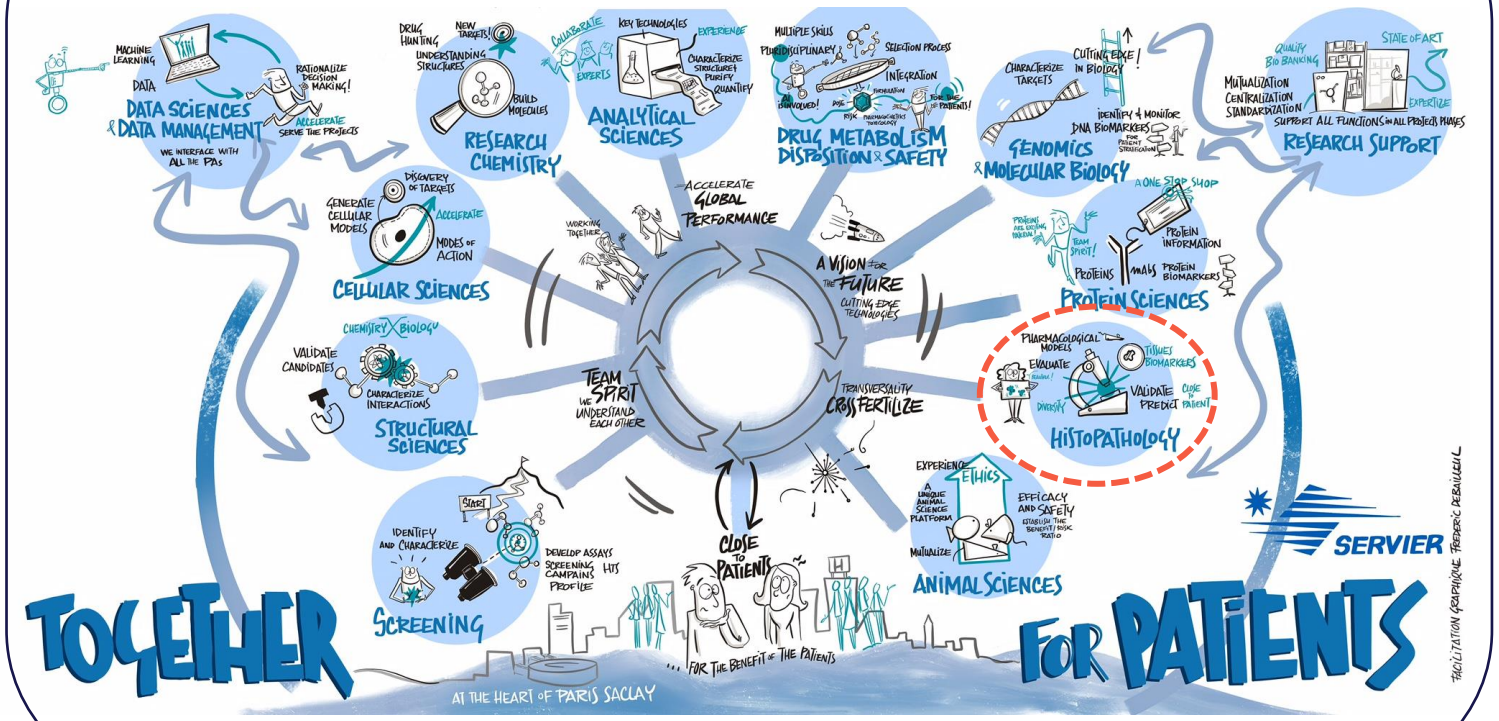


Servier Pharmaceutical Research – Paris Saclay Campus

3 THERAPEUTIC AREAS
(Project leaders)

- ONCOLOGY
- IMMUNO-INFLAMMATION
- NEUROSCIENCES

12 POLES OF ACTIVITIES (PAs)
(Team managers and technicians)



Requests in a tool
(Aim, deadline, type of data)

Histopathology Pole -> Projects involvement (Nov 2023)

Oncology | Immuno inflammation | Neurosciences

Research → **Translational Medicine** → **Clinical Development**

Early Research Phases | Late Research phases

CONCEPT | T2H | H2L | LO | PCC | PCD | Phase I | Phase II | Post AMM

Target Engagement / Drug Mechanisms of action

Models Characterization

Exploratory , predictive Target BMKs

Disease Understanding
Target identification & Validation

Characterization of Drug resistance

Expression profiling (normal & pathological tissues) of BMKs (Proteins, RNAs or DNAs in situ detection with IHC, RNAscope or FISH)

19 requests	8 requests	3 requests	5 requests	3 requests	2 requests	10 requests

30 projects / 50 requests

Histopathology Pole -> Organization (13 team members)

Head : Carole CHOTARD

Early Stage Manager

Johann RICHARD

Late Stage Manager

Erik MIRE

External Pathologists Network (Human/Animal expertise)

Dr Boivin
Human & Animal expertise

Dr Validire
Human expertise

Dr Stimmer
Animal expertise

Technical Team

- Sonia SIMOES 
- Mélanie COLLET 
- Soror GHALI (Engineer student) 
- *Open position (Technician Necropsy/Histology)*
- Karine ALBINET 
- Nelly SOURIOUX 
- Laurent RUTAULT 
- Nathalie RIDOUX 
- Tiffany ROBERT (March 2024)

Internal Pathologist (NCS) (Animal expertise)

- Flavia PASELLO dos SANTOS 

+ Jeremy LAVIGNE (postdoc) (Sept 2023) 

+ Daniela APOSTOL (trainee) (2 months)

Histopathology Pole -> Missions

Main activities

- **DEVELOP AND APPLY** Histological **and *in situ* tissue biomarker** detection methods *(human and animal samples)*
- **SUPPORT** preclinical model characterization
- **CHARACTERIZE** tissular expression profiles of targets and biomarkers at **DNA, RNA** or **protein** levels – Preclinical, Pharmaco-Toxicology and Clinical project
- **DEVELOP AND APPLY** automated Digital Image Analysis
- **ANALYSIS AND EXPERTISE** in Pathology (External and internal collaborations)
- **IDENTIFY TARGET ORGANS IN PRECLINICAL TOXICOLOGY** at tissular levels (Necropsy and Histology) in collaboration with the Toxicological pathologist
- **SCIENTIFIC AND TECHNICAL WATCH**, technological innovation for the characterization of biomarkers and acceleration of their application in translational research and clinical development

Histopathology Pole -> Expertises and workflow

NECROPSY / CONVENTIONAL HISTOLOGY/ Elec.M

- ***Necropsy :*** *Animal post-mortem examinations to determine the safety profile of a drug candidate*

- ***Histology :*** *Process and analysis of the Tissue morphology, and diagnosis*

- ***Electron Microscopy*** *sections preparation*

MOLECULAR HISTOPATHOLOGY

- ***FISH :*** *DNA detection on tissue*

- ***RNAscope :*** *mRNA detection on tissue*

- ***Immunohistochemistry (IHC) :*** *Single protein detection on tissue*

- ***Multiplex IHC*** *: Several proteins detection on tissue (OPAL and LUNAPHORE technologies)*

PATHOLOGISTS / Digital Image Analysis

External pathologists

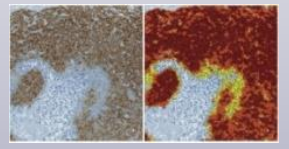
Dr Boivin	**Dr Stimmer**	**Dr Validire**
Human & Animal expertise	*Animal expertise*	*Human Expertise*

