



NEWS FROM THE RESERVOIRS

OLFACTORY MUCOSA

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University of Torino

DISCLOSURES

I have read and understood ICMJE policy on declaration of interest and I declare that in the past five years

- My Institution has received research grants from Abbvie, Gilead, BMS, Janssen-Cilag and ViiV;
- I received speaker's and consultancy honoraria from Gilead, Janssen-Cilag, MSD and ViiV.

WHY THE OLFACTORY MUCOSA?

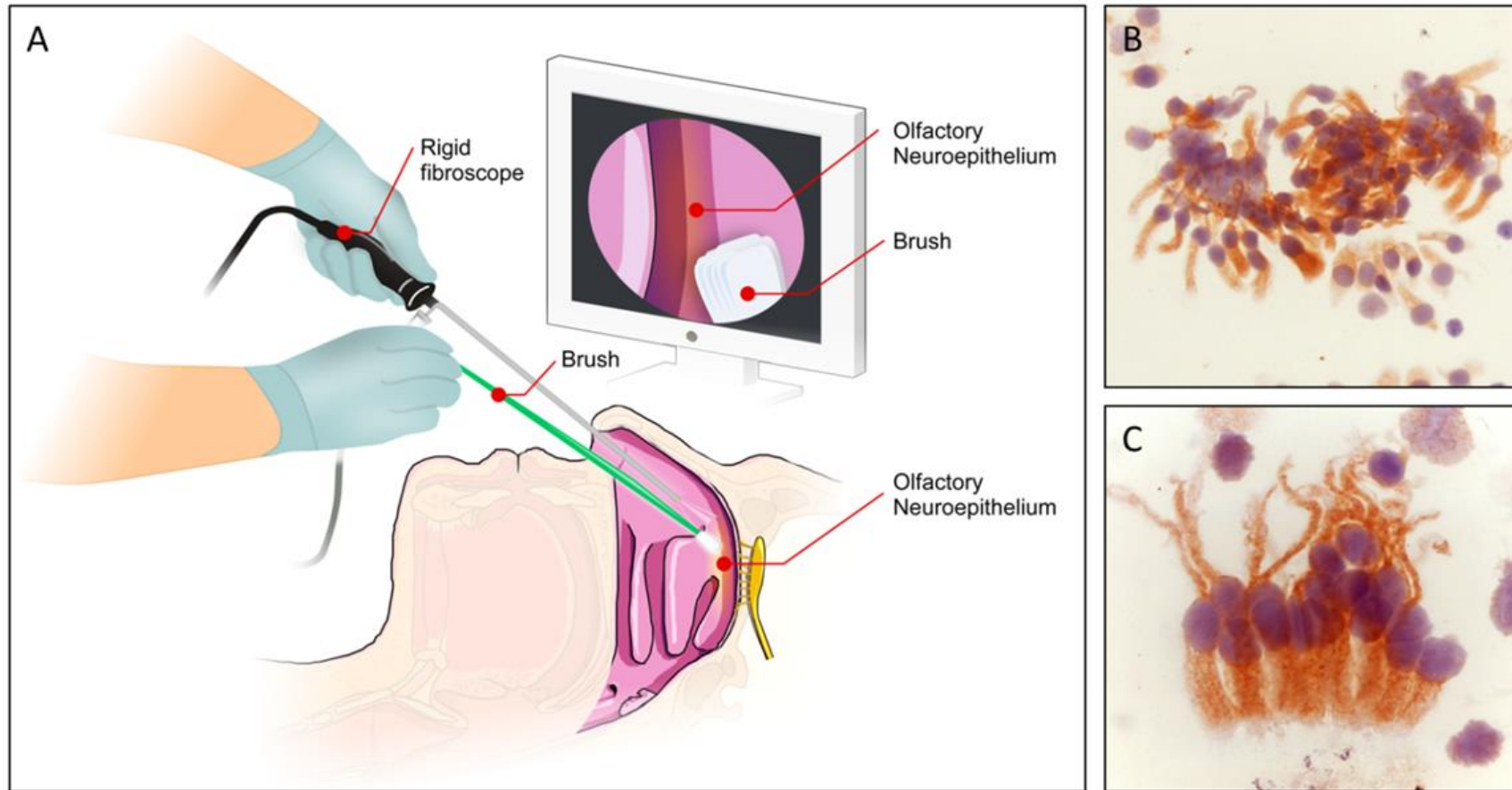
1. The other parts of the body were already taken...



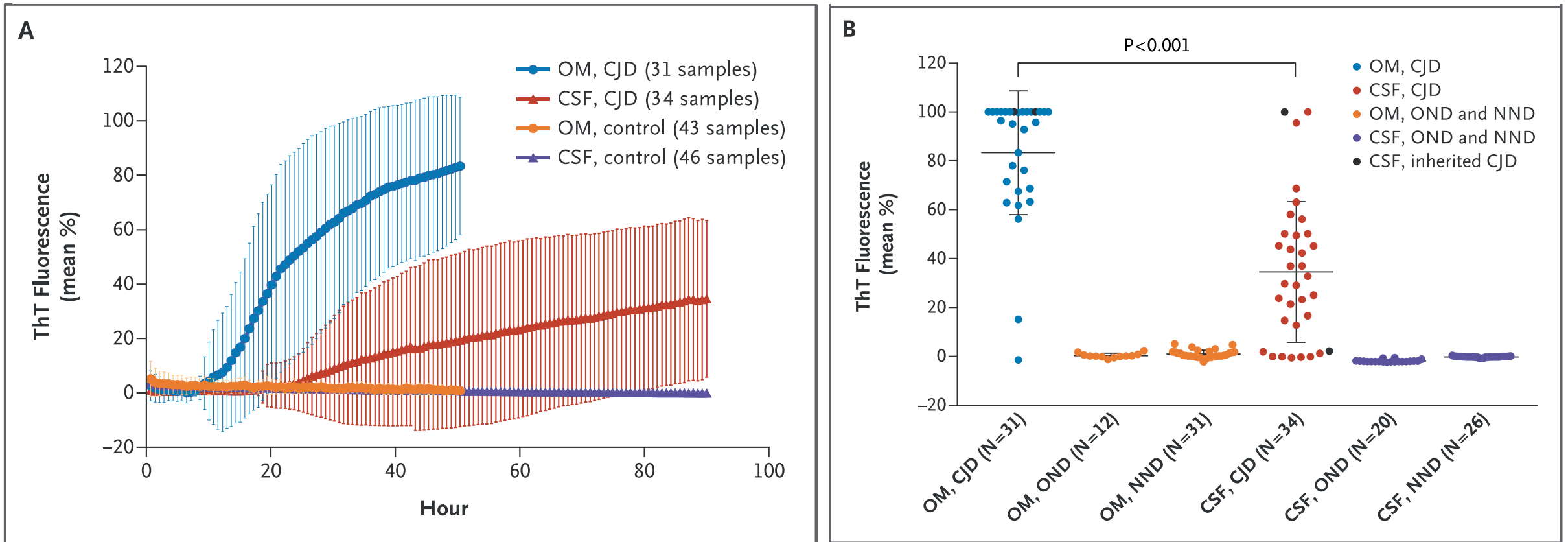
WHY THE OLFACTORY MUCOSA?

1. The other parts of the body were already taken...
2. Its use in Creutzfeld-Jacob disease

NASAL BRUSHING AND CREUTZFELD-JACOB DISEASE



NASAL BRUSHING AND CREUTZFELD-JACOB DISEASE



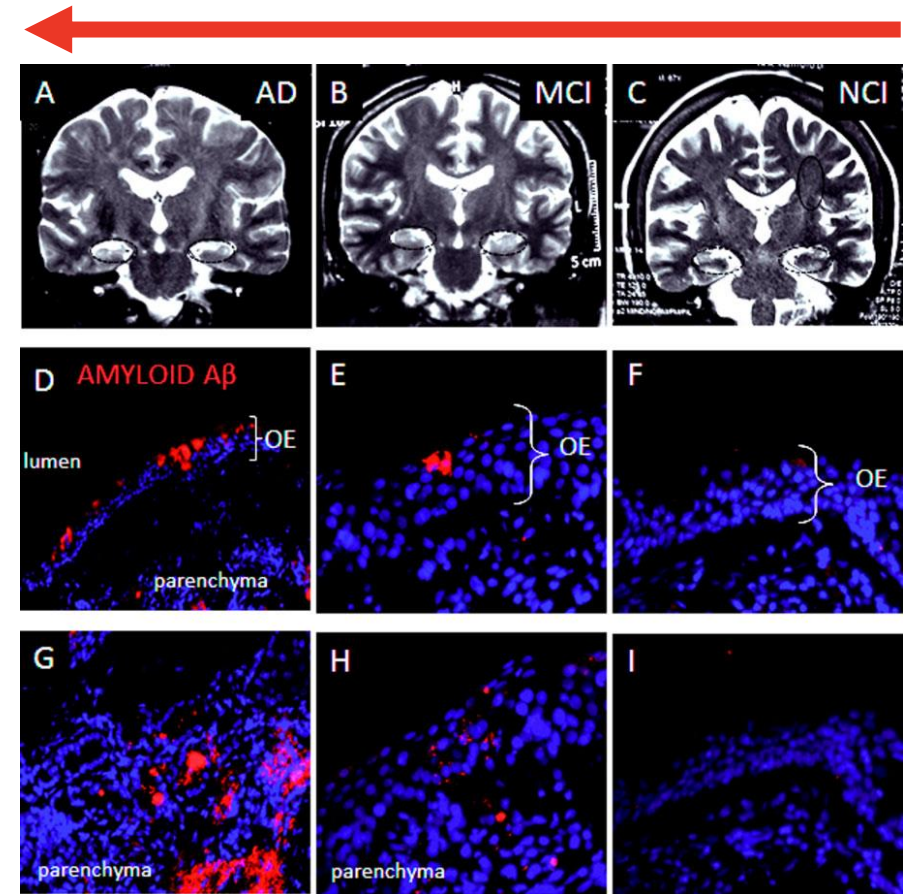
WHY THE OLFACTORY MUCOSA?

1. The other parts of the body were already taken...
2. Its use in Creutzfeld-Jacob disease
3. The previous attempts at using the OM in neurodegenerative disorders

ACCUMULATION OF PATHOLOGIC PROTEINS IN THE OM

Biopsy (associated with side effects such as bleeding, fistulization, etc)

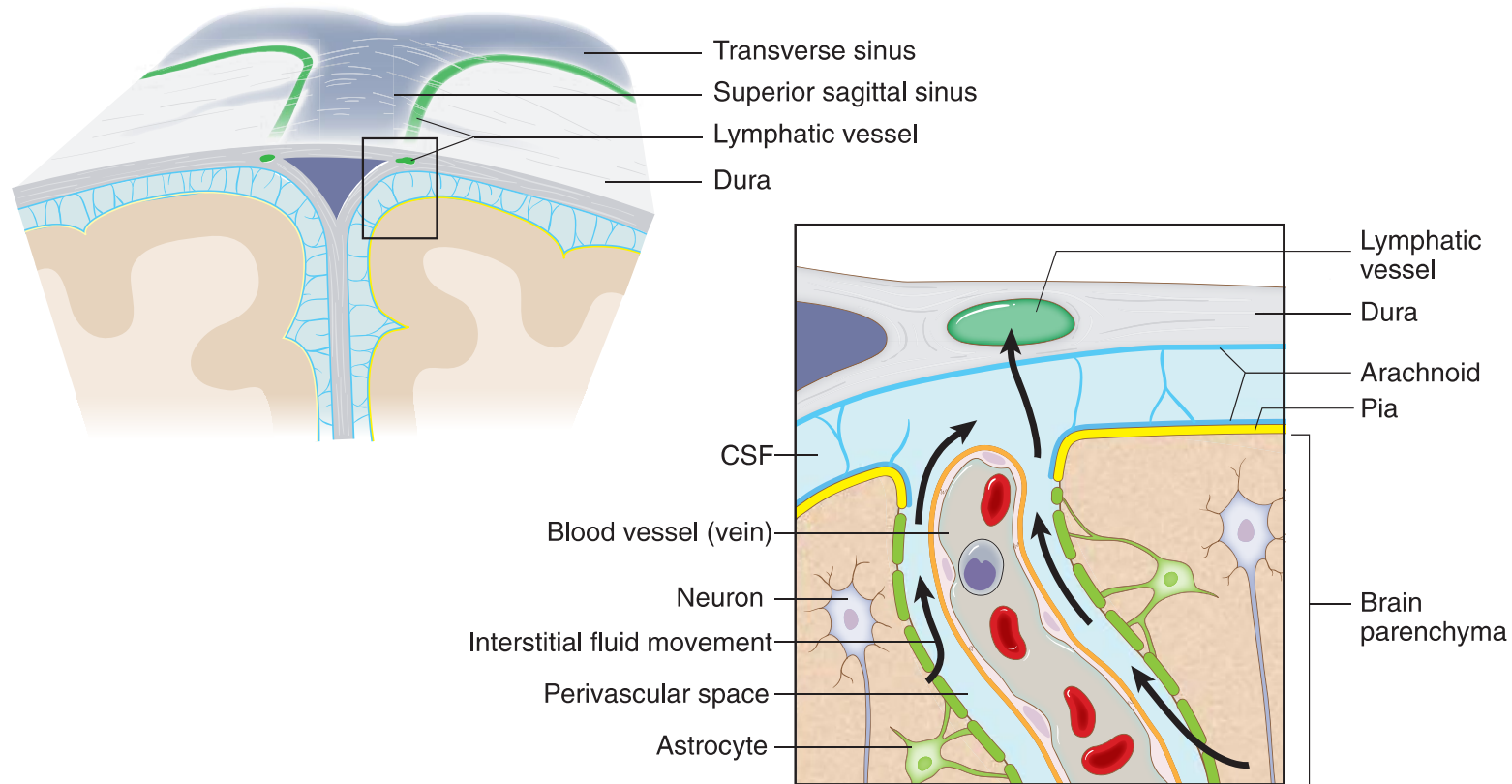
- Alzheimer's disease: **Beta-amyloid and Tau**
- Parkinson's disease: **Alpha-synuclein**
- Frontotemporal dementia: **Tau**



WHY THE OLFACTORY MUCOSA?

