OLFACTORY MUCOSA

THE RESERVE OF HIV RESERVOIRS?

Mattia Trunfio, MD
Department of Medical Sciences,
University of Torino

16th Residential Course
on Clinical Pharmacology
of Antiretrovirals

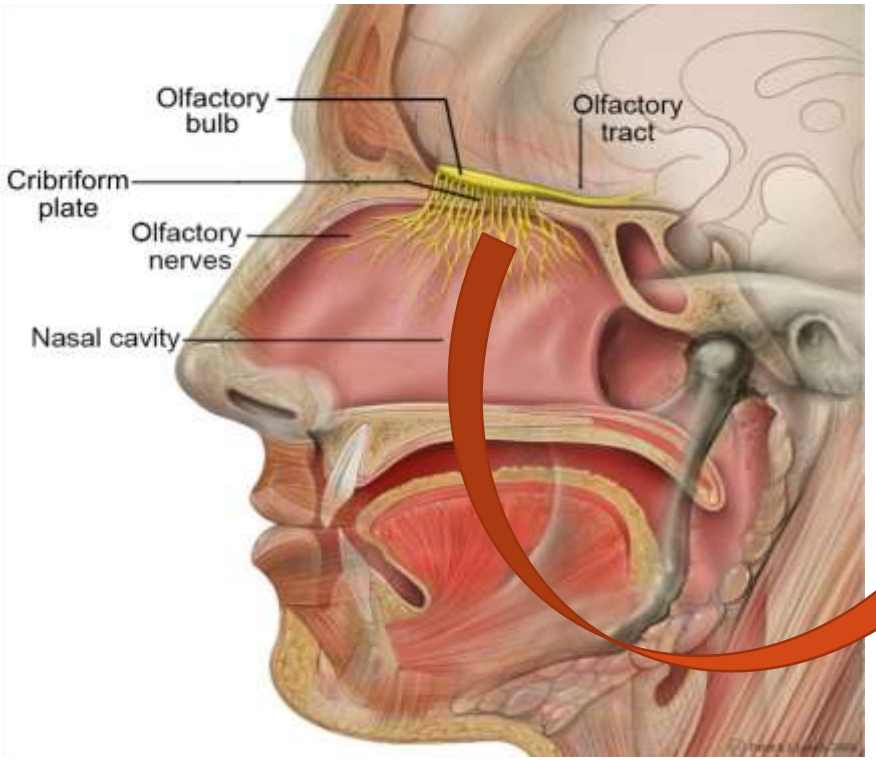
OUTLINE

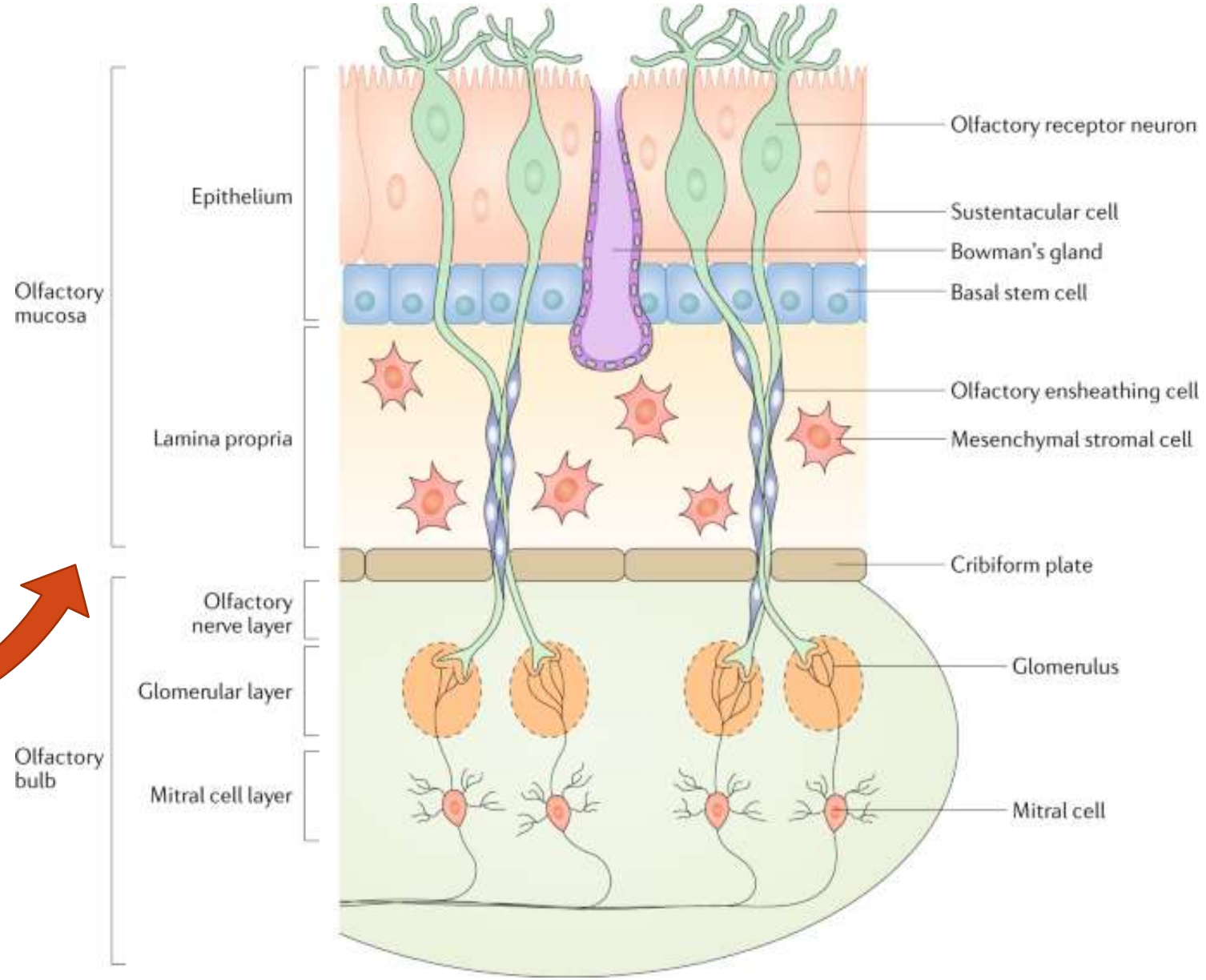
1. Is OM an HIV reservoir and could it be a surrogate for CNS reservoir?