Internal pathologist

Animal Expertise

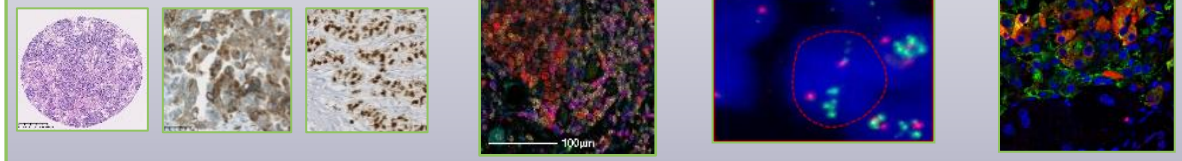
Dr Pasello dos Santos

Digital image analysis



SCANNER AND MULTISPECTRAL ANALYSIS

Histology / IHC /RNAscope	***IHC multiplex***	***FISH***	***Confocal Microscopy***



RESEARCH
AND DEVELOPMENT
Translational Medicine

SERVIER

Examples of projects where Digital Pathology is helping us to speed-up Biomarker detection (Machine and Deep-Learning approaches)

1. ***Chromogenic Immunohistochemistry***
- Preclinical model characterization (**Visiopharm Software**)
- Biomarker detection in multiple Diseases using Tissue Micro Arrays (TMAs) (**Visiopharm Software**)

2. ***Multiplex Immunohistochemistry***
- Akoya Technology (**HALO Software**)
- Lunaphore technology (**HALO Software**)

3. ***RNAscope***
- Toxicological biomarker detection (**HALO software**)

4. ***Deep-learning approach***
- Germinative centers in Human tonsil (**Visiopharm Software**)
- Purkinje cells in mouse brain (**Visiopharm Software**)

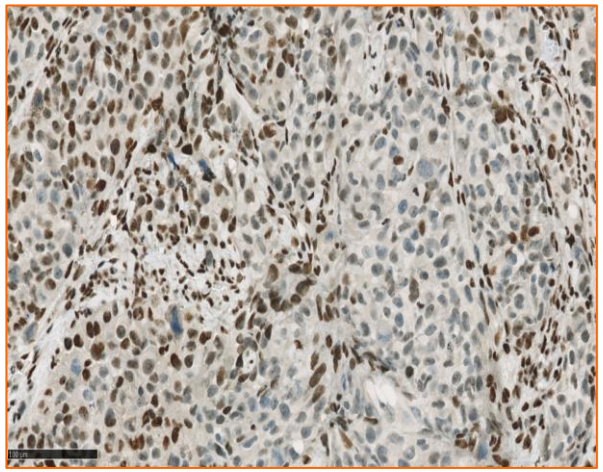
Histopathology pole -> Chromogenic Immunohistochemistry *Oncology project*

Laurent Rutault
Erik Mire

Ventana/Roche

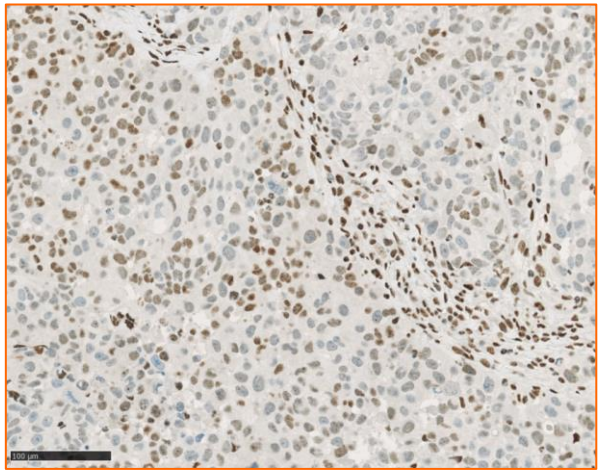
PRECLINICAL MODEL CHARACTERIZATION

PDAC PDX in mouse
Non TRT **anti-marker1**



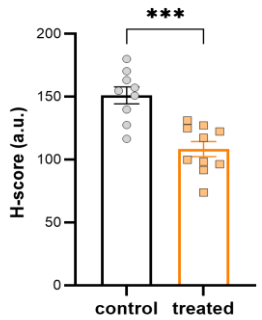
Histological score= 163

PDAC PDX in mouse
TRT target RNAi **anti-marker1**

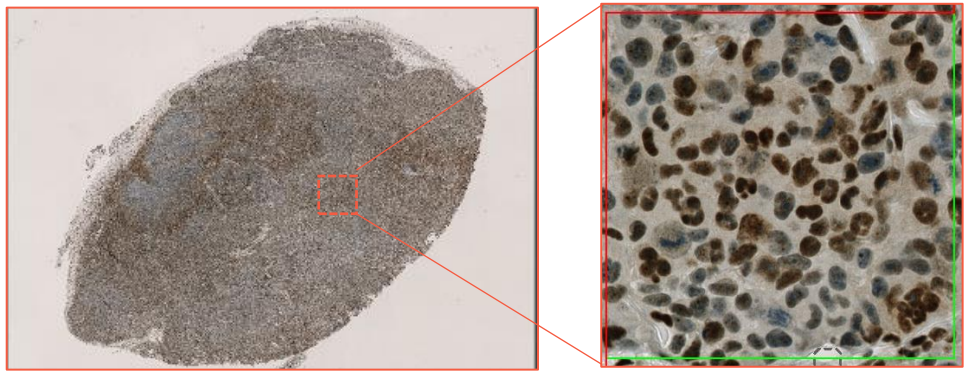


Histological score= 96

Marker 1 Hscore

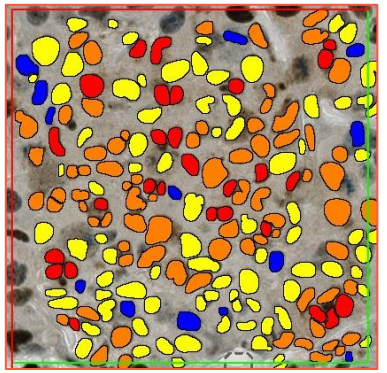


Quantification
with Visiopharm software

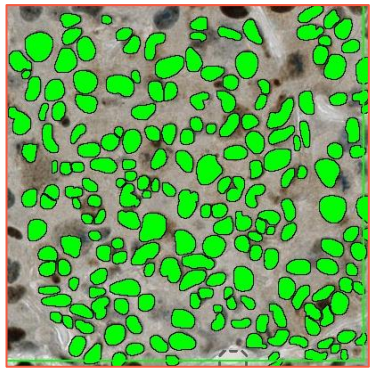


Nuclei
segmentation
(DL)

Negative
1+
2+
3+



Intensity based
classification
(ML)



- Quality evaluation of the H&E sections : Dr Boivin (Human Pathologist)
- Quantification using **Visiopharm software** (Machine & Deep Learning approaches)