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4. Data on the lymphatic drainage from the CNS

CNS “FUNCTIONAL” LYMPHATIC VESSELS

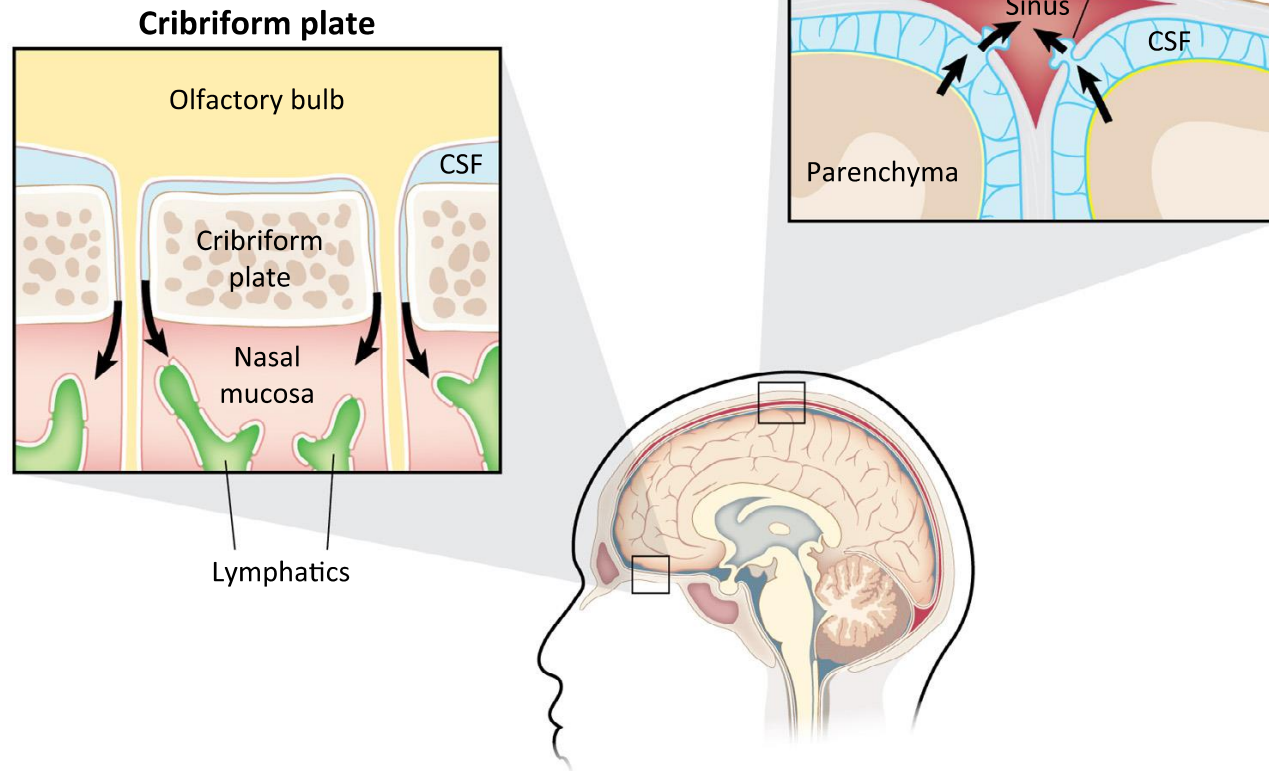


Special Issue: Neuroimmunology

Opinion

Revisiting the Mechanisms of CNS Immune Privilege

Antoine Louveau,¹ Tajie H. Harris,¹ and Jonathan Kipnis^{1,*}

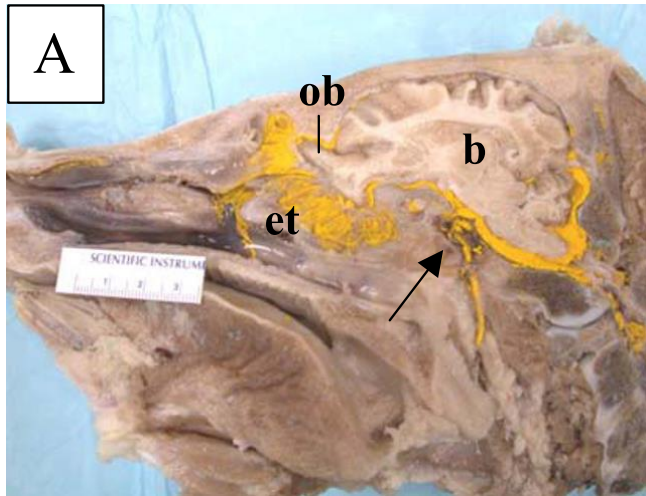


Trends in Immunology

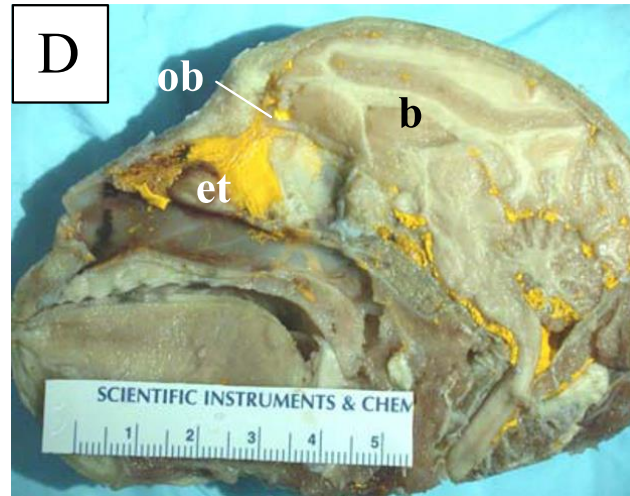
Figure 1. Paths of Cerebrospinal Fluid (CSF) Fluid Drainage. The CSF, flowing between the arachnoid and the pia mater of the meninges, can drain into the bloodstream through the arachnoid granulations located along the superior sagittal sinus and the transverse sinuses, or reach the lymphatic vasculature of the nasal mucosa by crossing the cribriform plate, localized under the olfactory bulbs, along the olfactory nerves.

LYMPHATIC DRAINAGE FROM THE CNS YELLOW MICROFILL IN THE CSF

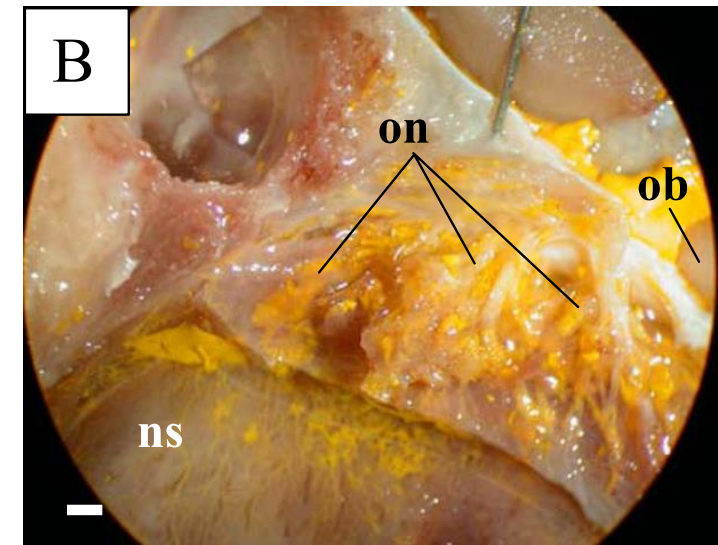
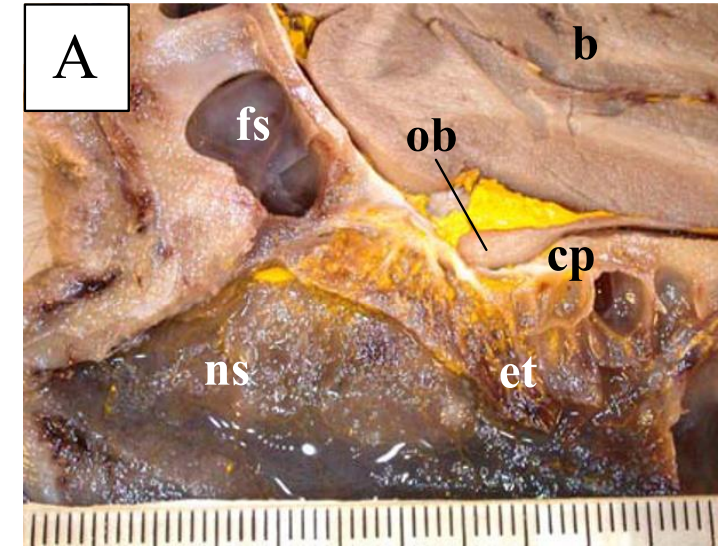
Pig

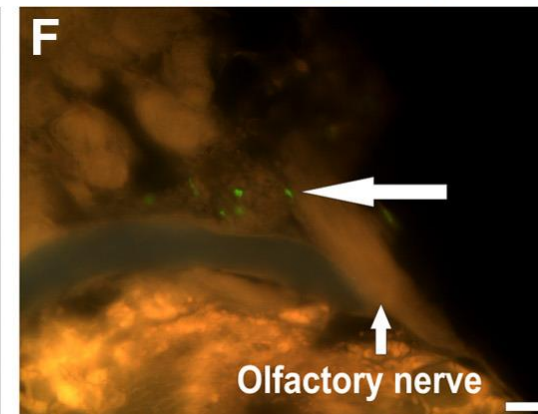
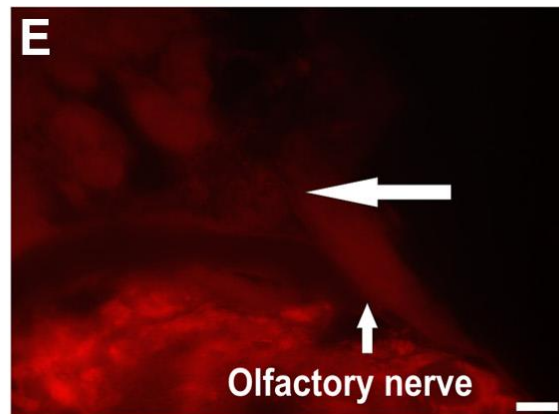
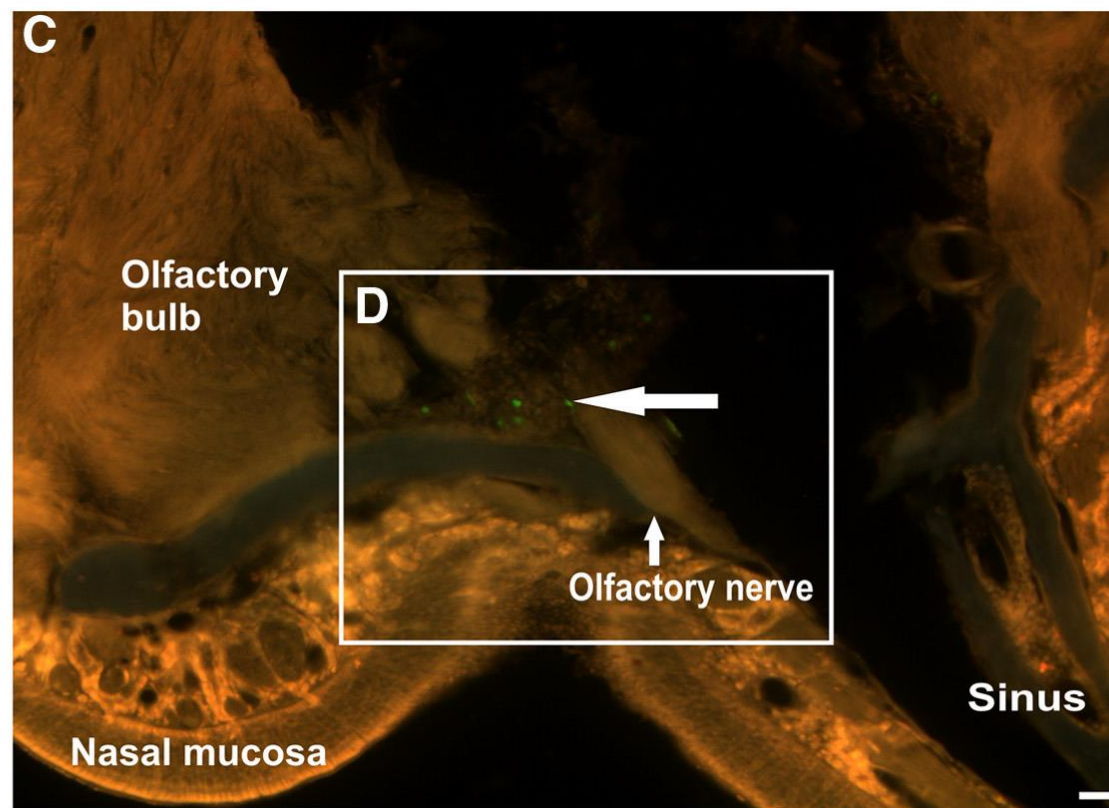
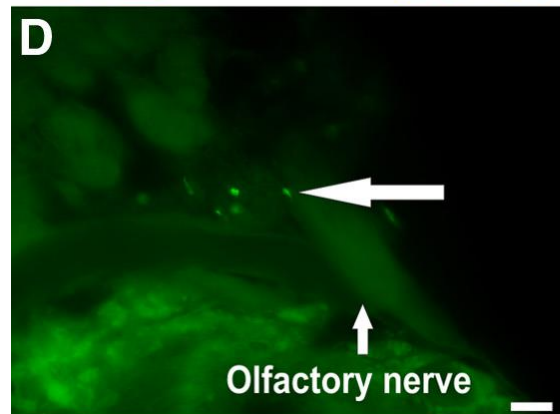
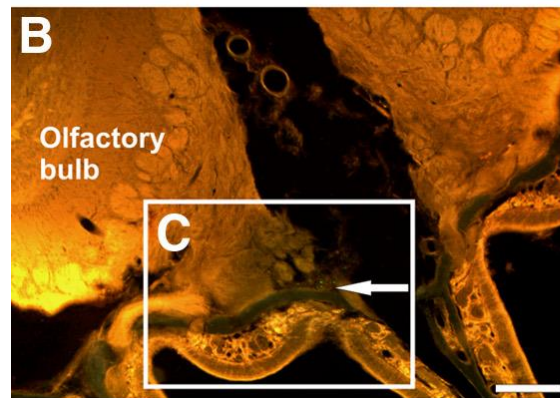
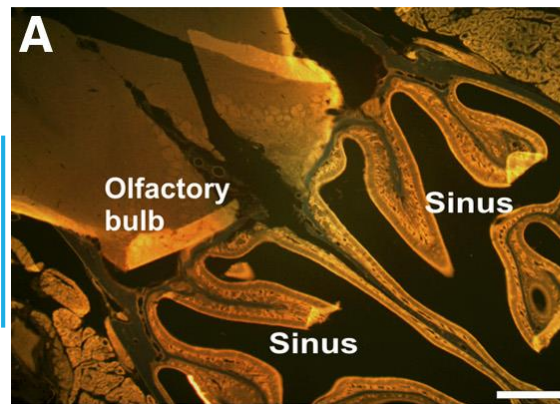