2. Do we need new reservoirs?

3. If yes, is OM a valuable candidate?

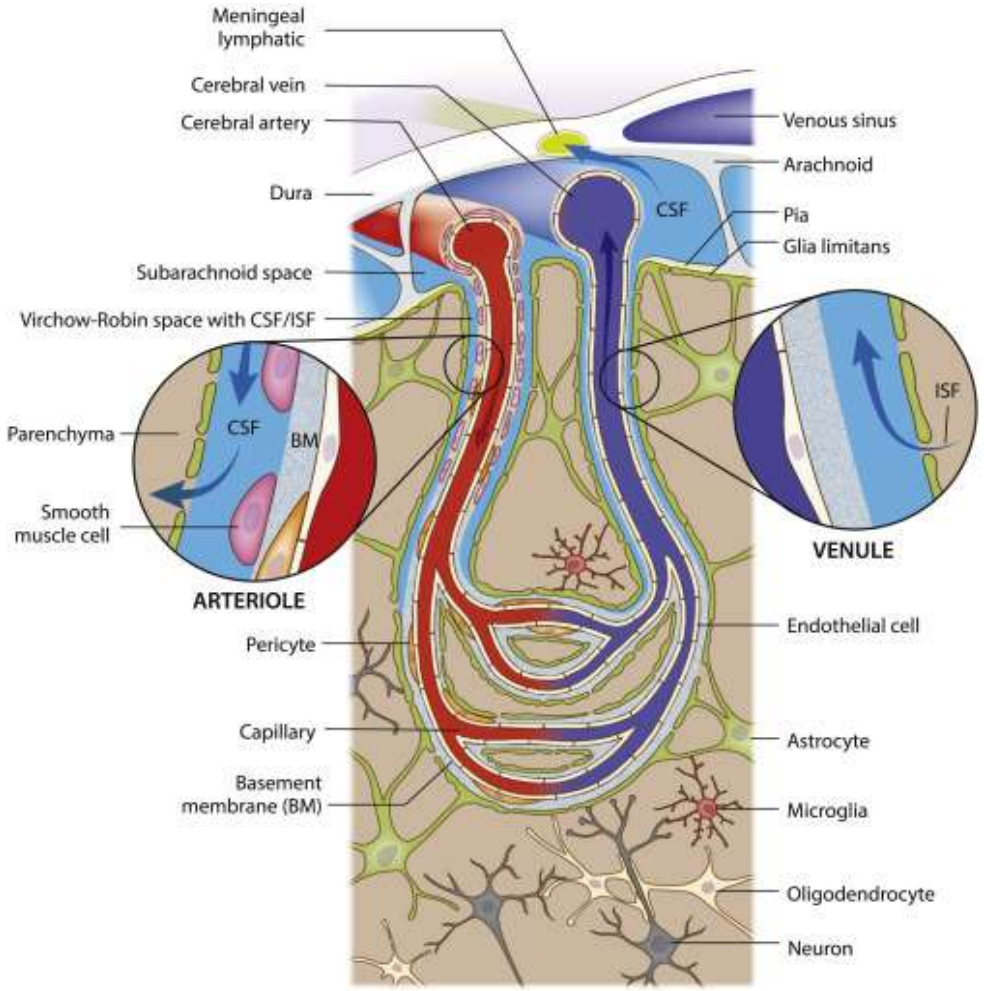
Olfactory Mucosa