RESEARCH
AND DEVELOPMENT
Translational Medicine

Histopathology pole -> Chromogenic Immunohistochemistry *Oncology project*

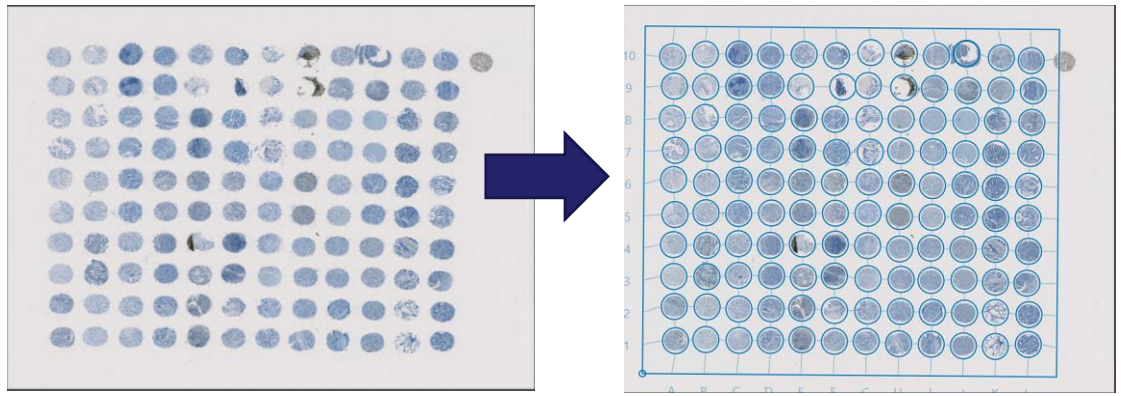
Nathalie Ridoux
Johann Richard

DISEASE UNDERSTANDING

TARGET IDENTIFICATION AND VALIDATION

Ventana/Roche Nanozoomer

Marker expression in Human Multi-cancer Tissue Micro Array (TMA)

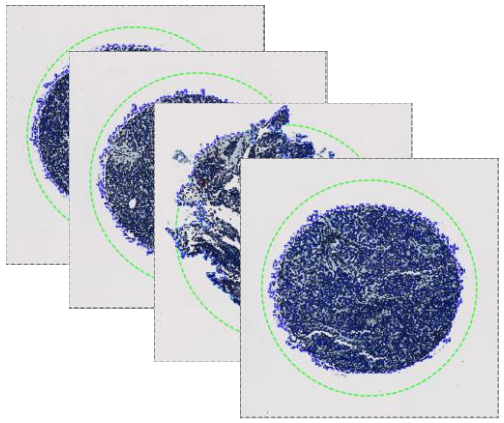


Original Image

Cores fitting

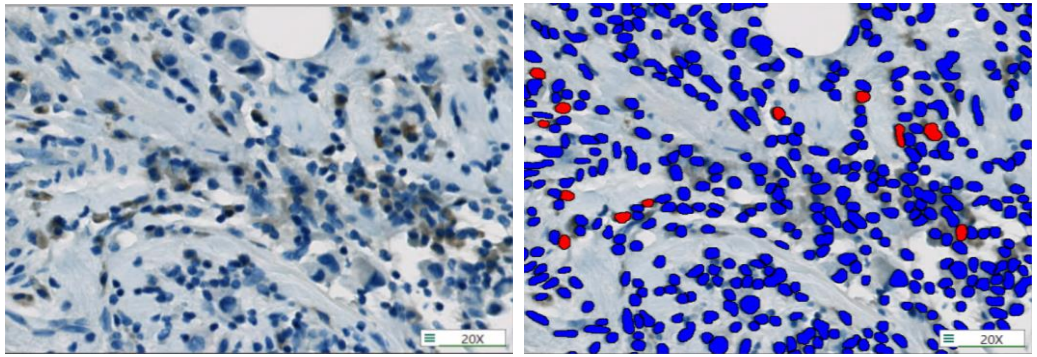
- Quality evaluation of the H&E sections : Dr Boivin (Human Pathologist)
- Speed-up quantification of protein marker using **Visiopharm Software**

Image analysis results per core (1 APP)



QC with possibility to redo analysis core per core

Positive cells density



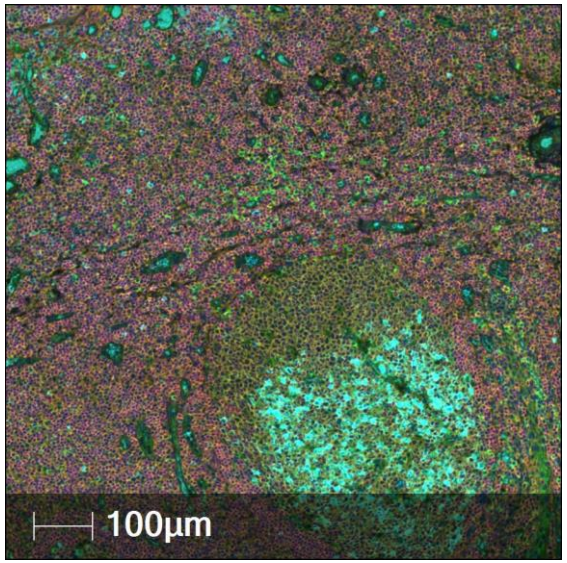
Histopathology pole -> Multiplex Immunohistochemistry – *Oncology project*

Mélanie Collet
Nathalie Ridoux
Karine Albinet
Johann Richard

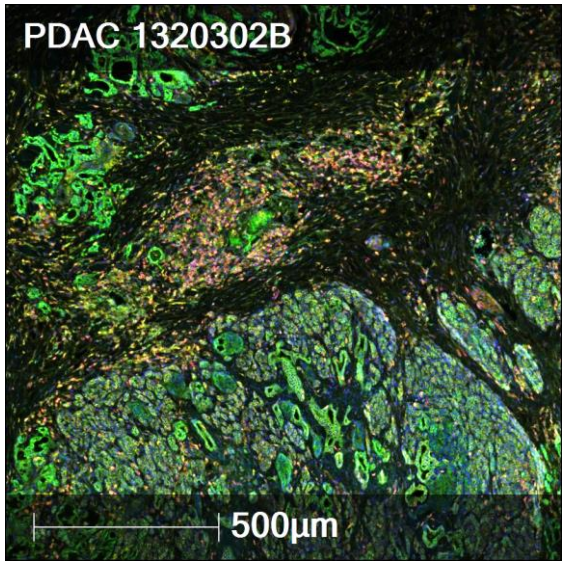
TARGET IDENTIFICATION AND VALIDATION

Ventana/Roche
PhenoImager (Akoya)

Human tonsil

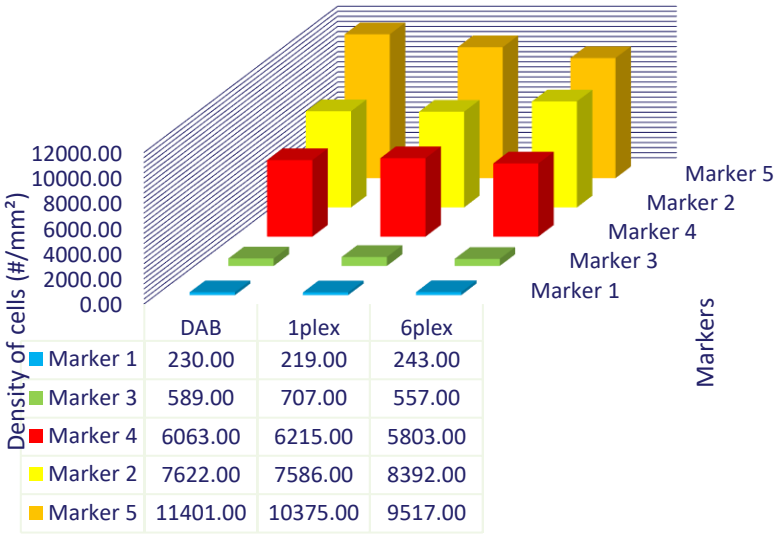


Pancreatic Cancer



DAPI
Marker 1 OPAL 480
Marker 2 OPAL 520
Marker 3 OPAL 570
Marker 4 OPAL 690
Marker 5 OPAL 620