Monkey



Human





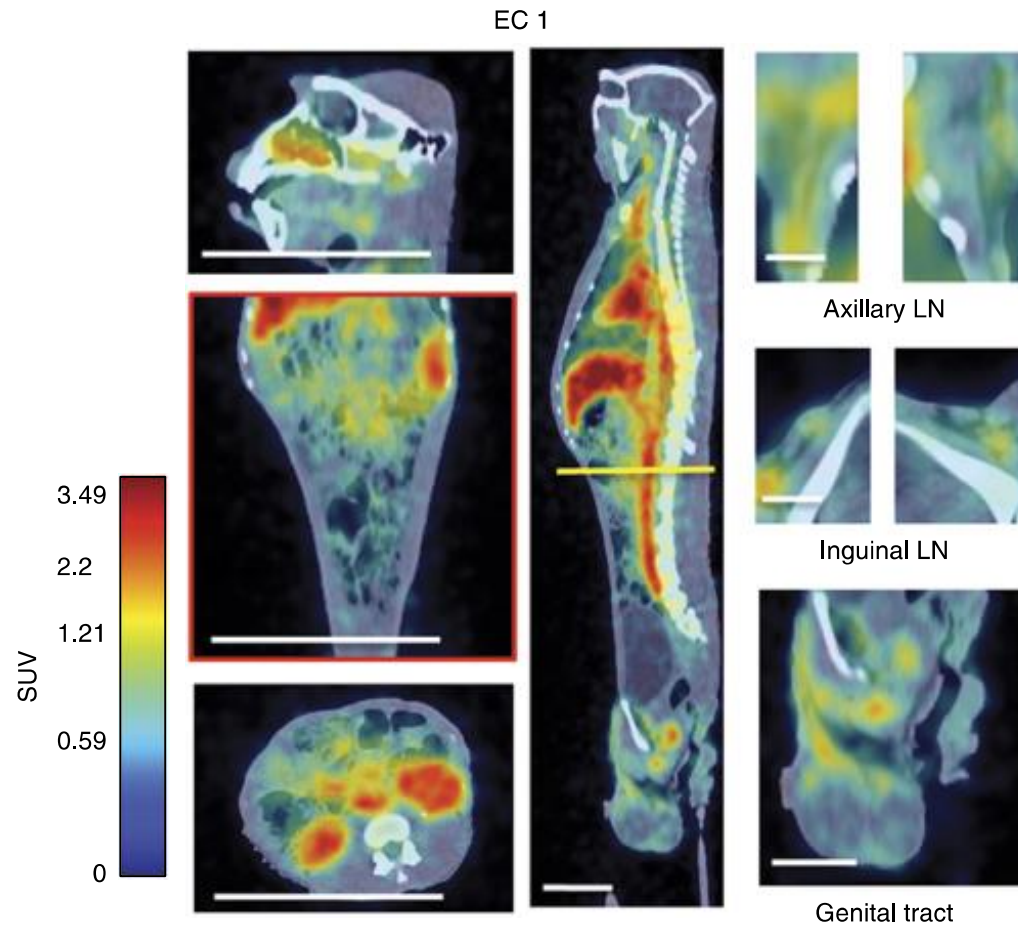
Monocytes migrated from the lesion site (endothorinal cortex) to **deep CLNs**, peaking in number at **Day 7**, but they were virtually absent in spleen and in superficial CLNs and inguinal LNs until Day 21 after lesion/injection. In whole-head sections, GFP monocytes were found attached to the **olfactory nerves and located within the nasal mucosa at 48 hpi**. Thus, monocytes are capable of migrating from lesioned brain areas to deep CLNs and use the cribriform plate as an exit route

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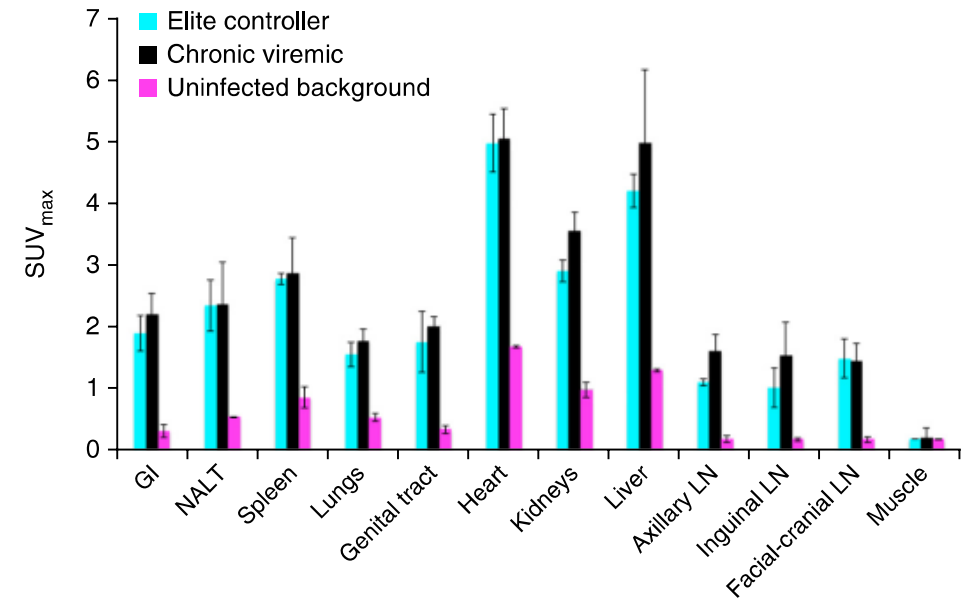
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2. Its use in Creutzfeld-Jacob disease
3. The previous attempts at using the OM in neurodegenerative disorders
4. Data on the lymphatic drainage from the CNS
5. Data on the NALT in SIV-infected macaques

NASAL ASSOCIATED LYMPHOID TISSUE (NALT)

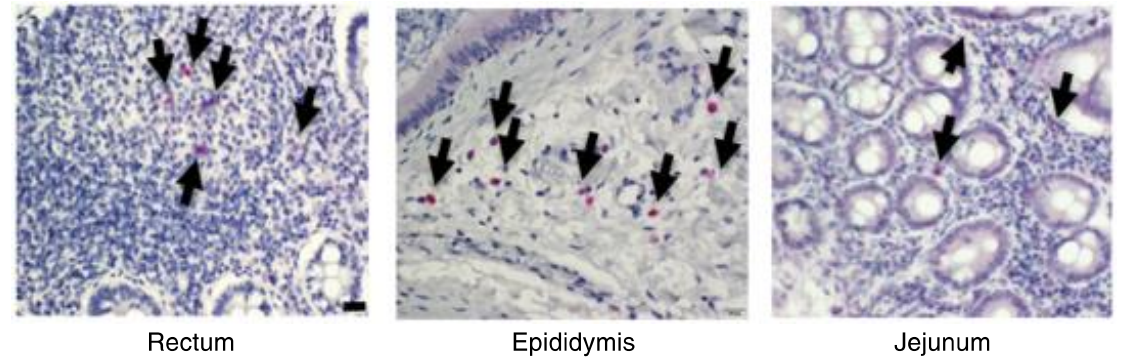
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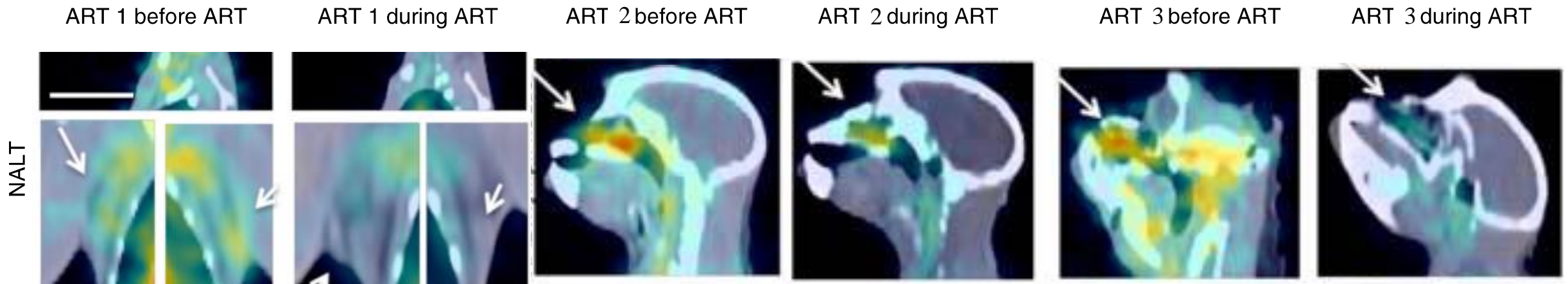
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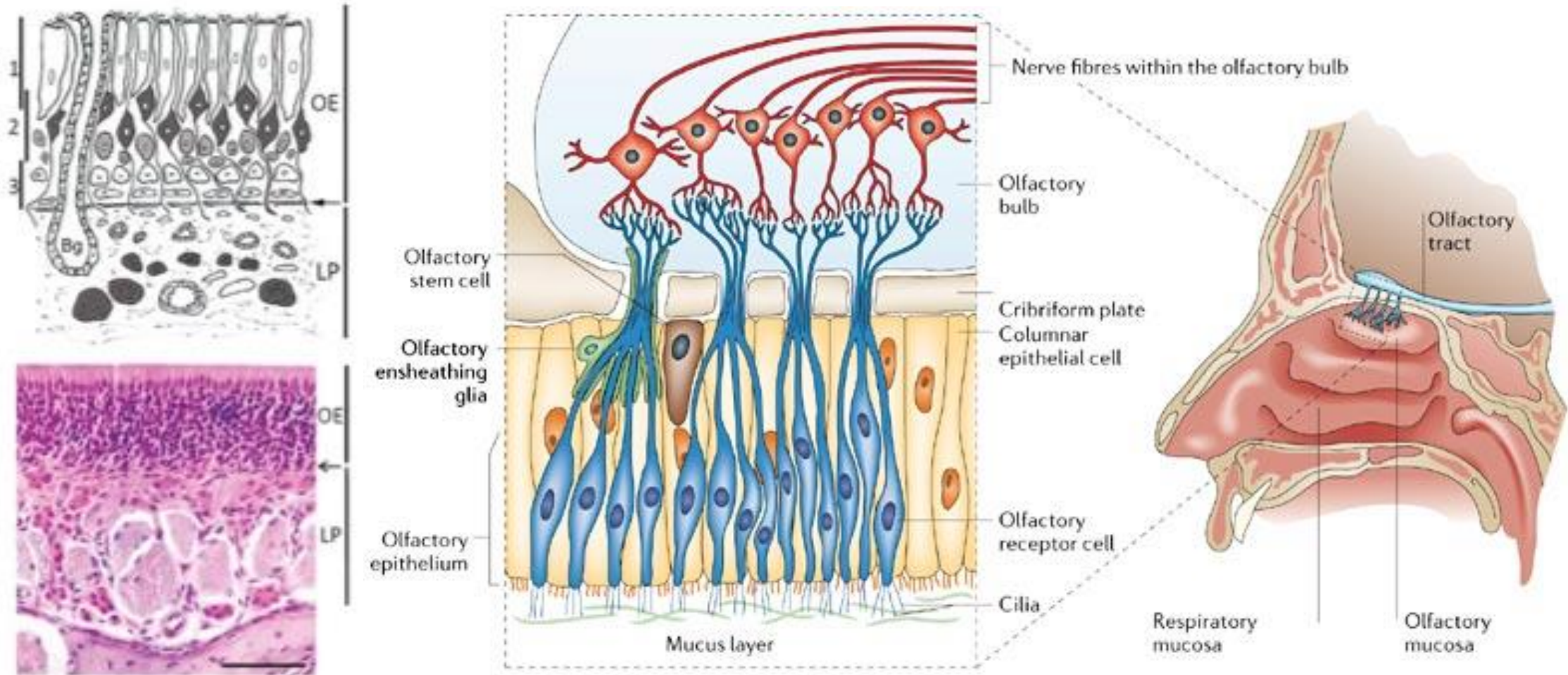
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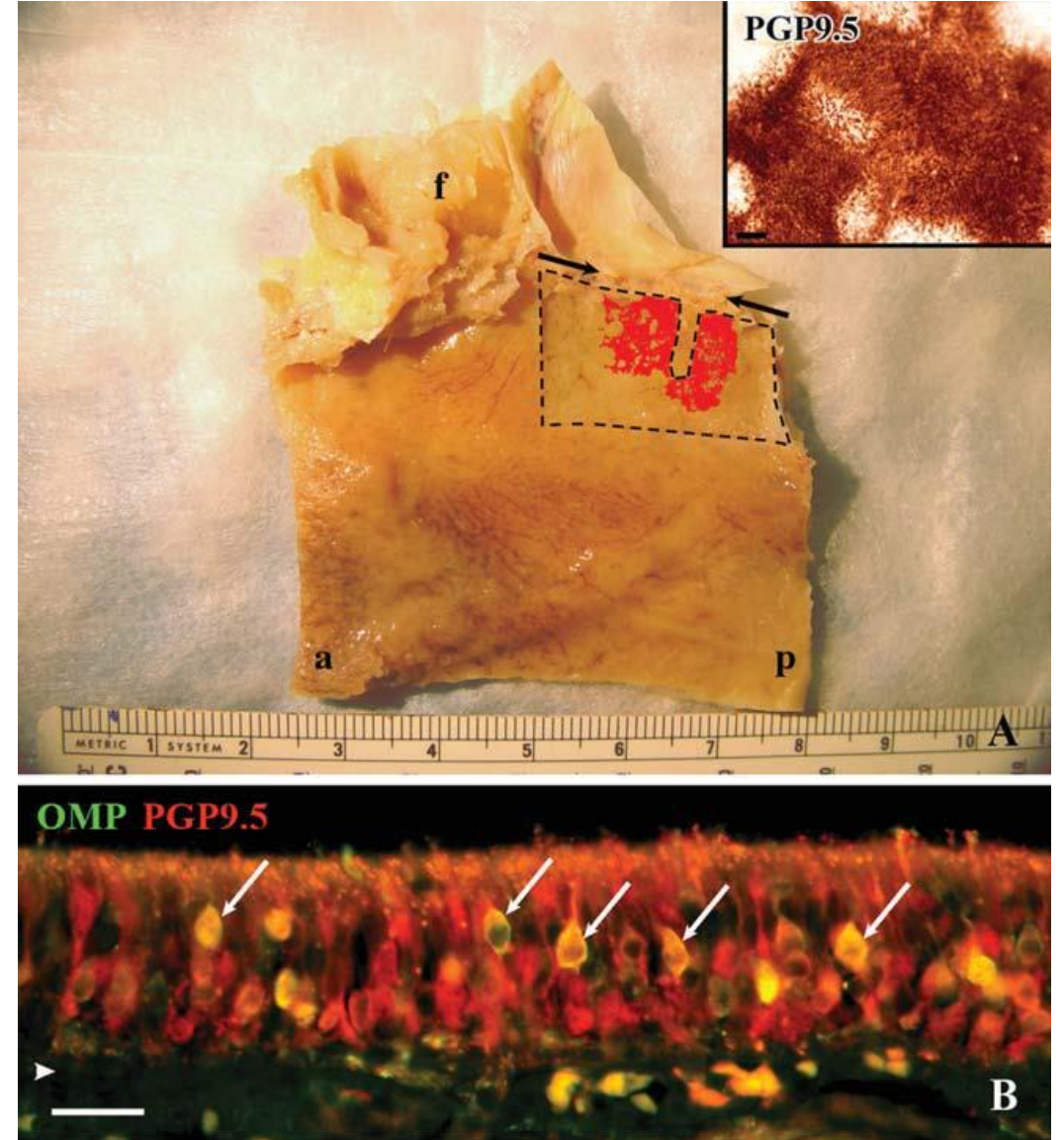
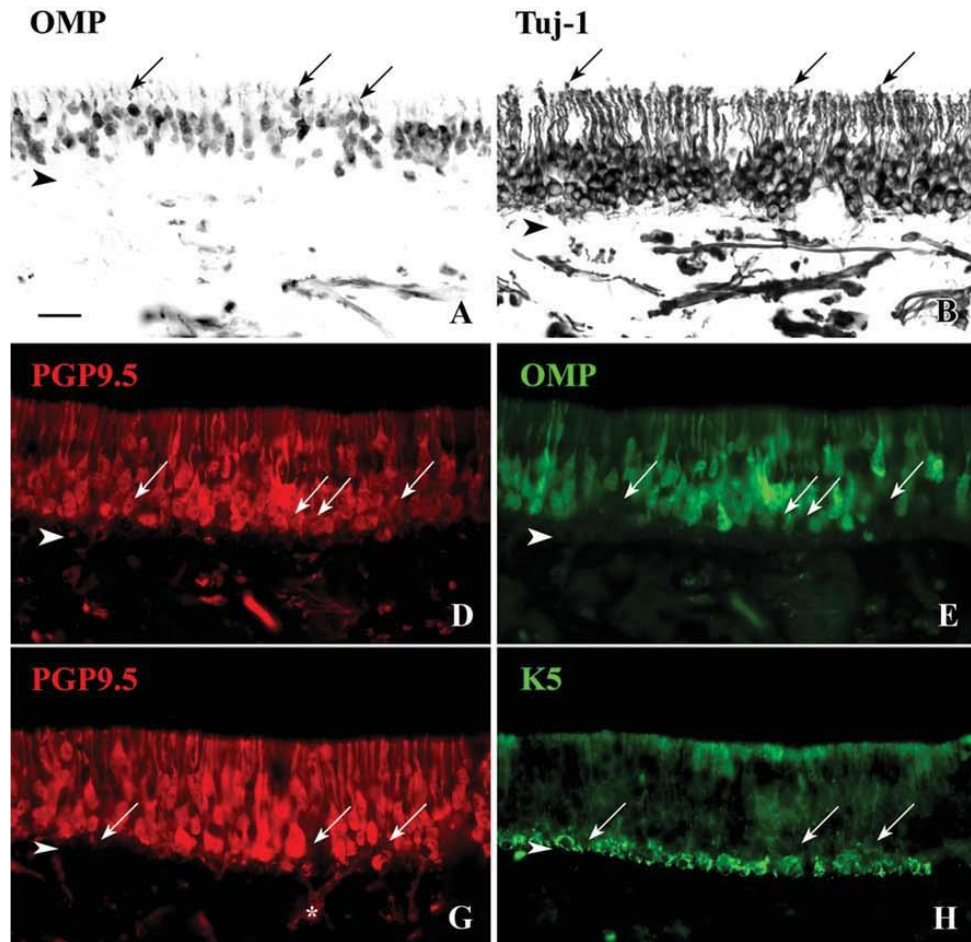
NASAL ASSOCIATED LYMPHOID TISSUE (NALT)