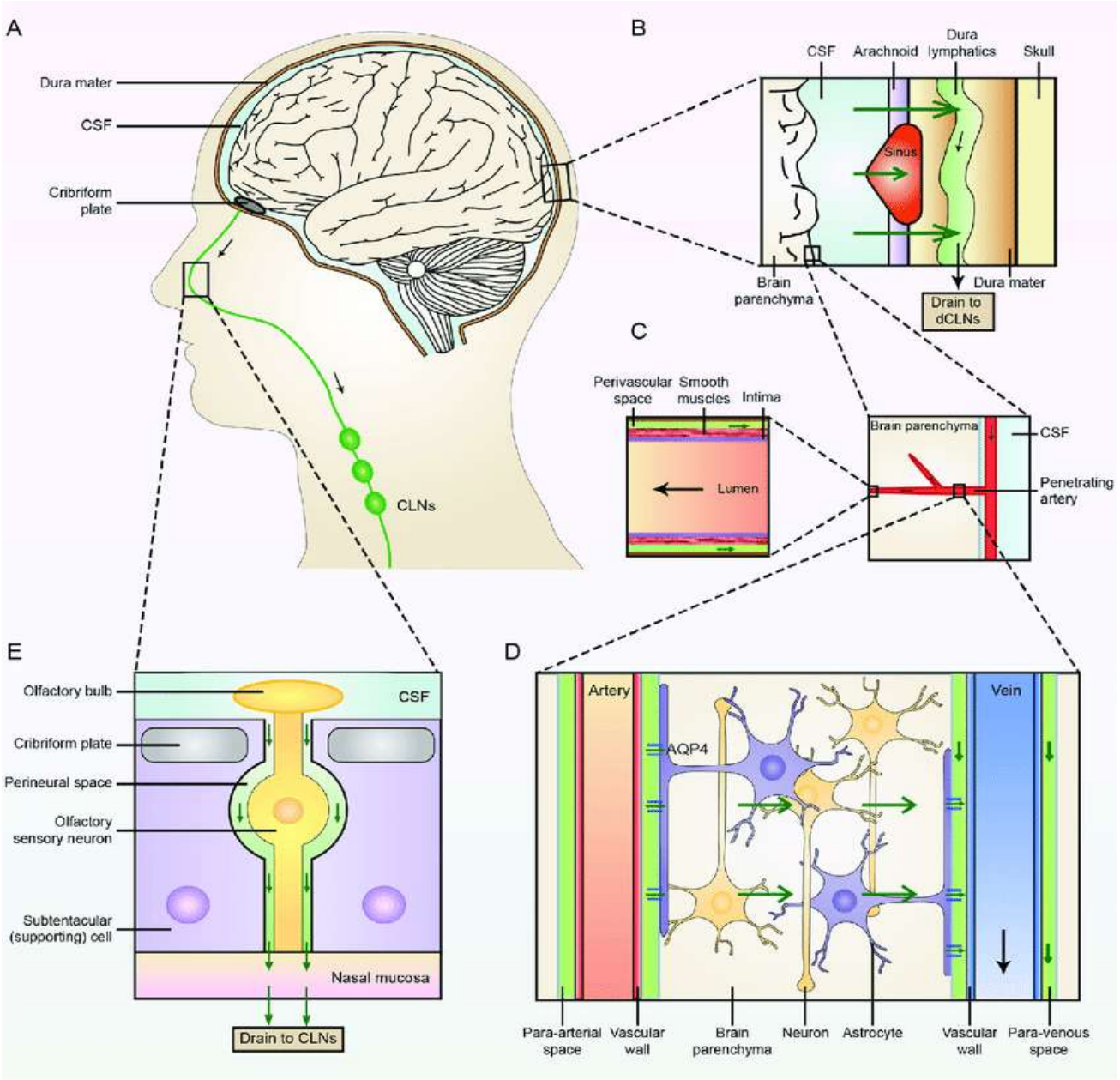




Lindsay LS et al, Nature Rev Neurol 2020

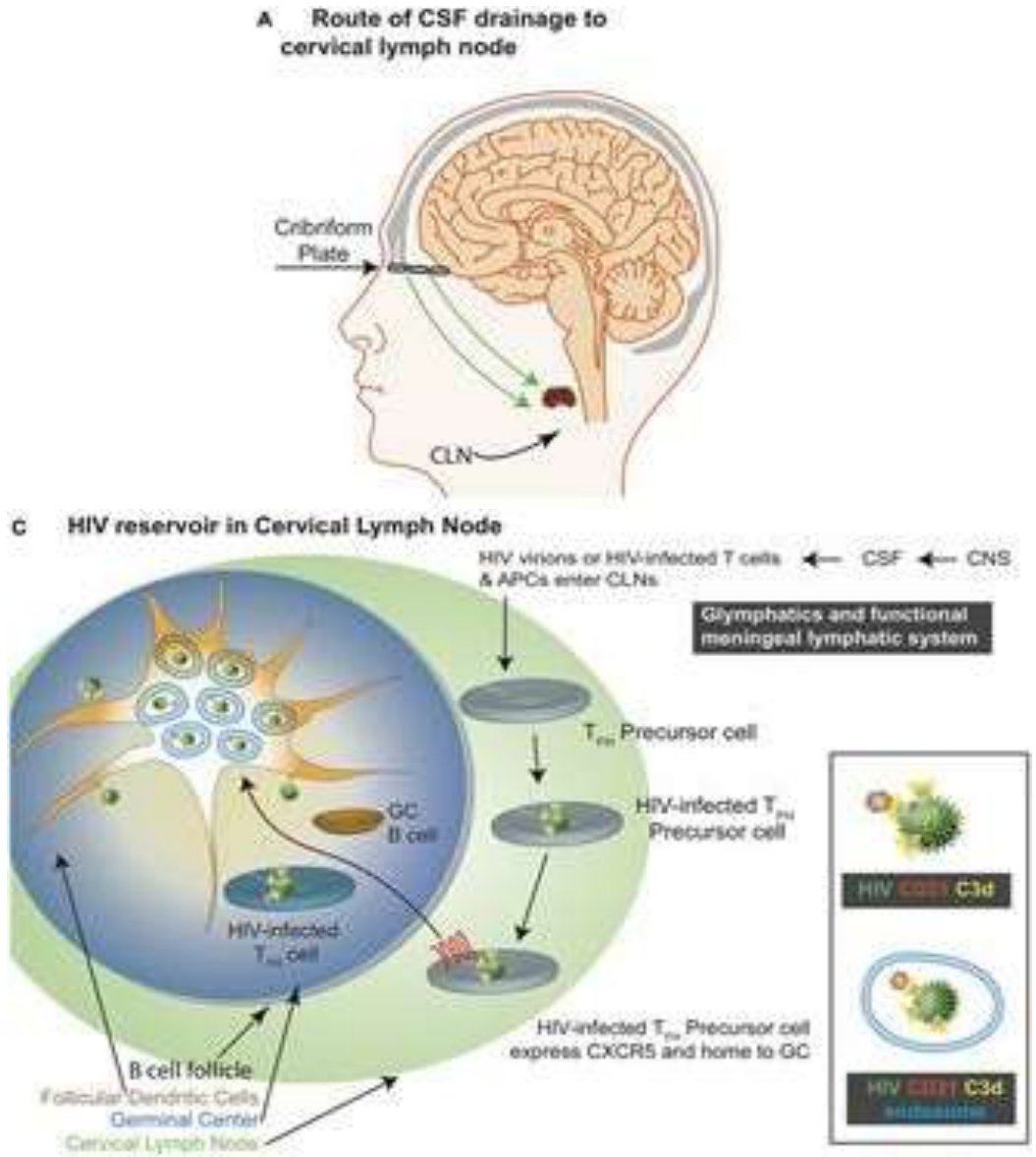
Glymphatic and Meningeal Lymphatic System