Graphic of each marker's density on Human Tonsil for validation



	DAB	1plex	6plex
Marker 1	230.00	219.00	243.00
Marker 3	589.00	707.00	557.00
Marker 4	6063.00	6215.00	5803.00
Marker 2	7622.00	7586.00	8392.00
Marker 5	11401.00	10375.00	9517.00

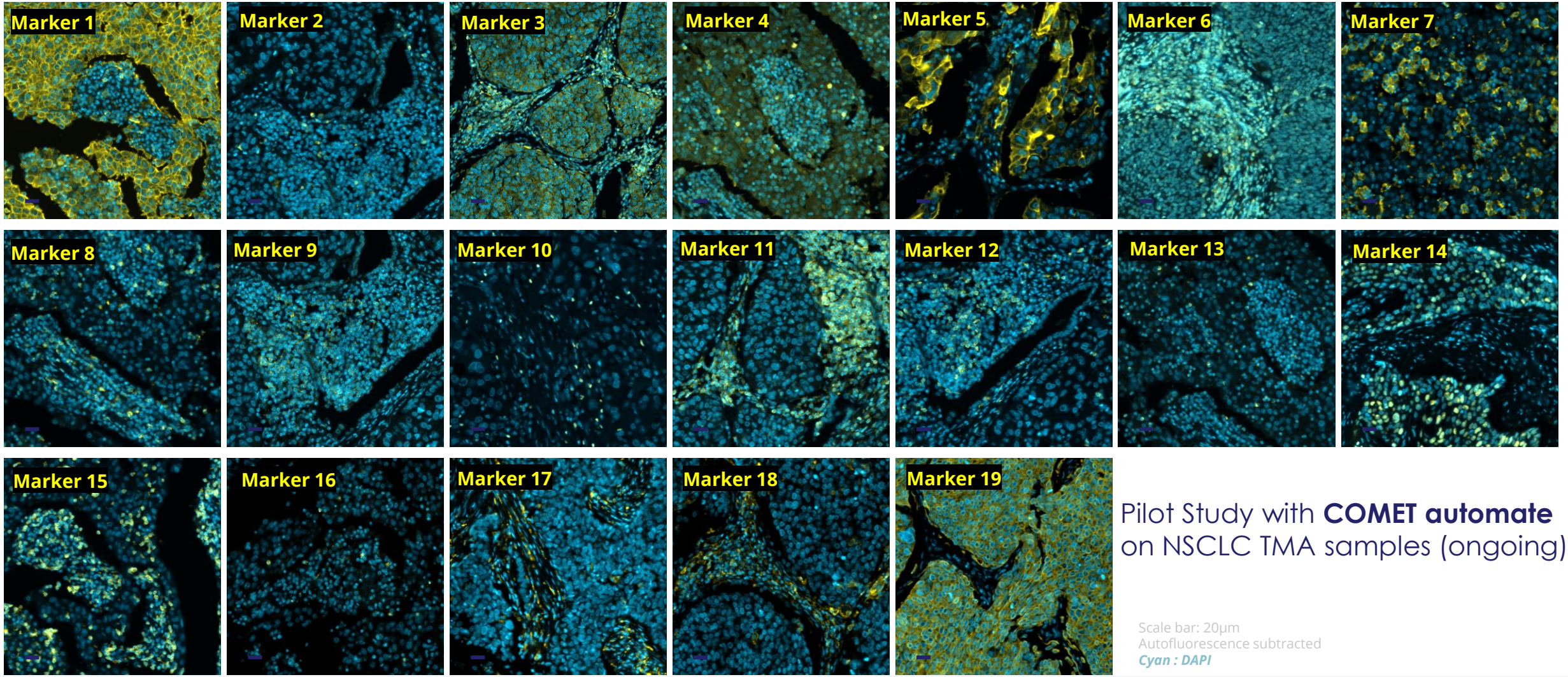
- Quality evaluation of the H&E sections : Dr Boivin (Human Pathologist)
- Quantification of the biomarkers (**density**, **percentage of expression and co-expression**) using **HALO software** with **Spatial distribution** of the biomarkers in the tumor microenvironment

Collaboration with the Genomics platform for Spatial Transcriptomics

Histopathology pole -> Multiplex Immunohistochemistry – *Immuno-Oncology project*

LUNAPHORE Team

TARGET IDENTIFICATION AND VALIDATION



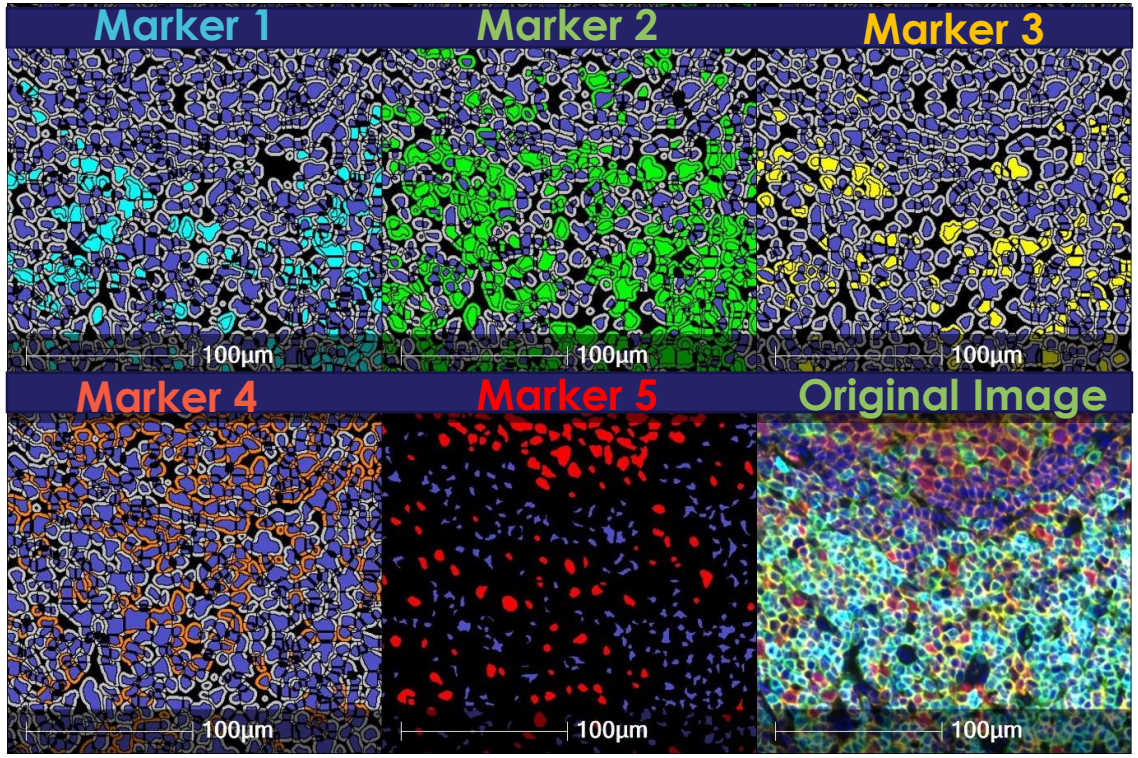
Pilot Study with **COMET automate** on NSCLC TMA samples (ongoing)