STRUCTURE OF THE OM



MICROSCOPY OF THE OM



THE SOLFAMU STUDY

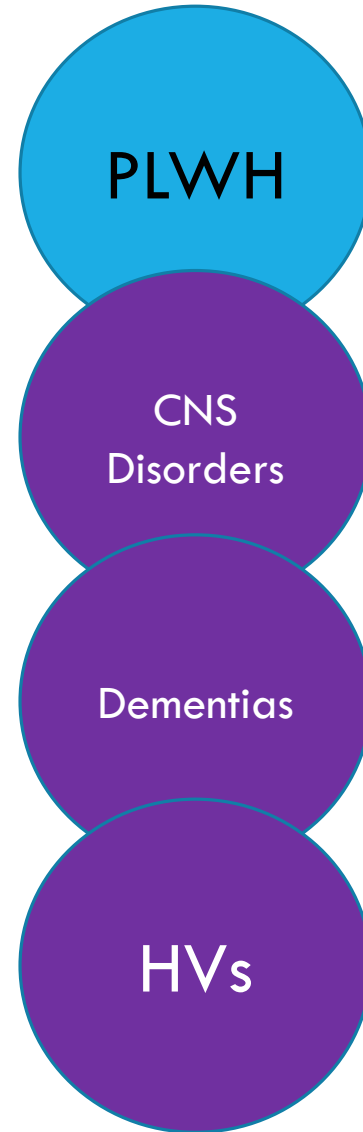
STUDY OF NASAL BRUSHING COLLECTED **OLFACTORY MUCOSA**
SAMPLES IN THE DIAGNOSIS OF HUMAN ENCEPHALOPATHIES
NCT02951559

1. Primary Outcome :

- Diagnostic concordance with gold standard diagnostic procedures

2. Secondary Outcomes:

- Side effects of the nasal brushing
- Cytology and immunohistochemistry
- OM markers of neuronal damage and amyloid deposition
[tau, p-tau, 1-42 beta amyloid, S100beta, alpha synuclein, TDP-43 on cell blocks and supernatant]

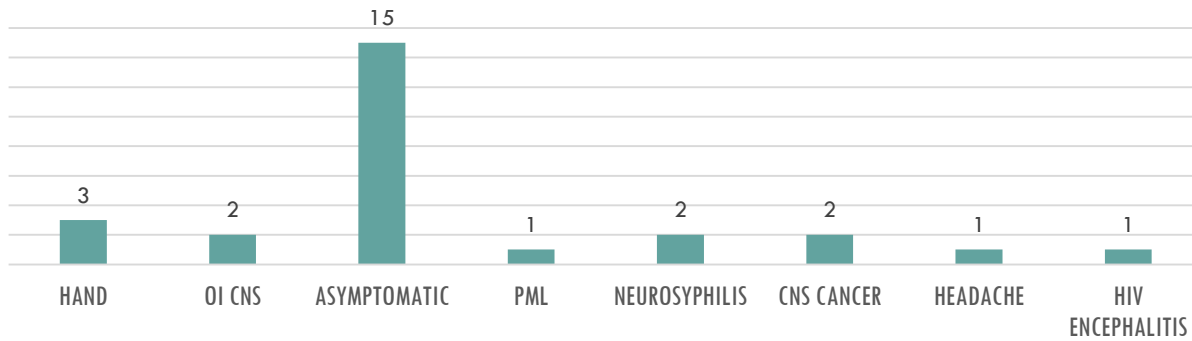


BASELINE CHARACTERISTICS

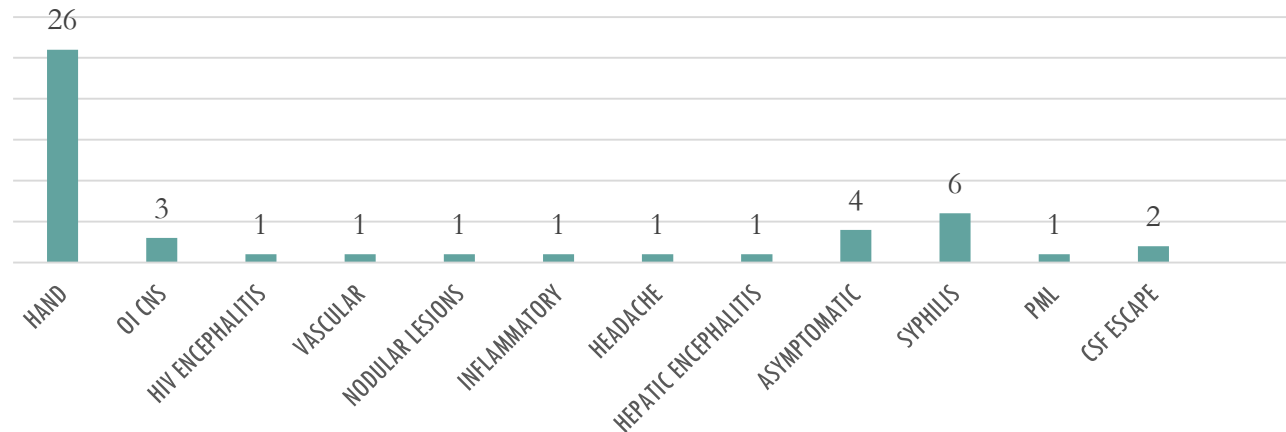
	HIV +	HIV -
	n (%) or median (IQR)	
n	90	32
Male gender	64 (71.9%)	19 (59.4%)
Age (years)	51 (45-57)	47.6 (36.1-60.7)
• Caucasian	78 (88%)	30 (93.75%)
• South American	6 (6.7%)	1 (3.15%)
• African	5 (5.6%)	1 (3.15%)
BMI (Kg/m²)	23.5 (19.8-26.6)	----
HCV+	14 (15.7%)	0
HBV+	9 (10.1%)	1 (3.15%)
Cirrhosis	4 (4.5%)	0
• MSM	32 (35.9%)	----
• MSW/WSM	27 (30.3%)	
• IVDU	17 (19.1%)	

DIAGNOSIS

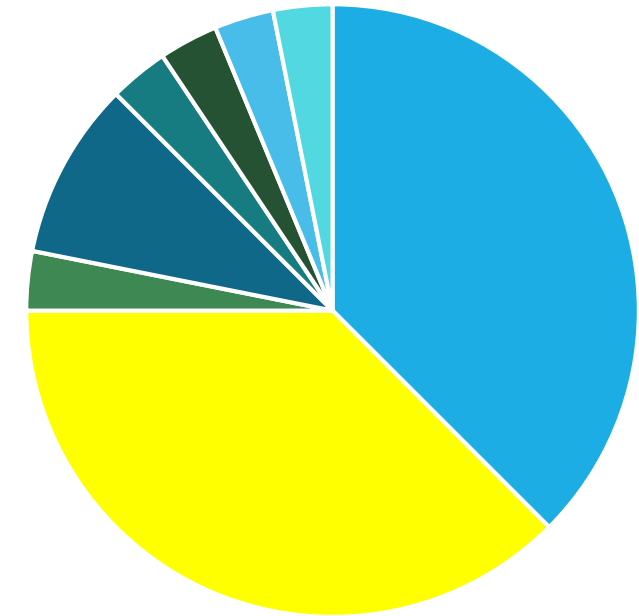
PLWH naive



PLWH on treatment

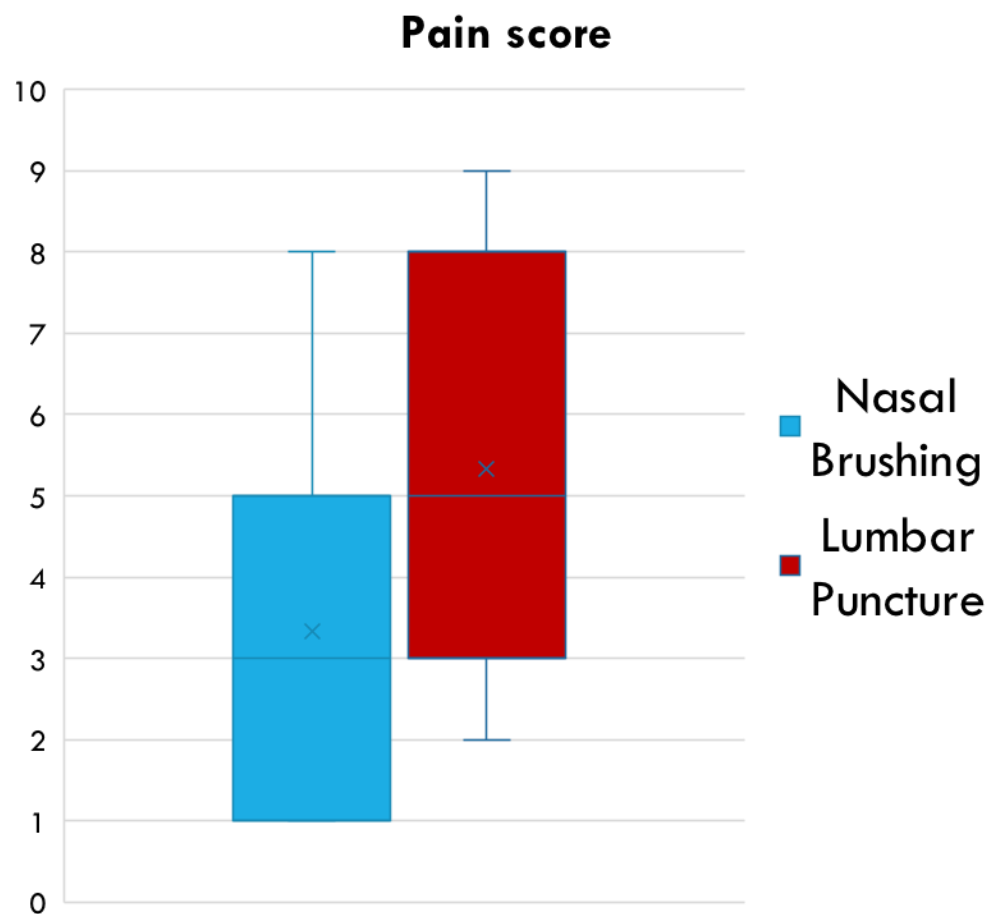


HIV-

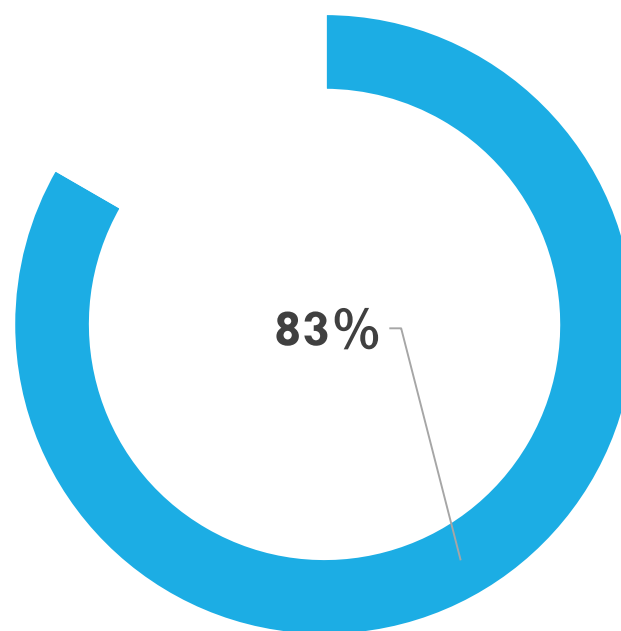


- Aseptic meningitis
- PML
- Creutzfeldt-Jacob
- Uveitis
- Dementia
- Healthy control
- ALS
- Autoimmune Encephalitis

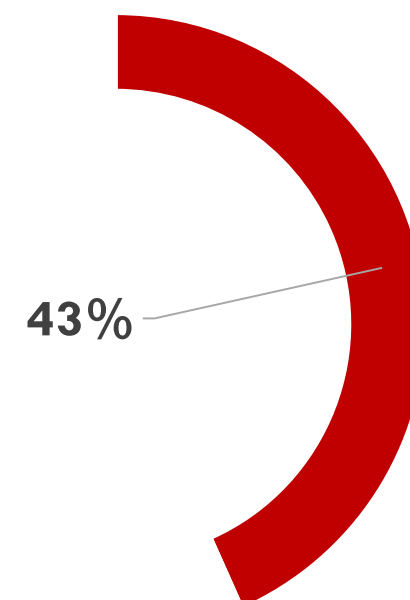
TOLERABILITY



Yearly test?