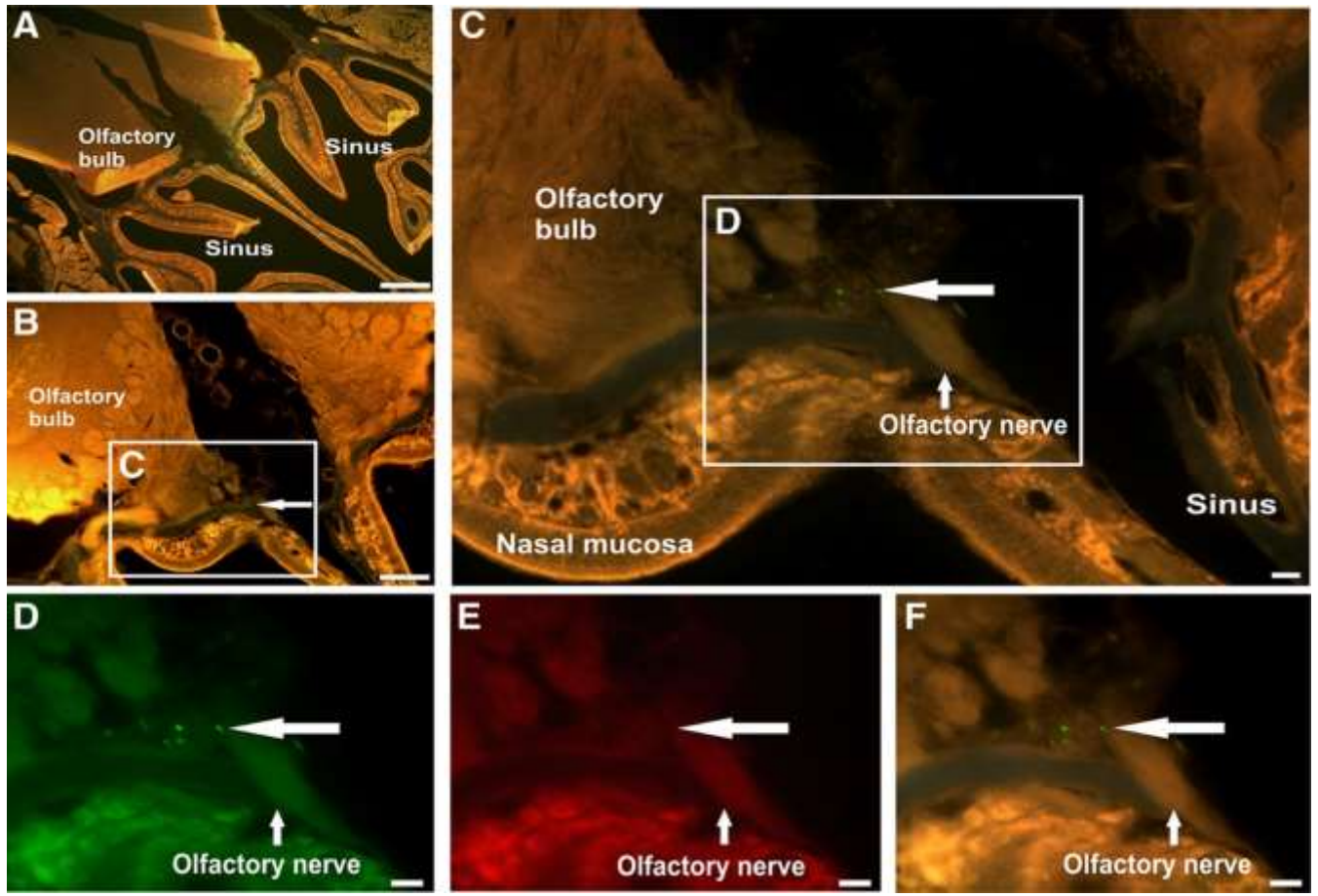




Da Mesquita S et al, Neuron 2018
Bao-Liang S et al, Adv Neurobiol 2017

OM as bridge between CNS and Deep Cervical Lymph node

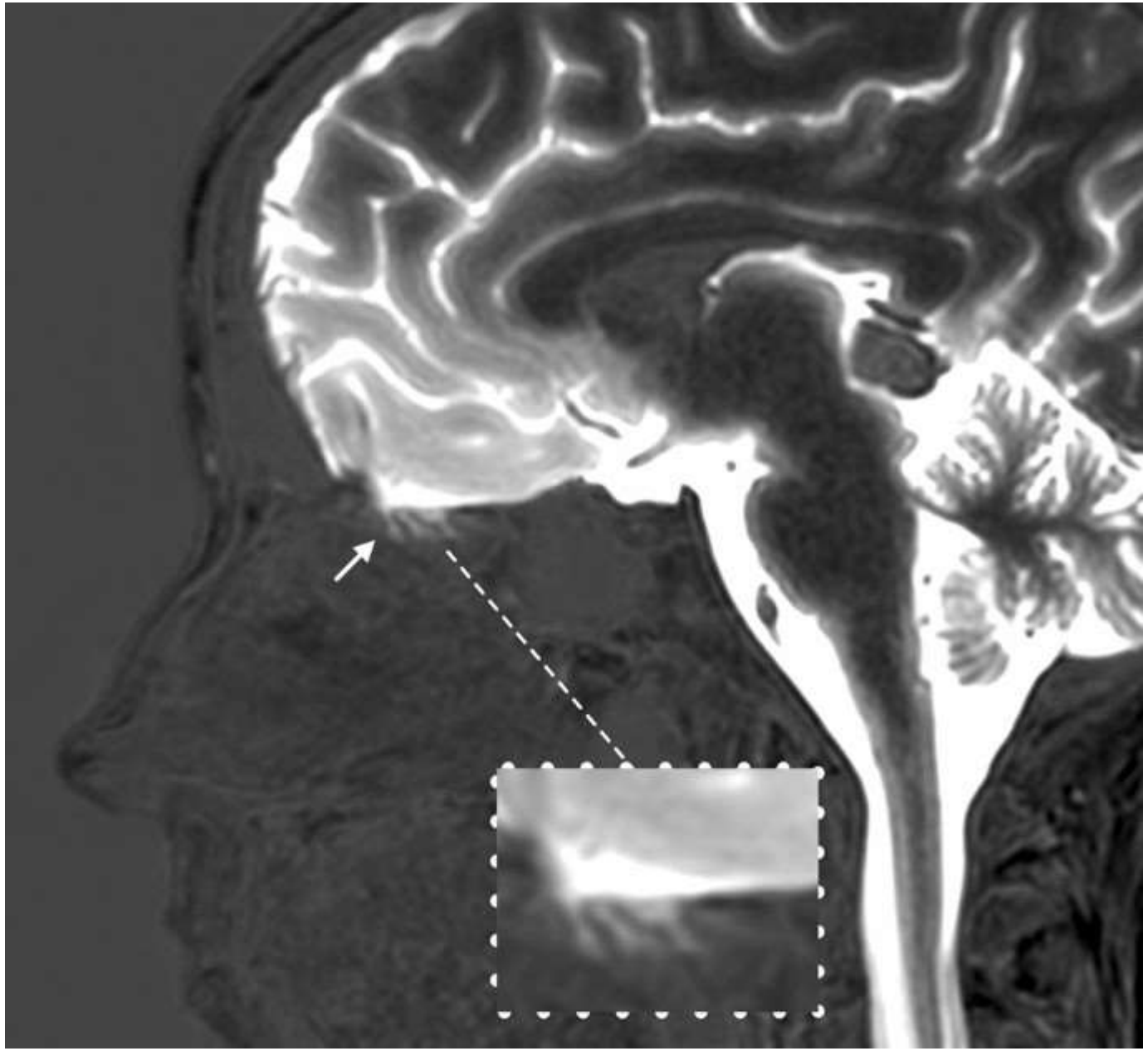




Rajnish SD et al, Front Immunol 2018
Kaminski M et al, J Leukocyte Biol 2012

In vivo assessment of cerebrospinal fluid efflux to nasal mucosa in humans

- Animal studies consistently suggest that the cribriform plate and nasal lymphatic vessels are crucial for molecular clearance from CSF