Scale bar: 20µm
Autofluorescence subtracted
Cyan : DAPI

Histopathology pole -> Multiplex Immunohistochemistry – *Immuno-Oncology project*

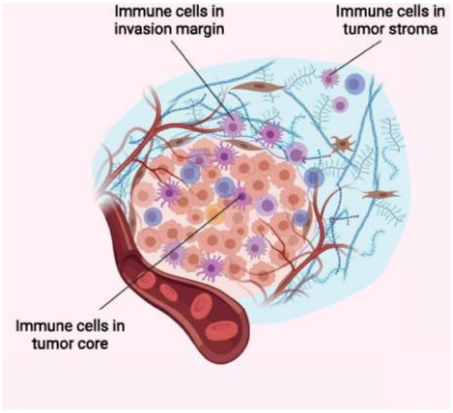
PROPATH

TARGET IDENTIFICATION AND VALIDATION

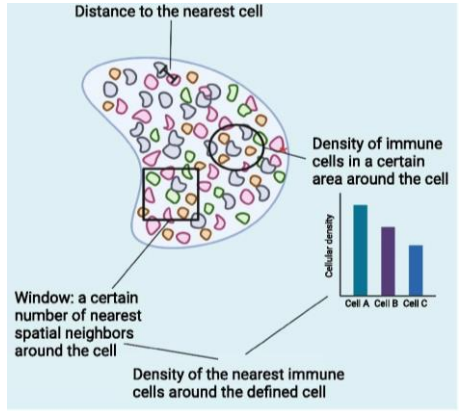


➢ Spatial cell distribution evaluated by quantification (**HALO software**) (ongoing)

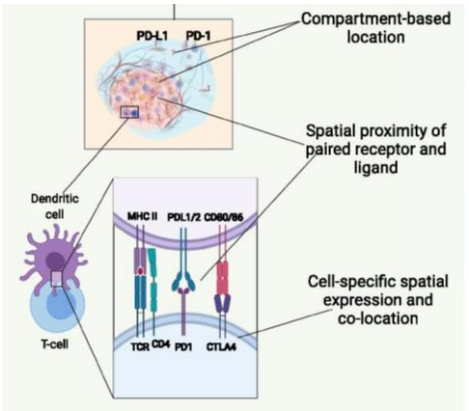
LOCATION



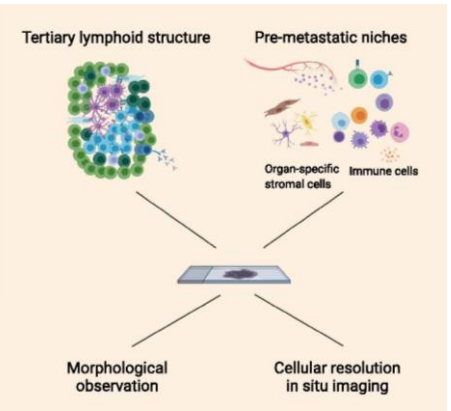
DISTANCE



DISTRIBUTION



SPECIAL PATTERNS

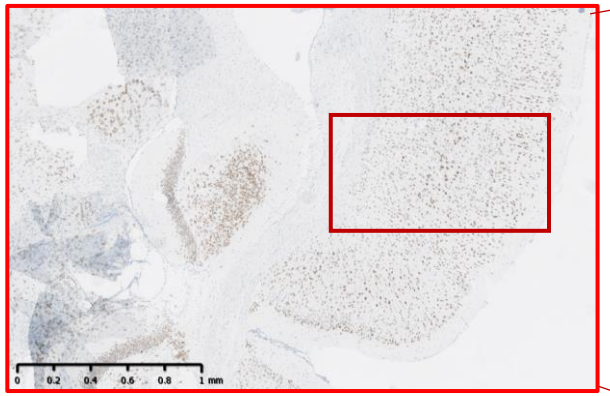


Histopathology pole -> RNAscope - *Neurosciences project*

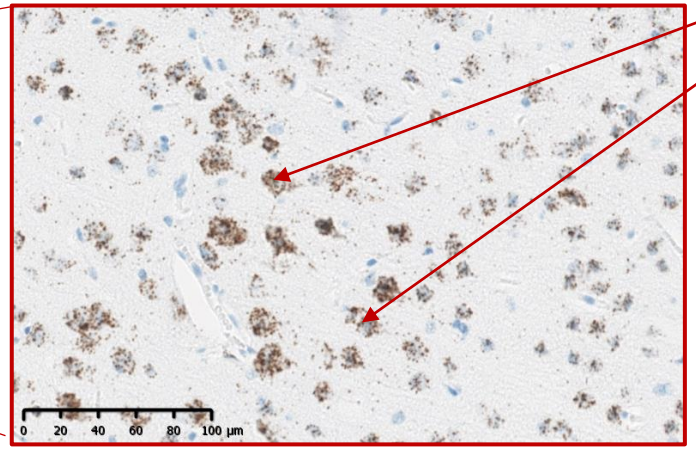
Sonia Simoes
Karine Albinet
Nelly Sourioux
Erik Mire