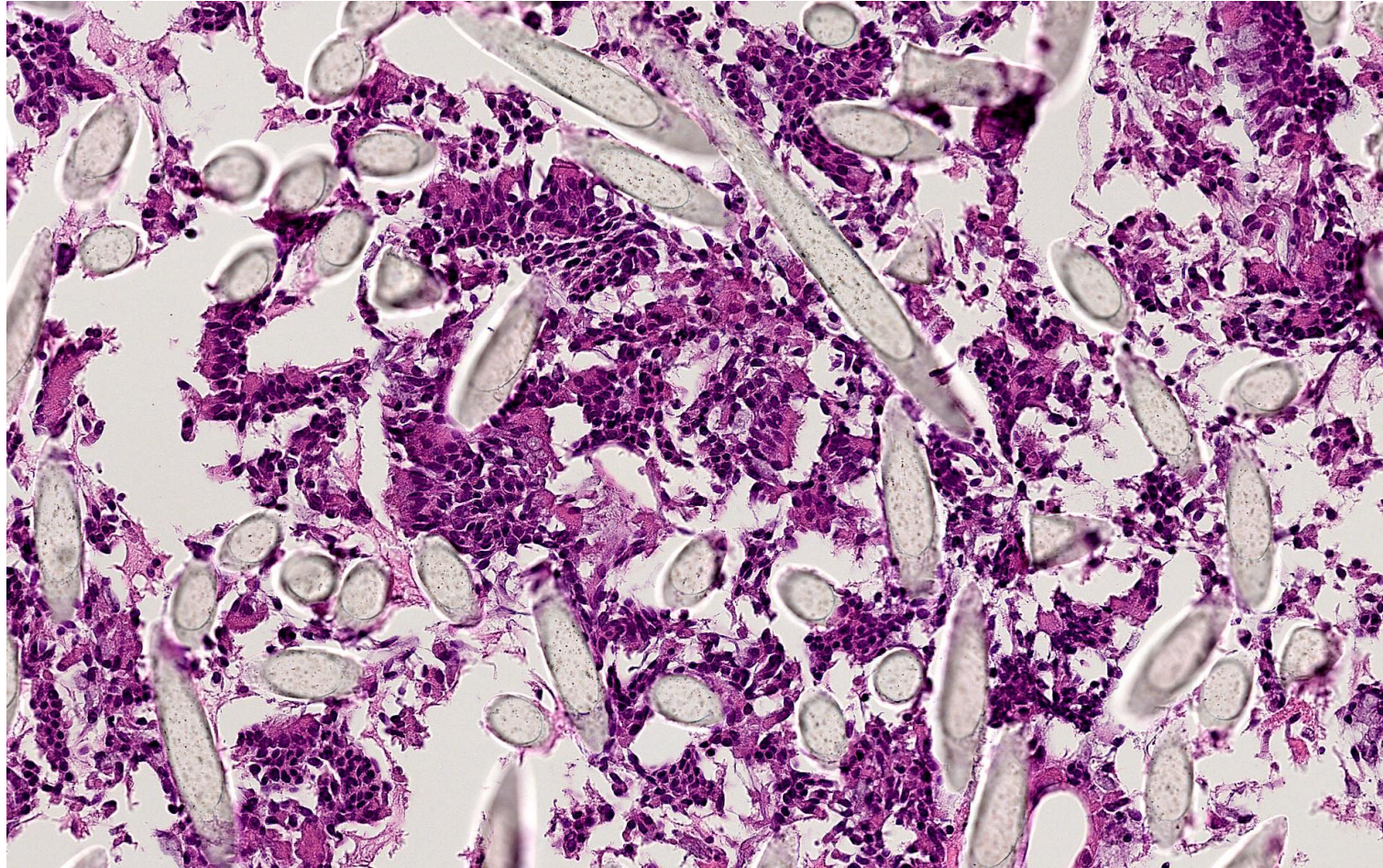
Yearly test?