- Consecutive magnetic resonance imaging during 48 h after intrathecal administration of a tracer was performed in 24 patients
- Despite a strong enrichment of tracer in CSF spaces nearby the cribriform plate, there was no significant enrichment of CSF tracer in nasal mucosa



Melin E et al, Scient Rep 2020

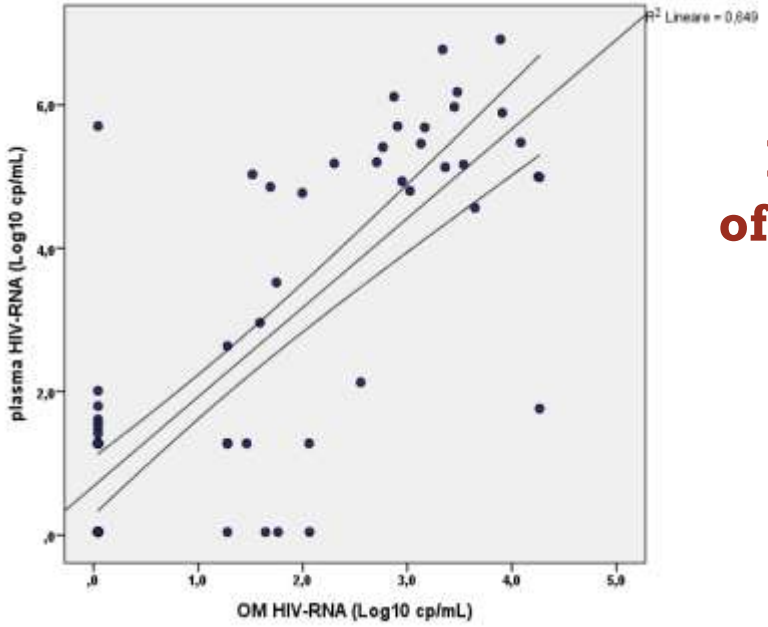
HIV-1 detection in the OM of HIV-1-infected participants

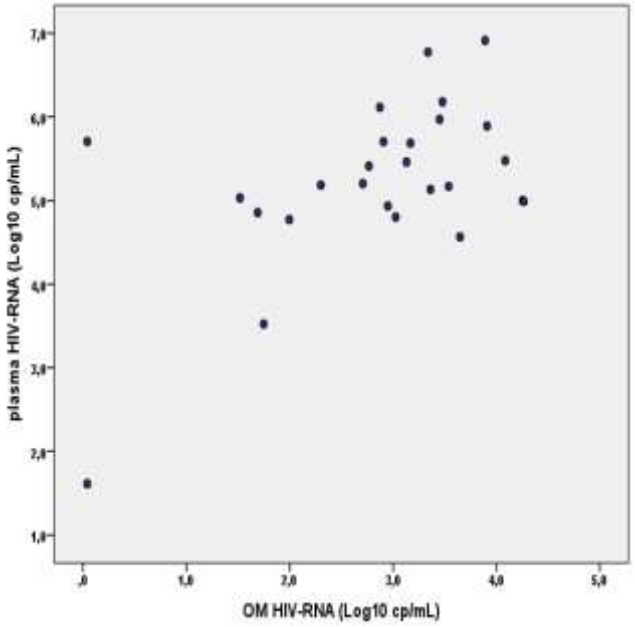
Study population (79)