Ventana/Roche
Nanozoomer

VALIDATION OF TOXICOLOGICAL BIOMARKERS

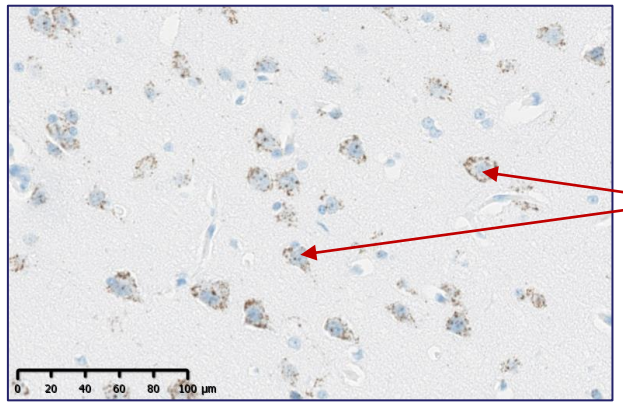


Marker 1 mRNA



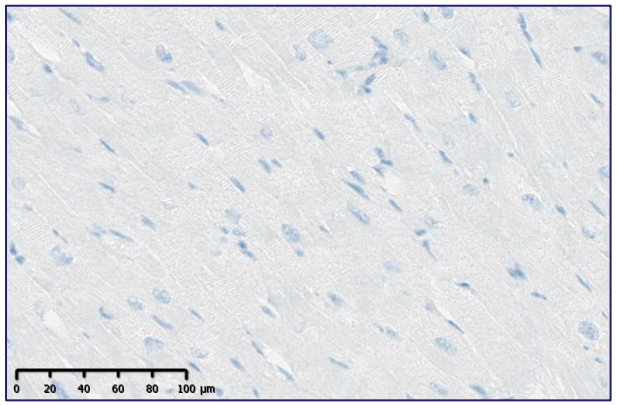
Marker 1 mRNA

Marker 1 positive neurons in **mouse** Cortex



Marker 1 mRNA

Marker 1 positive Neurons in **Cynomolgus monkey** Cortex



Marker 1 mRNA

No expression of Marker 1 in **Cynomolgus monkey** heart

Histopathology pole -> RNAscope - *Neurosciences project*

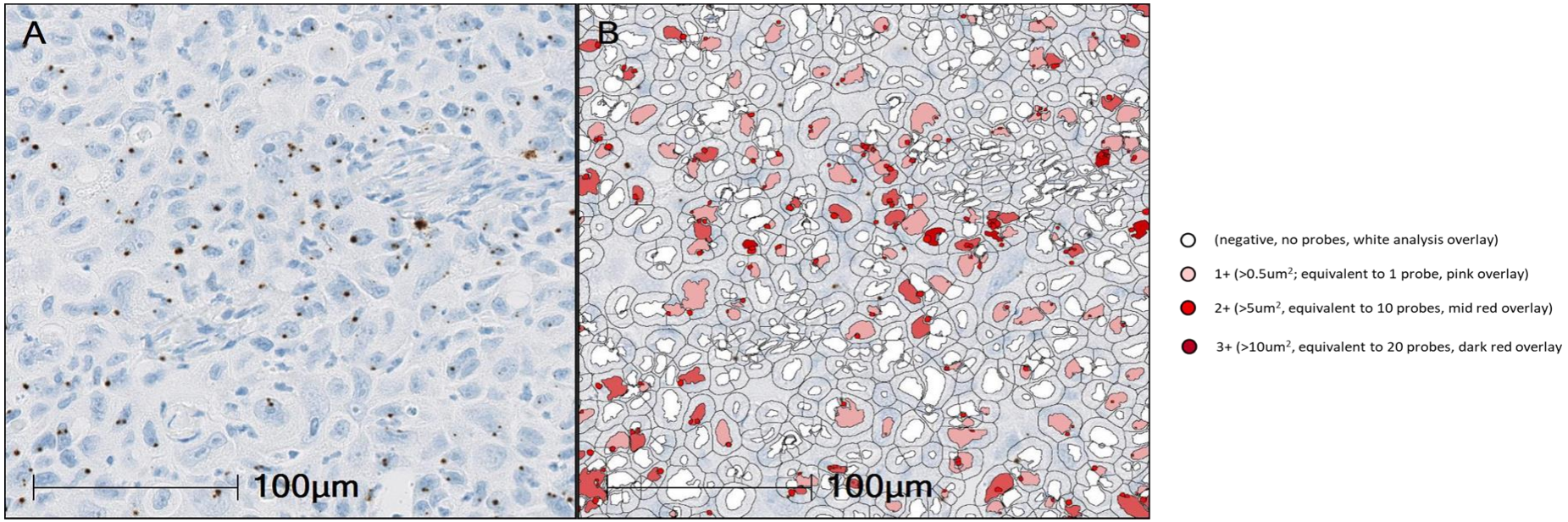
Oracle Bio

VALIDATION OF TOXICOLOGICAL BIOMARKERS

- Quality evaluation of the H&E sections and Marker 1 mRNA distribution : Dr Pasello Dos Santos (Toxicologist Pathologist)
- Speed-up quantification of mRNA marker in several species and organs using **HALO software** (Machine Learning)

Original Image

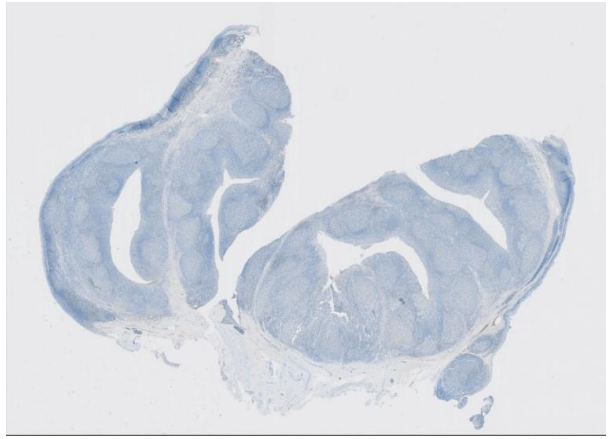
HALO quantification



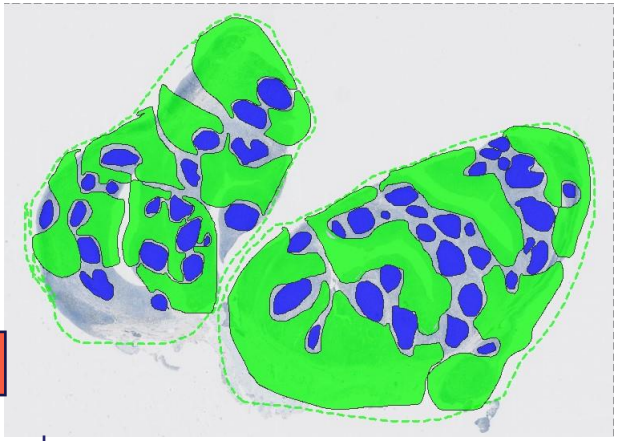
H-score = (1x %cells Low) + (2x %cells Medium) and (3x %cells High)

Histopathology pole -> Deep learning approach (Visiopharm software)