CELLS

H&E

CD4+



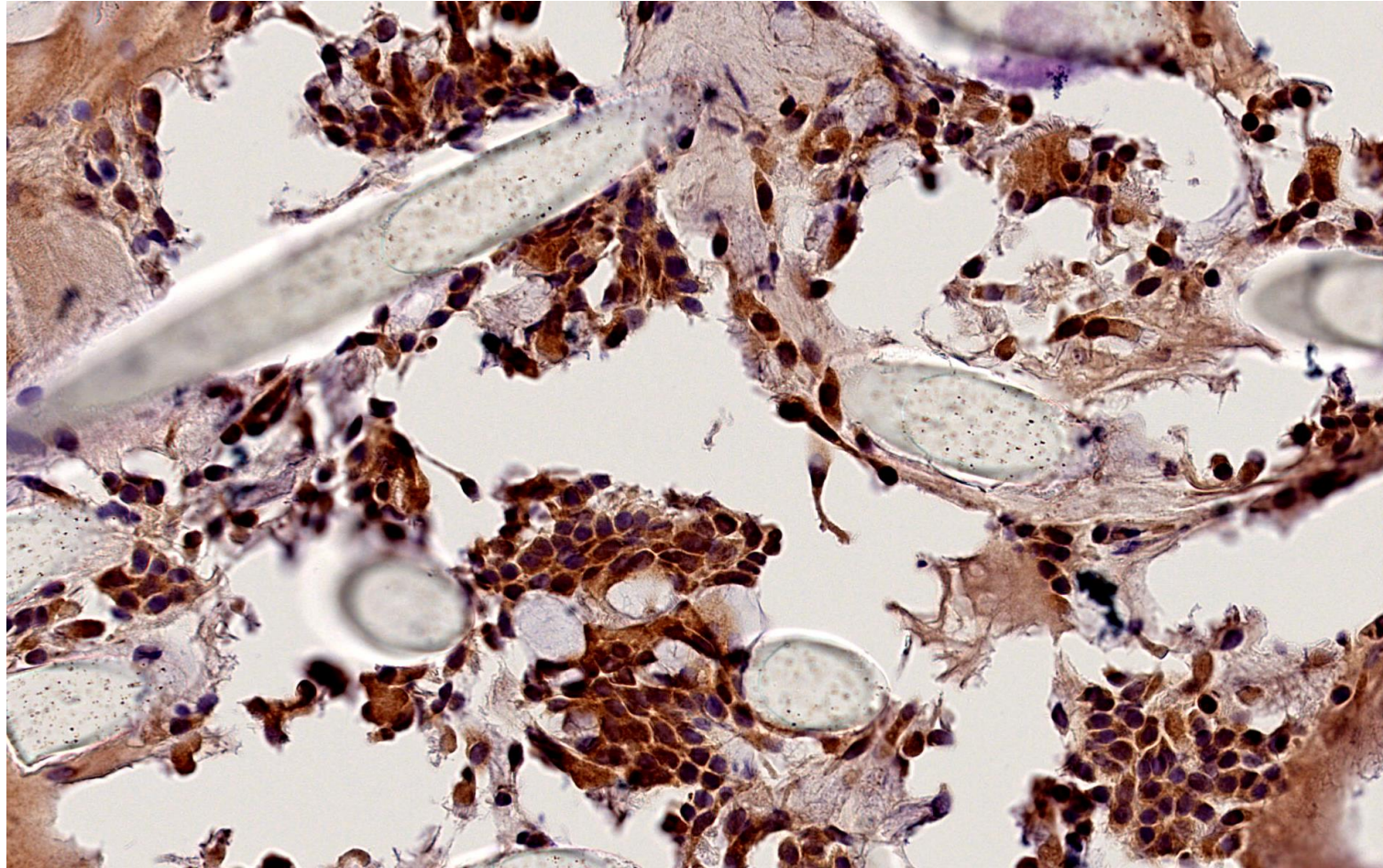
OMP

CD8+

CELLS

H&E

CD4+



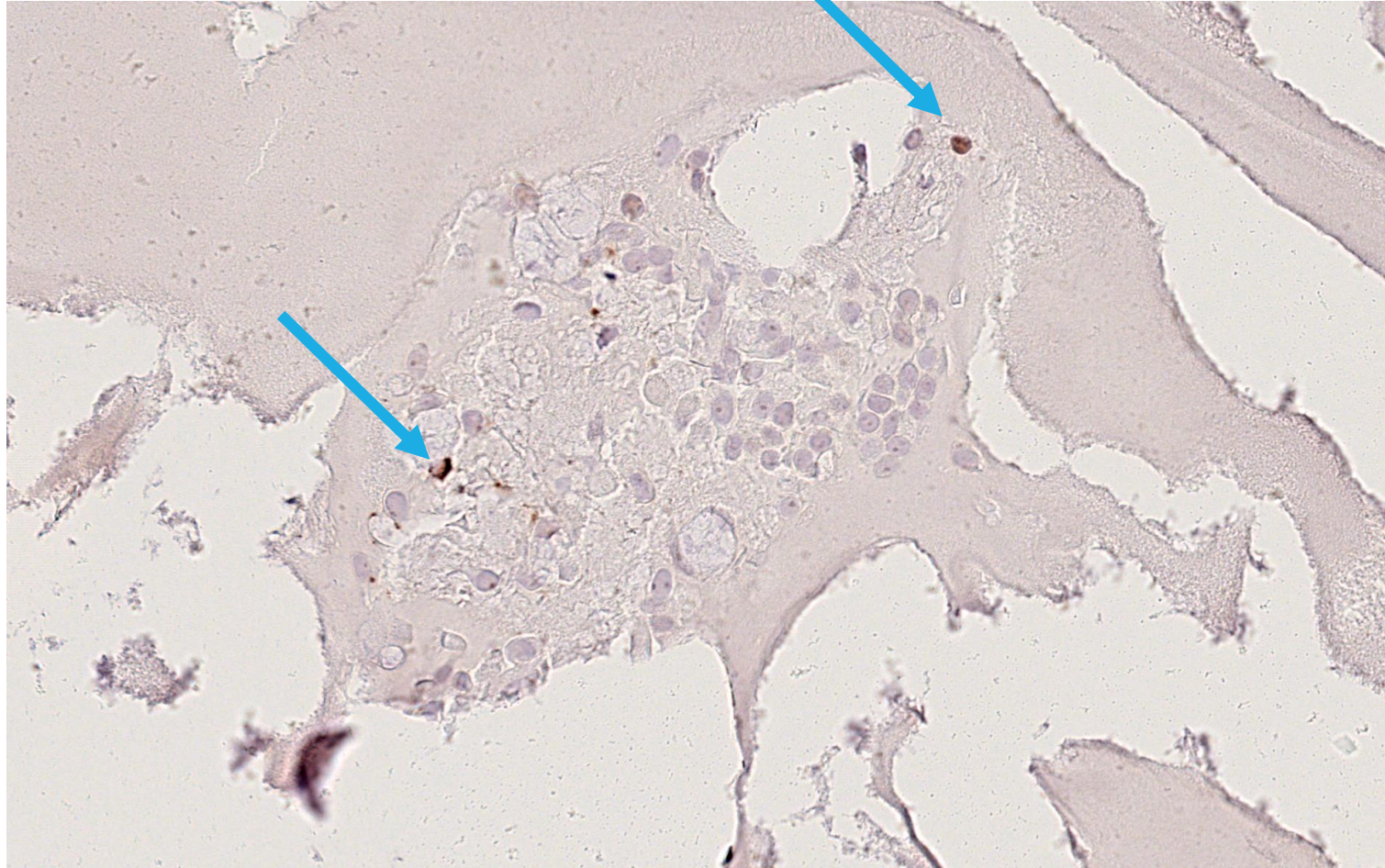
OMP

CD8+

CELLS

H&E

CD4+



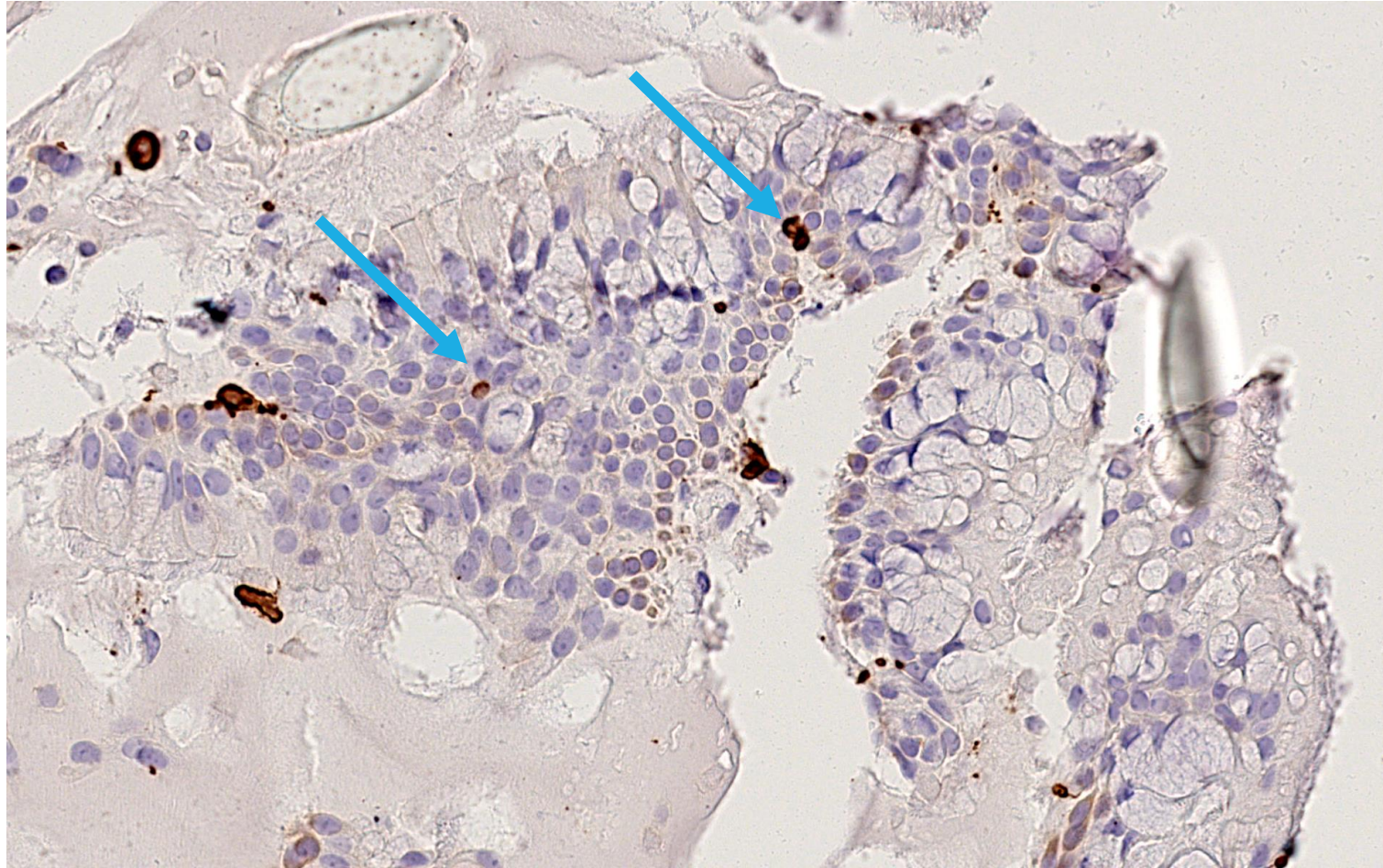
OMP

CD8+

CELLS

H&E

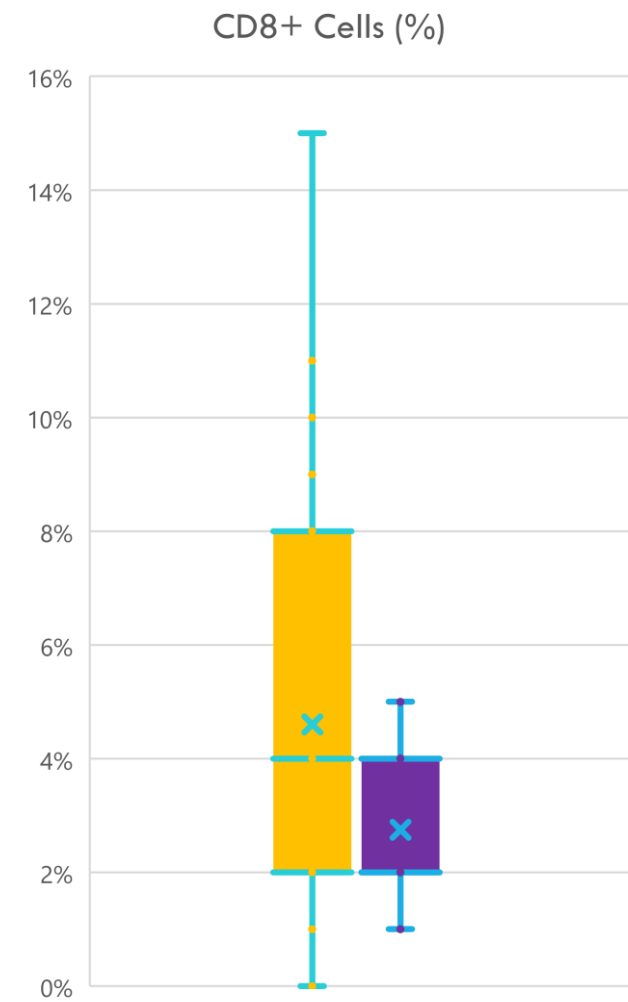
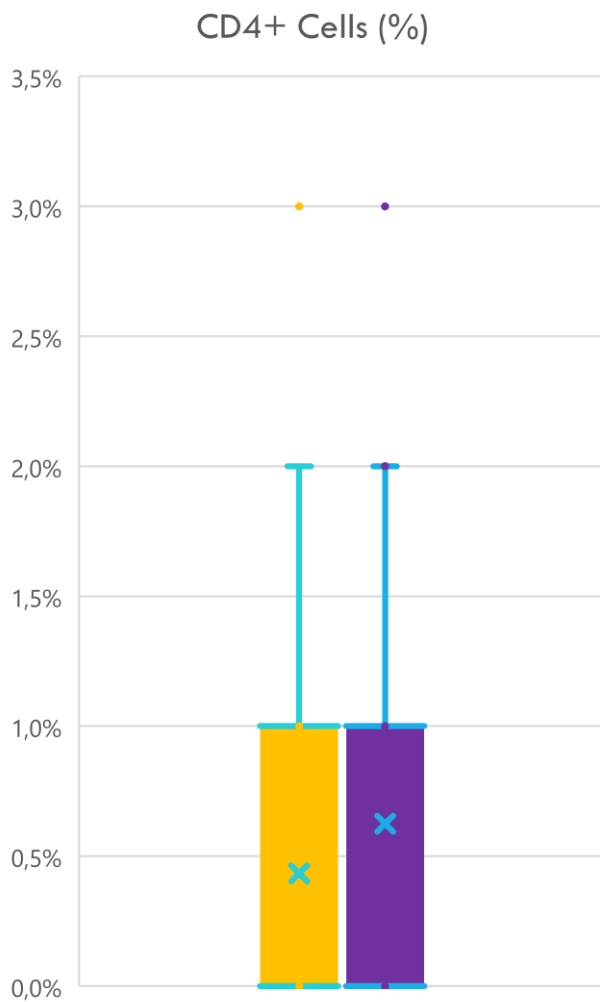
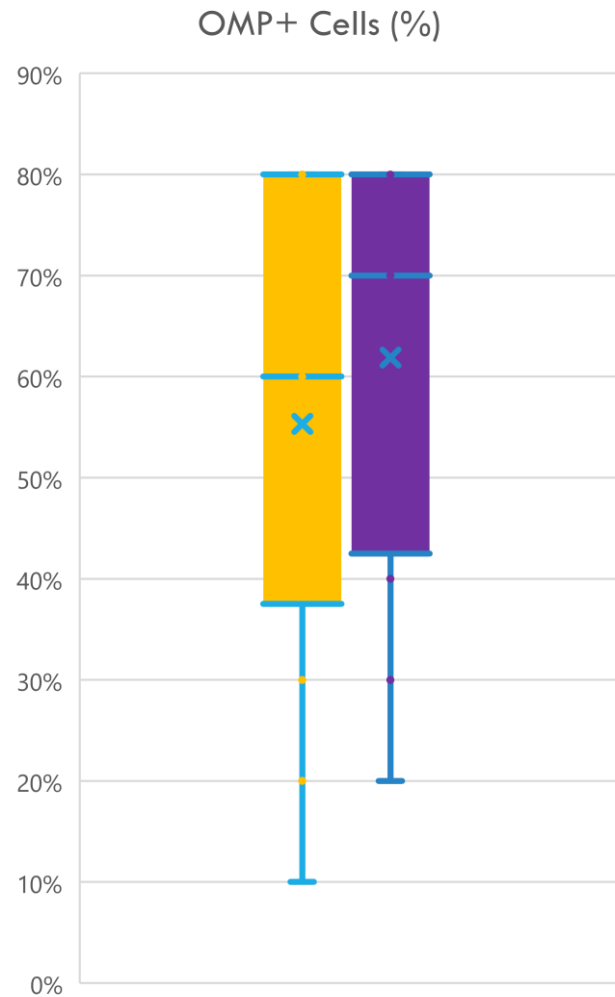
CD4+



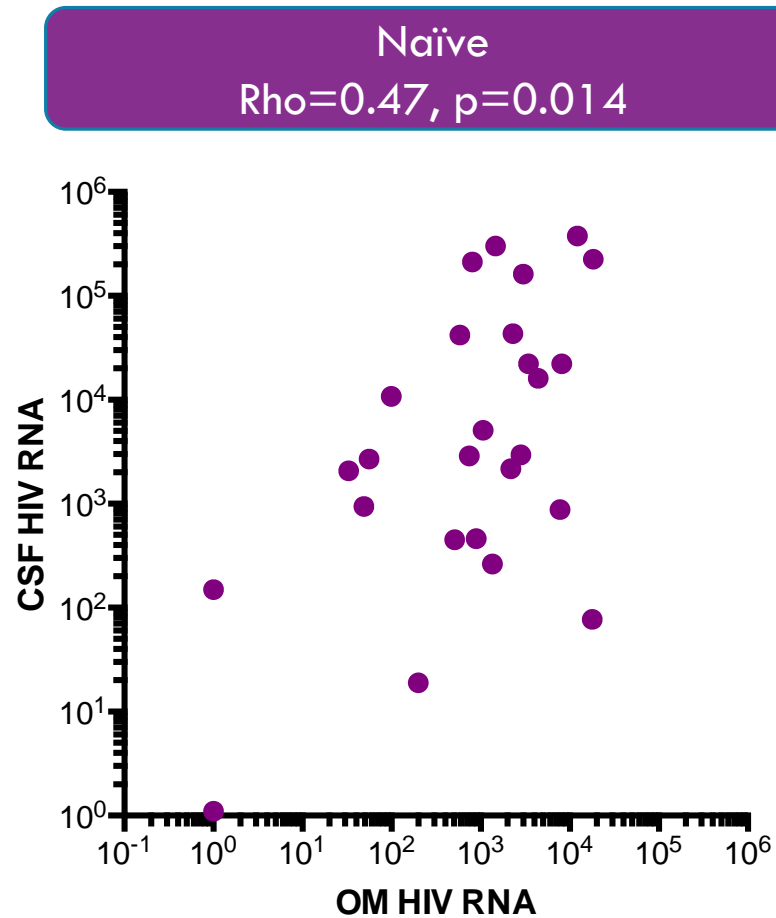
OMP

CD8+

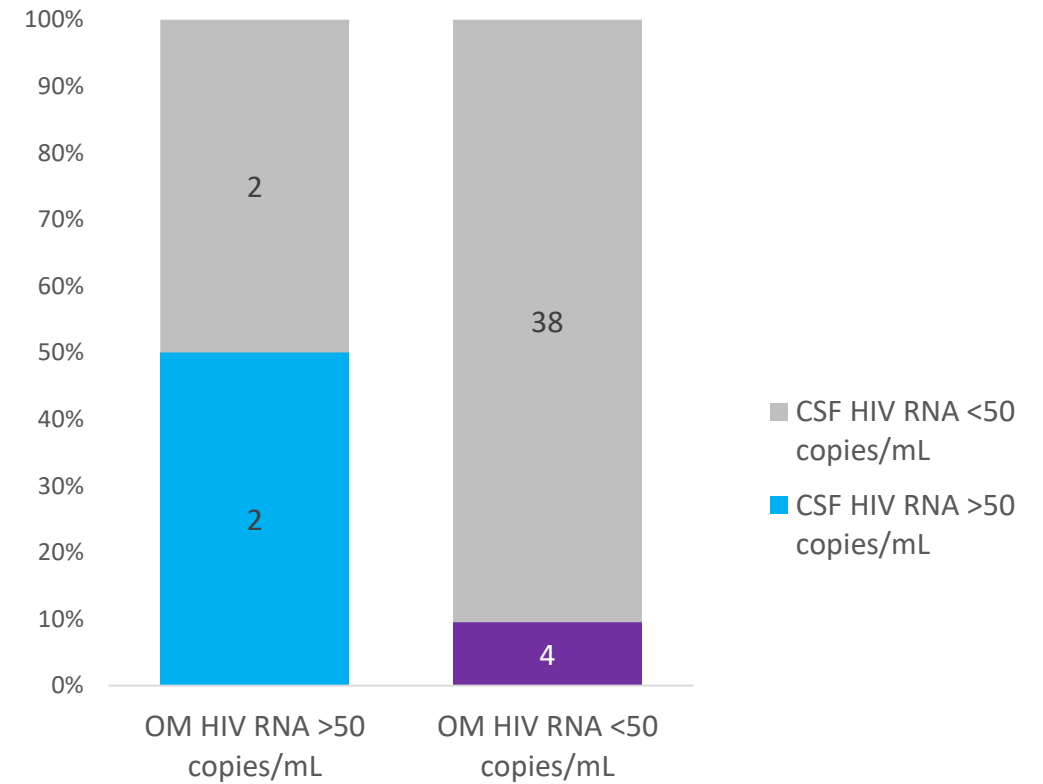
IMMUNE-HISTOCHEMISTRY



HIV RNA

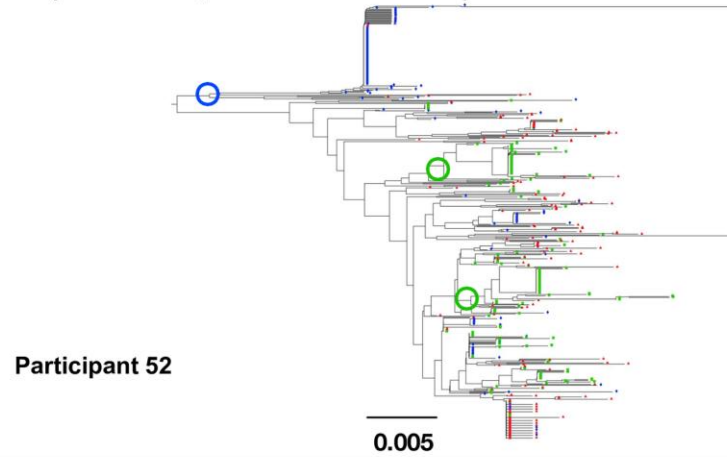


Plasma HIV RNA < 50 copies/mL
p=0.022, OR 9.5 (1.03-86.96)