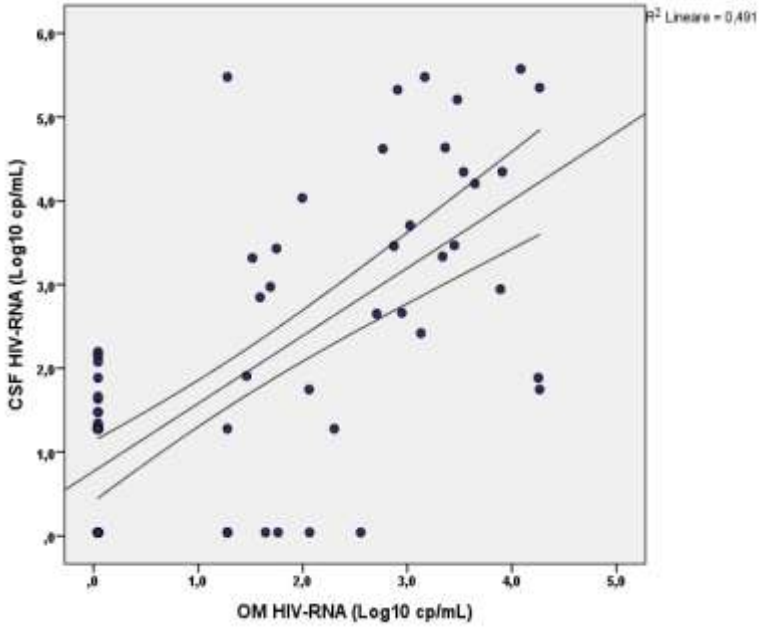
Naive only (27)