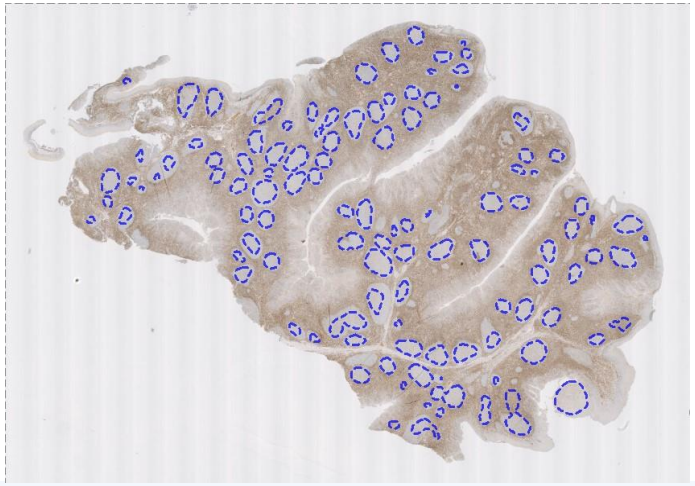
Mélanie Collet
Johann Richard

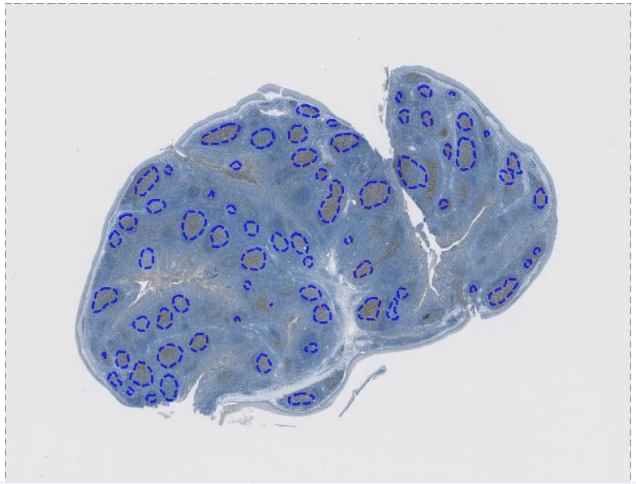


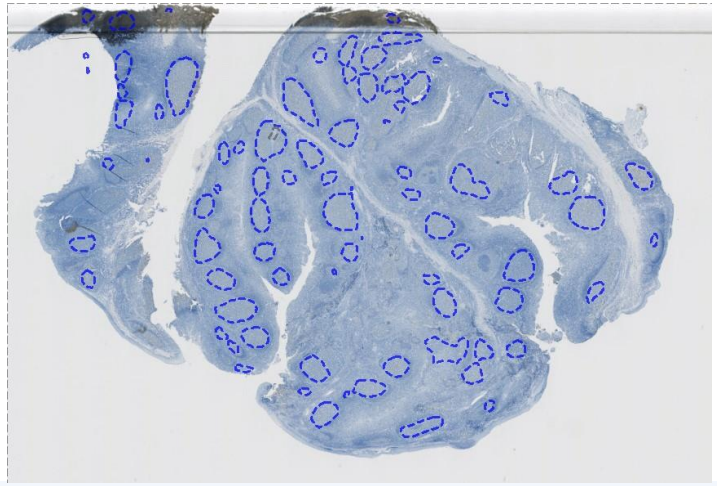
Manual annotation of germinative centers in human tonsils and deep learning training (Visiopharm)



Automatic detection of germinative centers in human tonsils for all type of stained slides (H&E, IHC, RNAscope, ...)

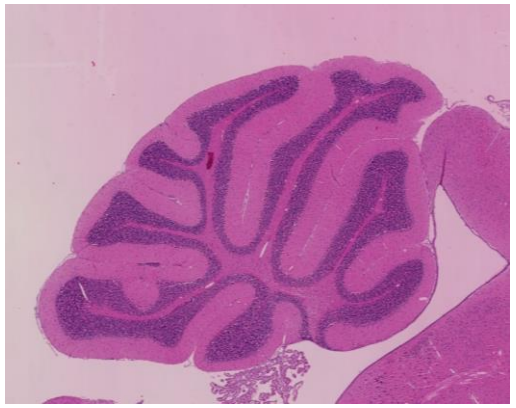




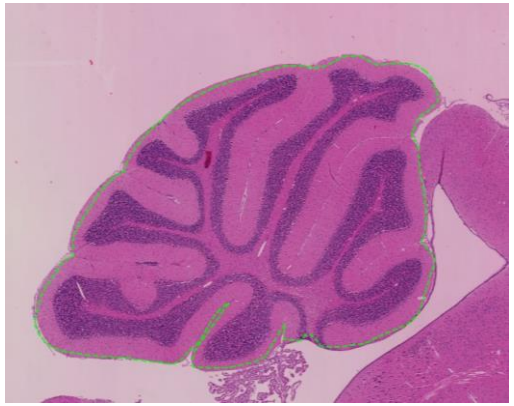


Histopathology pole -> Deep learning approach - *Neurosciences project*

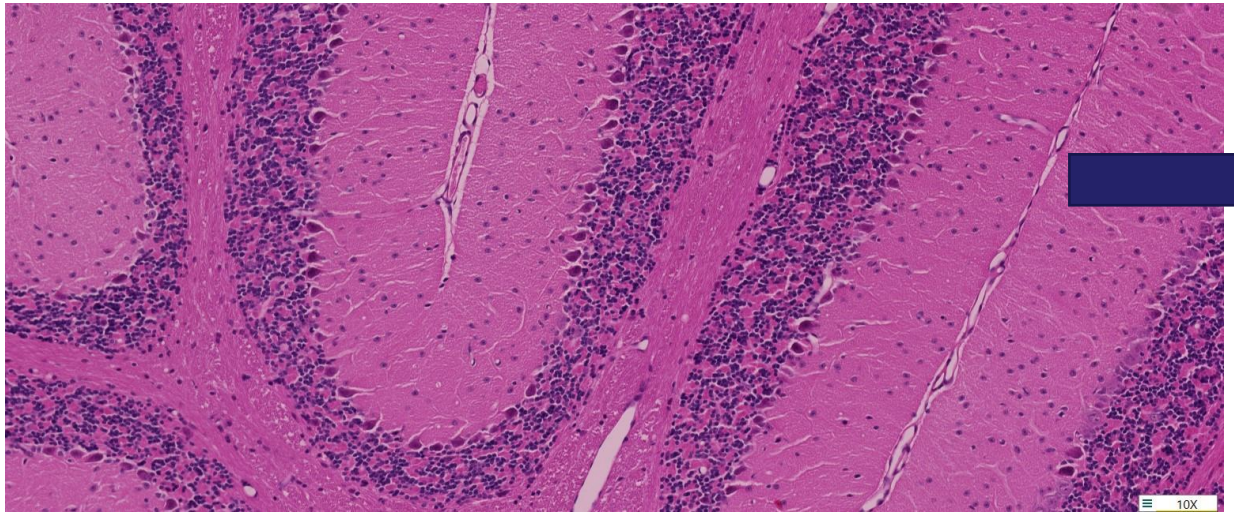
Erik Mire
Johann Richard

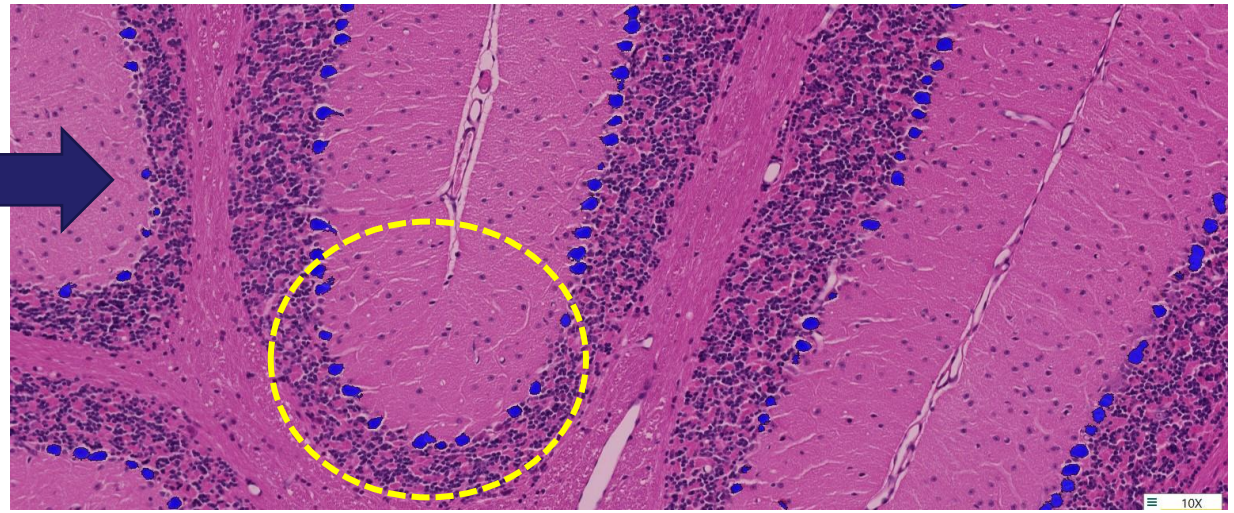


Manual annotation of mouse cerebellum



Automatic detection of Purkinje cells and quantification (Deep learning **Visiopharm software**) (ongoing)