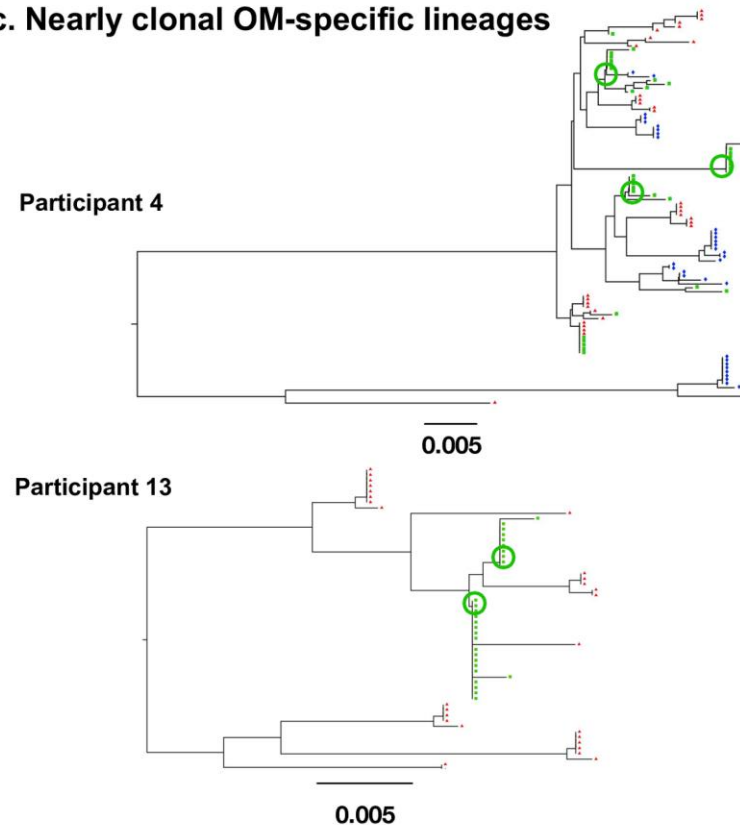


GENETIC DIVERSITY IN THE OM/CSF/SERUM

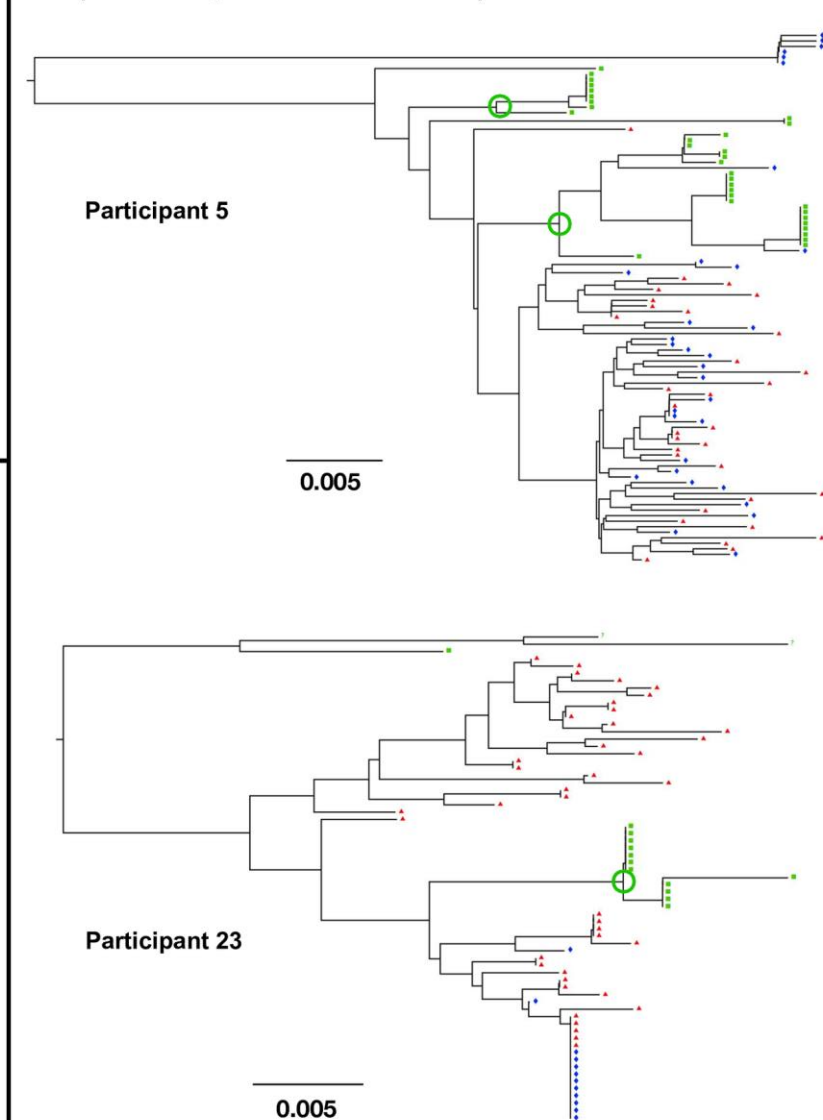
a. Diverse CSF-specific lineage
(CSF compartmentalization)



c. Nearly clonal OM-specific lineages



b. Diverse OM-specific lineages
(OM compartmentalization)



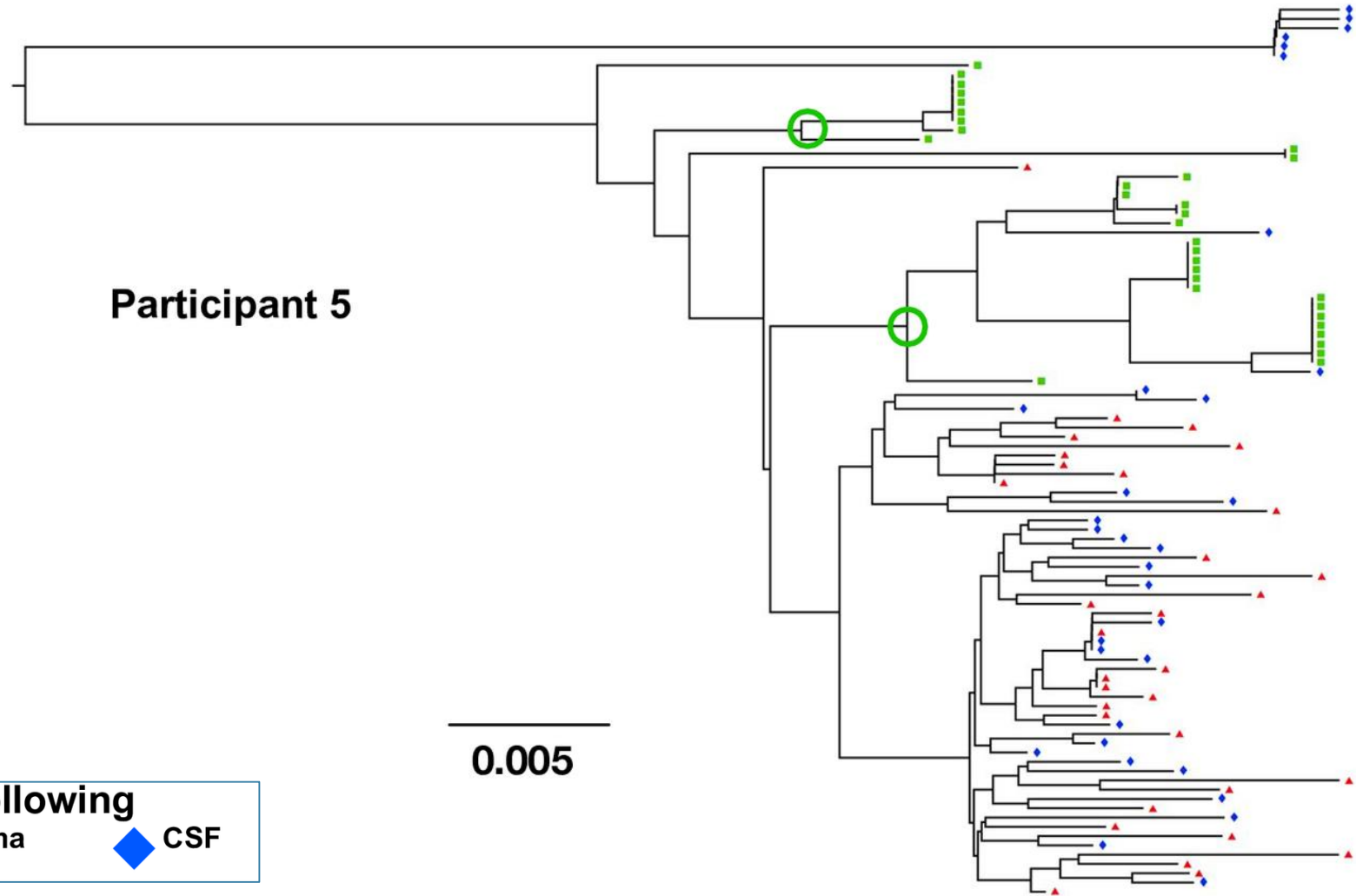
Sequences were derived from the following
types of samples: ■ OM ▲ Plasma ◆ CSF

GENETIC DIVERSITY IN THE OM/CSF/SERUM

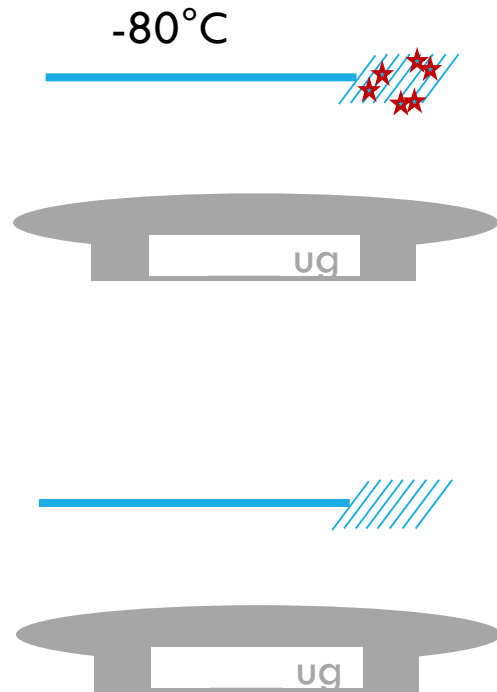
Participant 5

0.005

Sequences were derived from the following
types of samples: ■ OM ▲ Plasma ◆ CSF



PK ON OLFACTORY MUCOSA

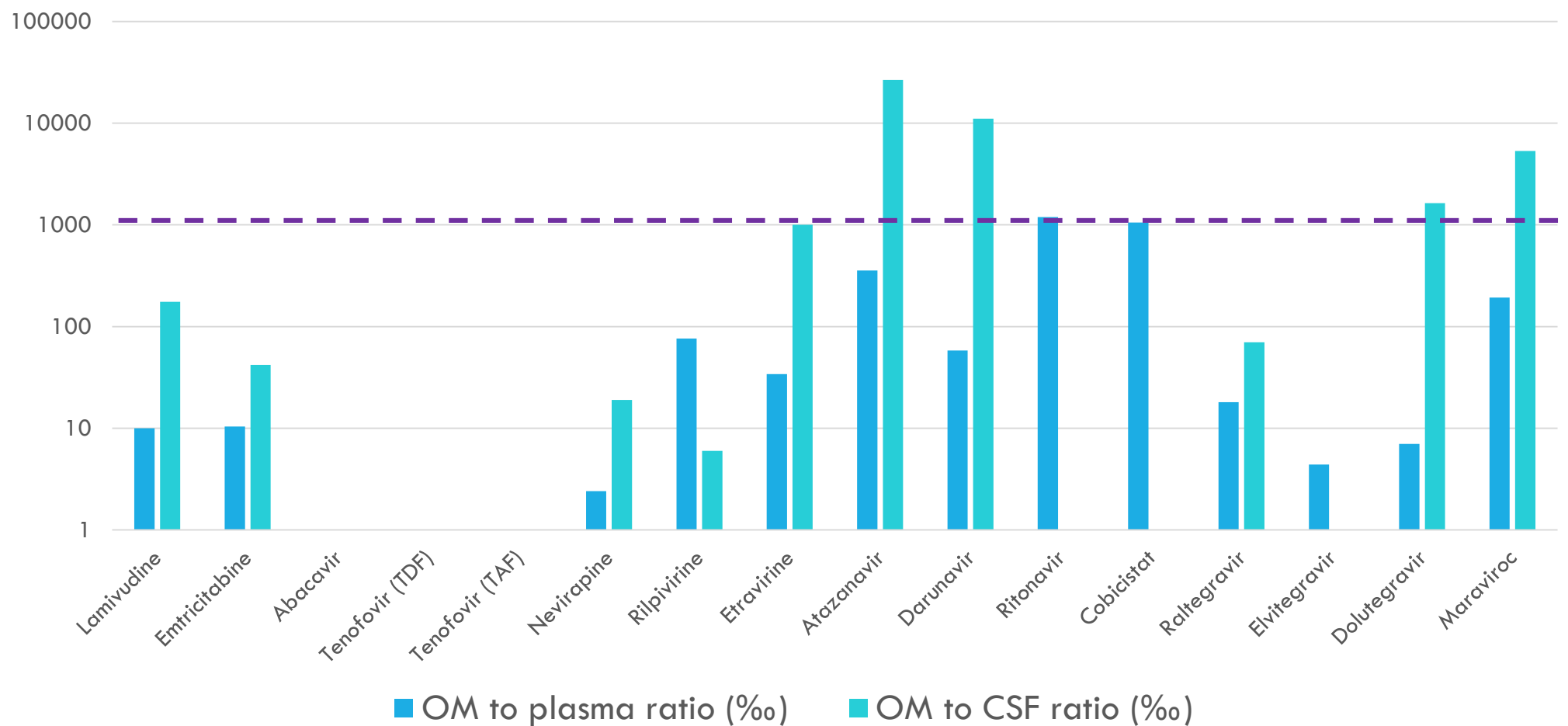


- 40 μl of internal standard (marked with stable isotopes) working solution plus 500 μl of water:methanol solution (30:70 v:v);
- vortex-mixed 10 sec and sonicated for 10 minutes at 40°C ;
- The dry extracts were dissolved in 110 μL of water:acetonitrile:acetic acid (94.9:5:0.1 v:v:v) solution and 10 μl of acid phosphatase (0.5 X) and incubated for 1 hour at 37°C