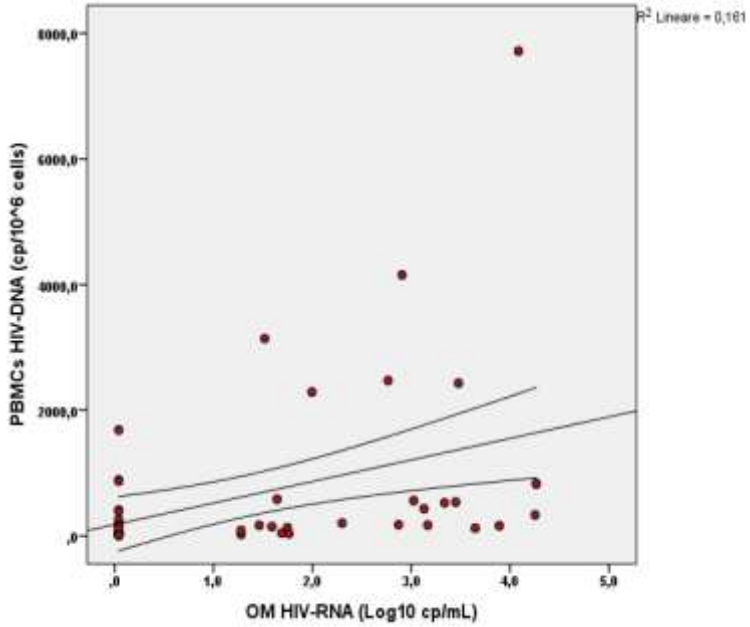
Plasma

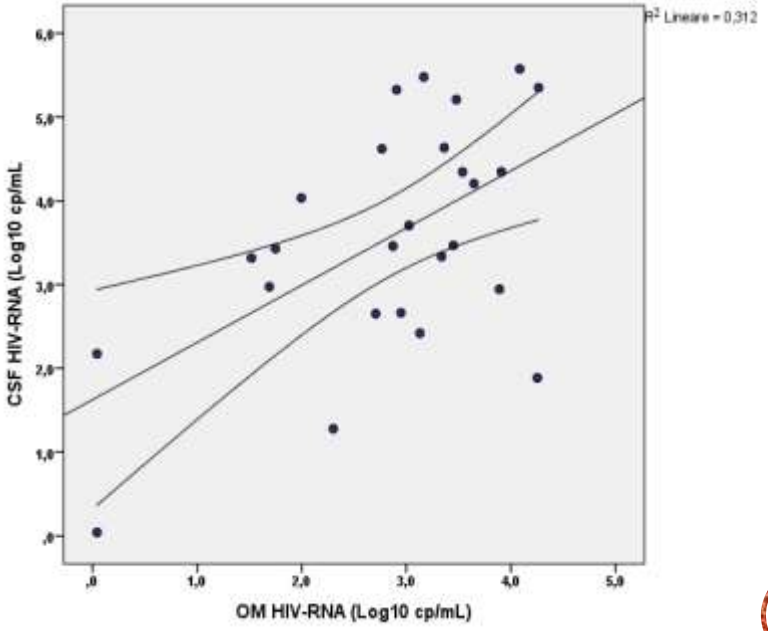
CSF







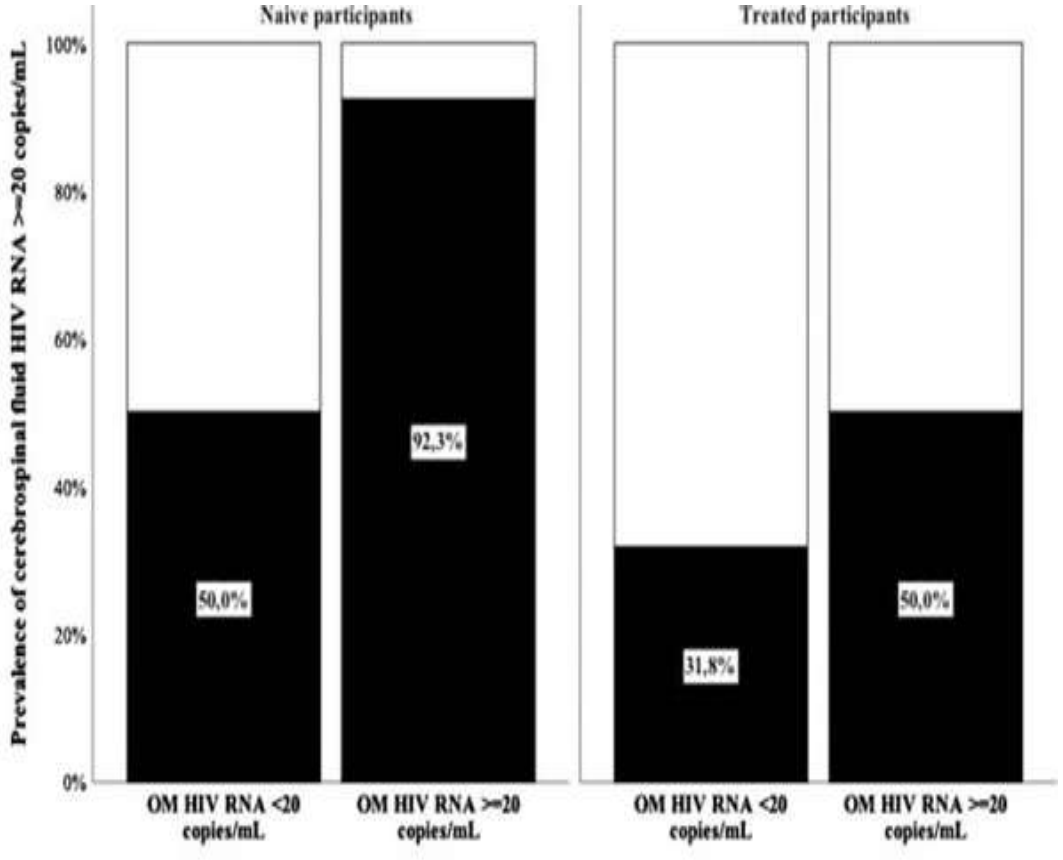