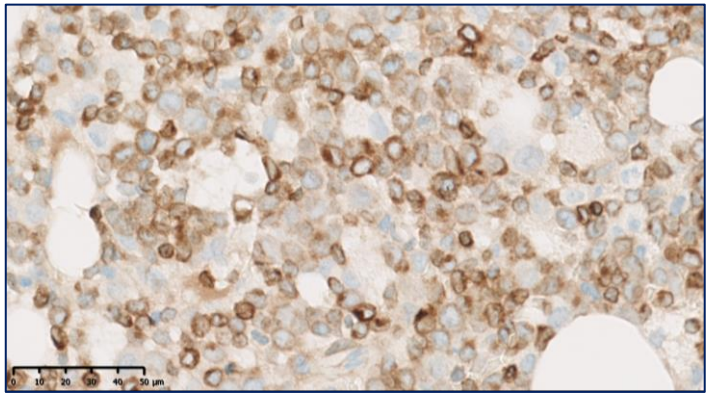


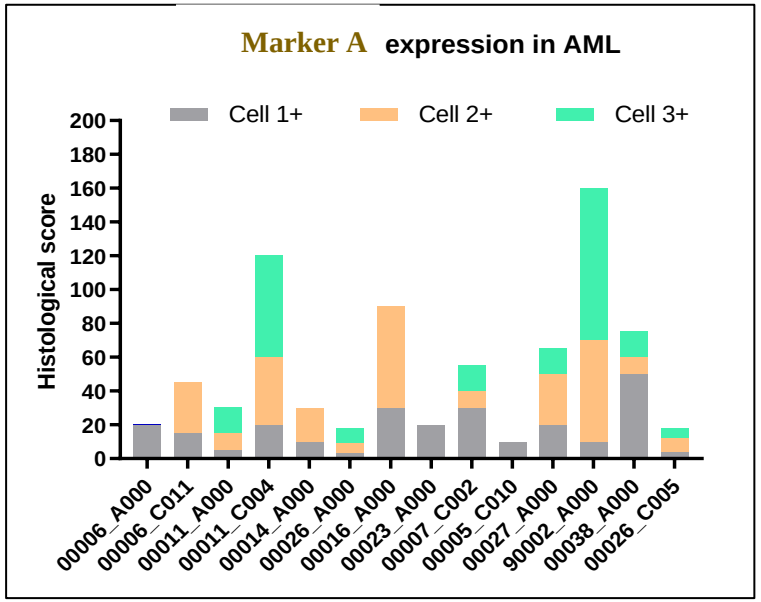
Histopathology pole ->Chromogenic Immunohistochemistry – *Oncology clinical study*

Karine Albinet
Nathalie Ridoux
Erik Mire

EVALUATE EFFICACY OF A TREATMENT AND TARGET ENGAGEMENT

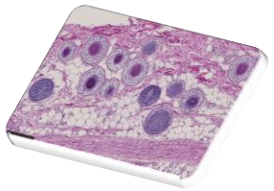
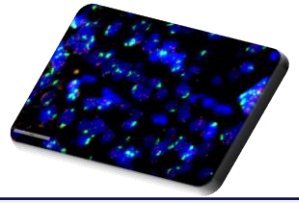
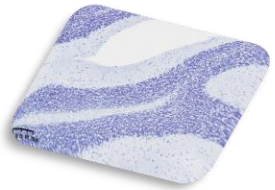
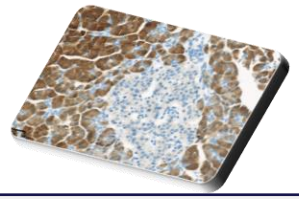
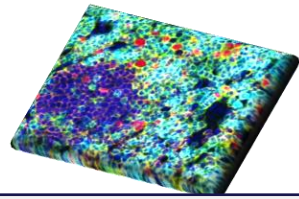
Marker A protein expression in AML





- Quality evaluation of the H&E sections : Dr Boivin
- Quantification of the biomarkers before stratification of patients : Dr Boivin
- No involvement so far of AI in our internal clinical studies

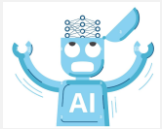
Conclusions & Perspectives

✓ Work closely with pathologists for the quality evaluation of our tissue sections, validation and distribution of the biomarkers in the tissue.

Two aspects of Digital Pathology in our workflow :

1. Computer based virtual slides review by the pathologist instead of a microscope (Slides digitalization, Calopix web application to visualize scanned microscope slides)



2. Artificial Intelligence (AI) techniques : Machine Learning (ML) and Deep Learning (DL) models

Contributions of AI in our workflow :

✓ Reduce variability observed in Manual Pathology and improve biomarker measurement.

✓ Allows more complex analysis of a large amount of data (Multiplex IHC to evaluate expression, co-expression and distribution of several markers)

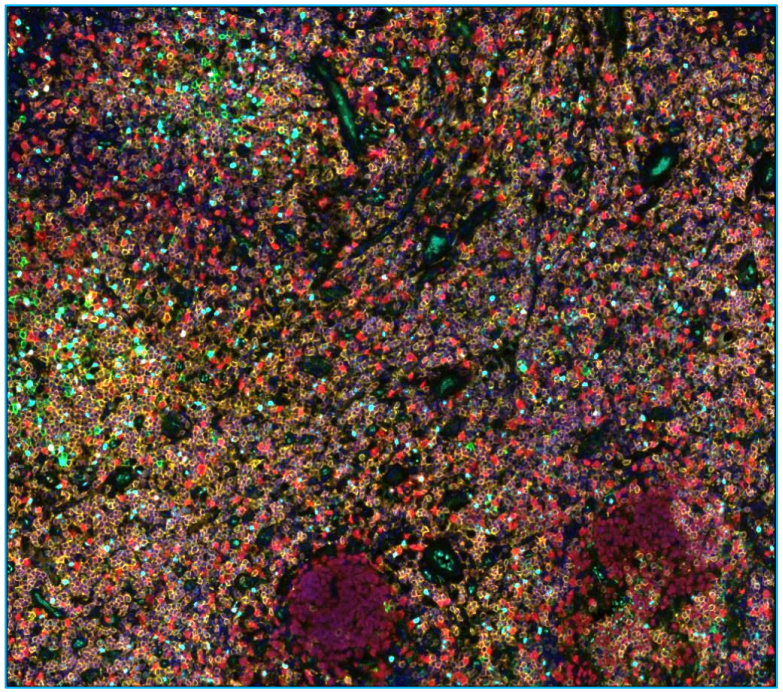
✓ Contribution to decrease the amount of tissue requested (multiplex IHC), specially for biopsies

✓ Evaluate a bigger amount of biomarkers in Disease Understanding in a shorter time (TMAs, automatic Image Analysis)

✓ Use more and more deep-learning approaches in the future

✓ Evaluation of virtual stainings for futures projects to decrease the amount of work and use of tissue.

A BIG THANK YOU

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