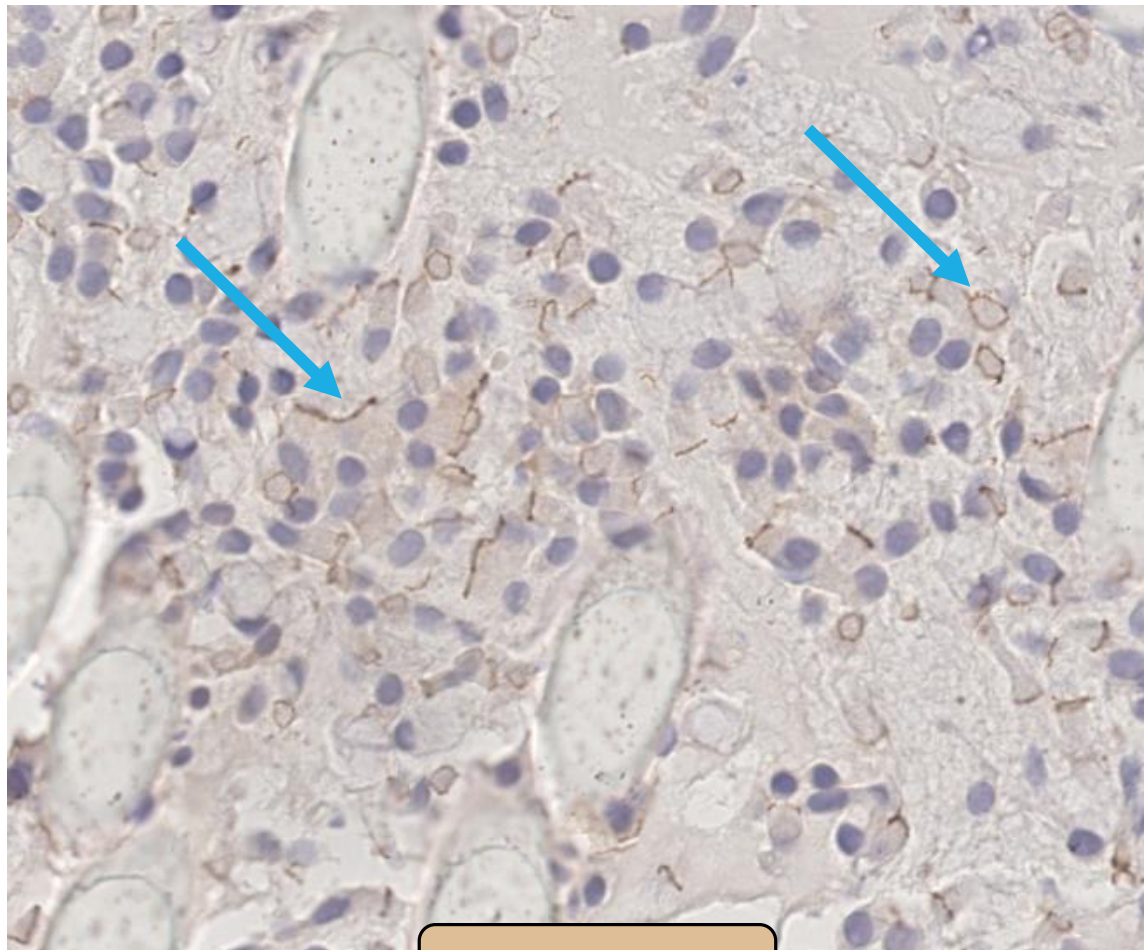


HPLC/MS-MS

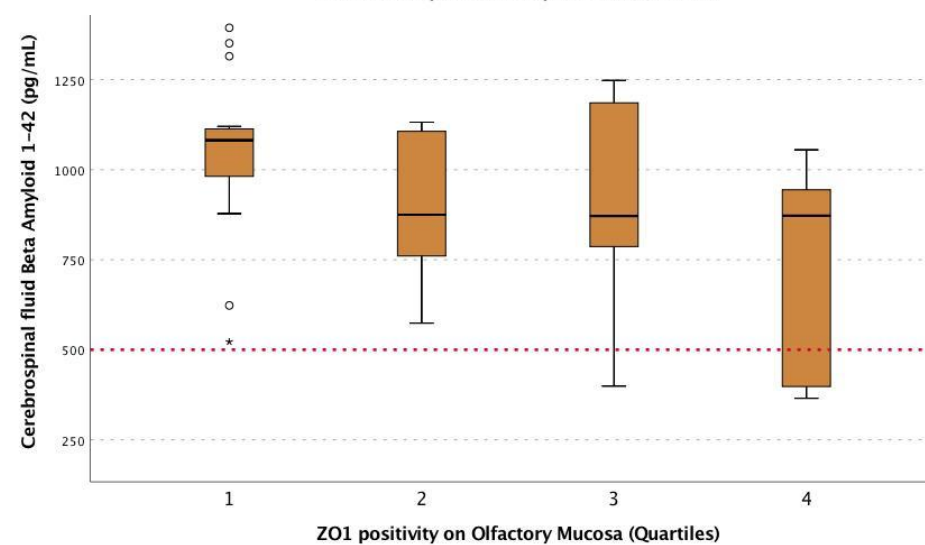
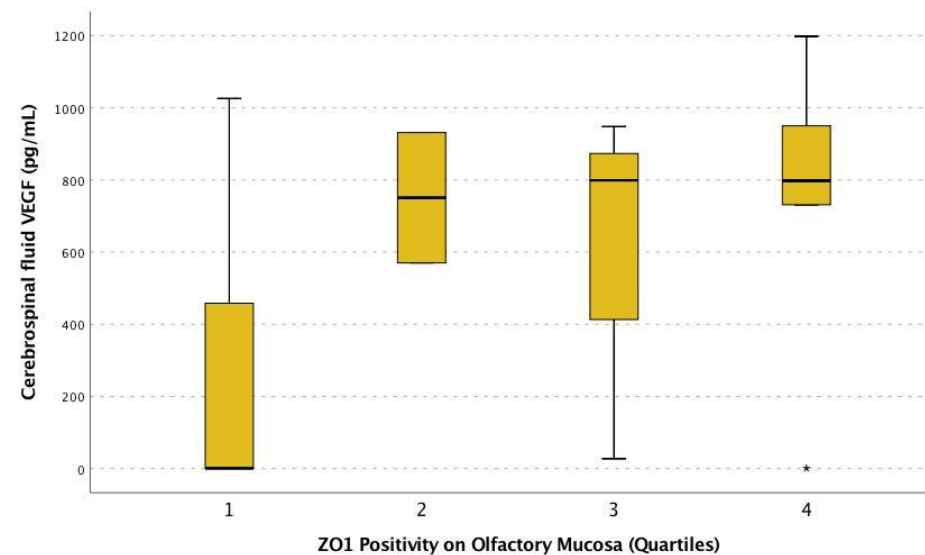
PK ON OLFACTORY MUCOSA (2)

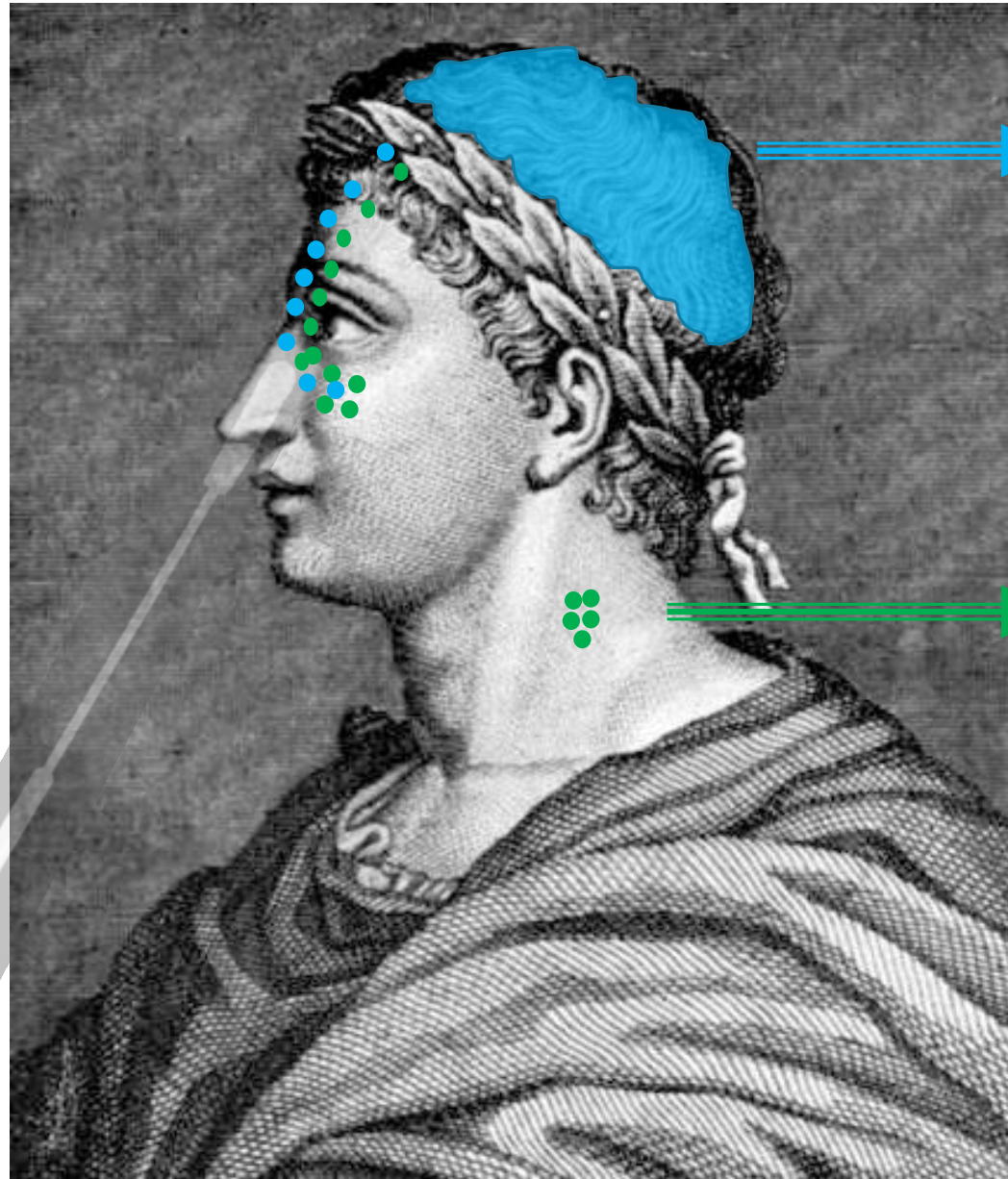


BLOOD BRAIN BARRIER STATUS ON THE OM



Zo-1 MoAb



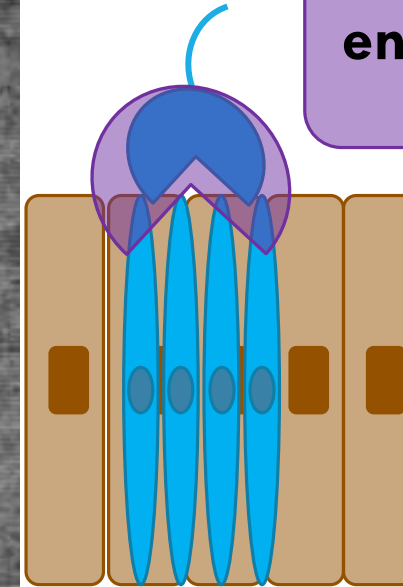
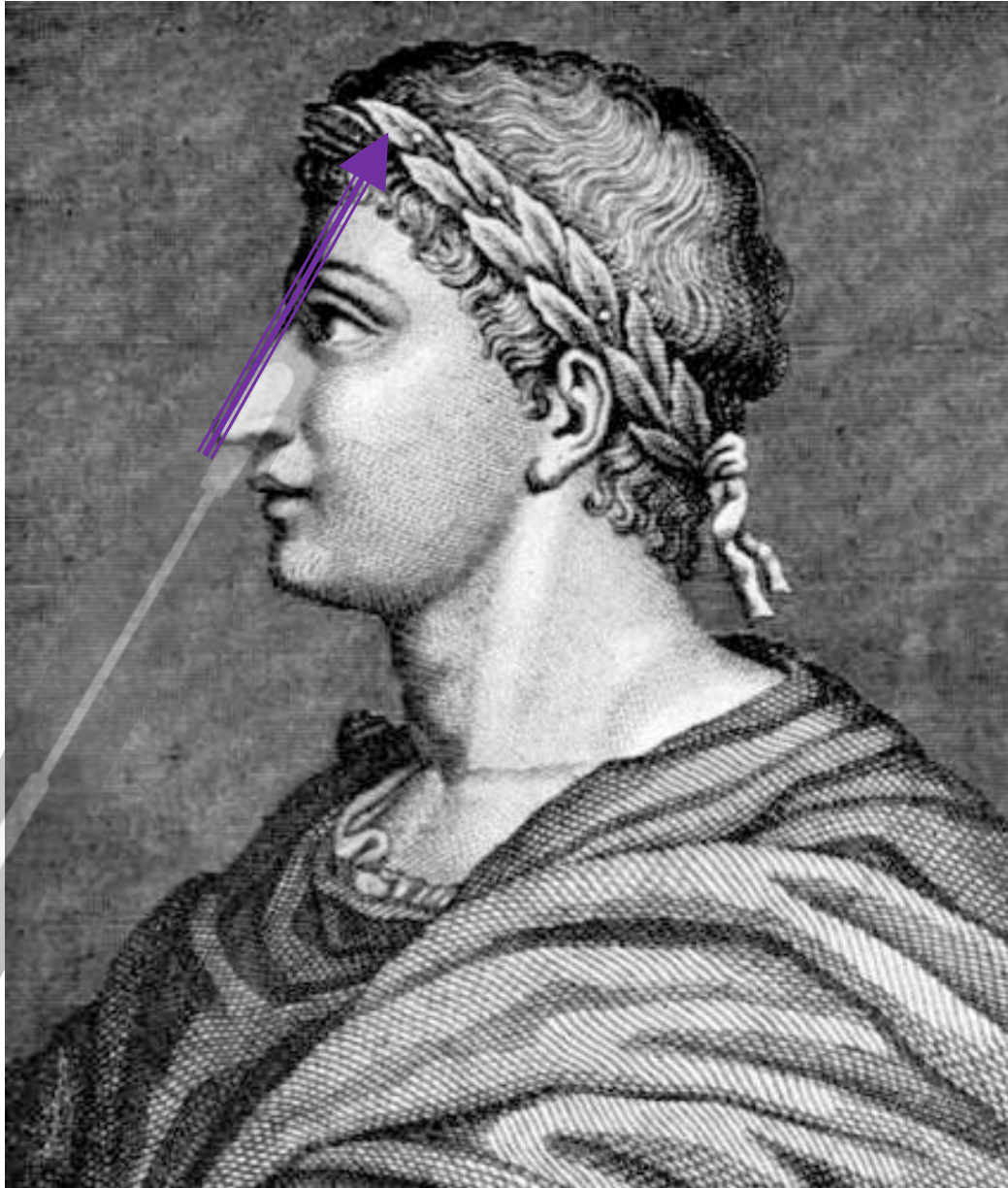


Infos from the Brain
Pranchyma??

Infos from the
Lymphatic system??

Way of entry of
bugs into the CNS?

Influenza
HSV-1?
HHV-6
Cryptococcus



Olfactory
ensheathing glial
cells

ONGOING STUDIES

- *Env* deep sequencing on OM, nostrils, **tonsils** and CSF
- **Metagenomics** on CSF and OM
- Tau, p-tau and Beta₁₋₄₂ on CSF and OM
- **Targeted Proteomics** → AD vs. HAND

ACKNOWLEDGEMENTS



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