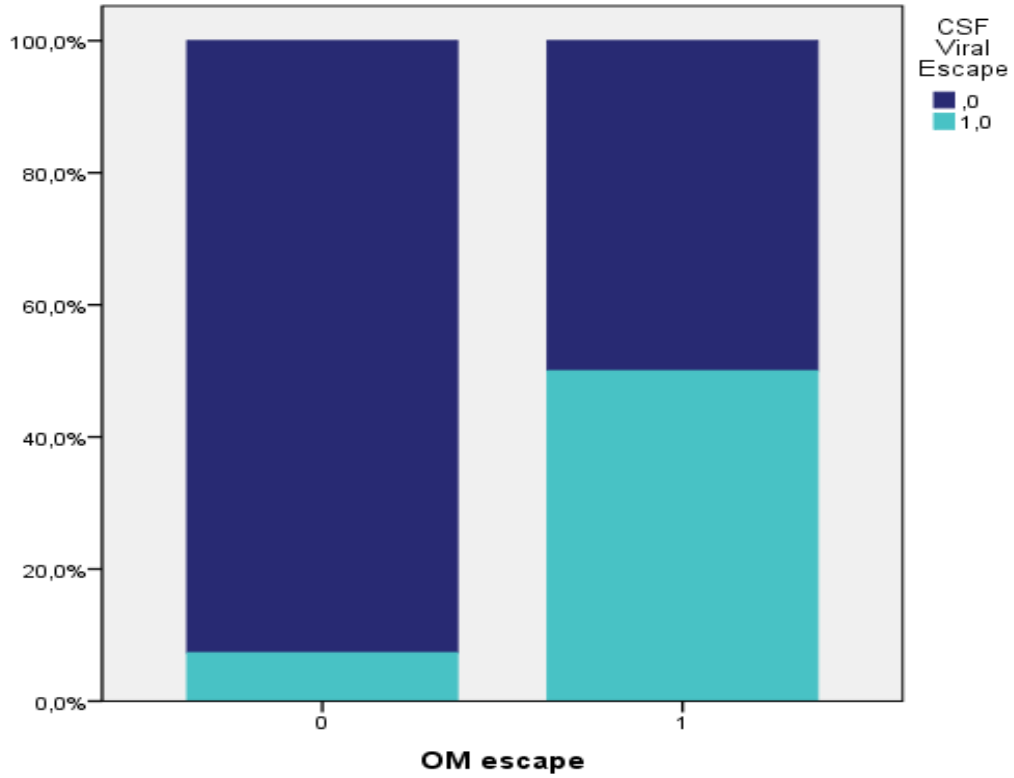


updated from Bertero L et al, AIDS 2019

HIV-1 detection in the OM of HIV-1-infected participants

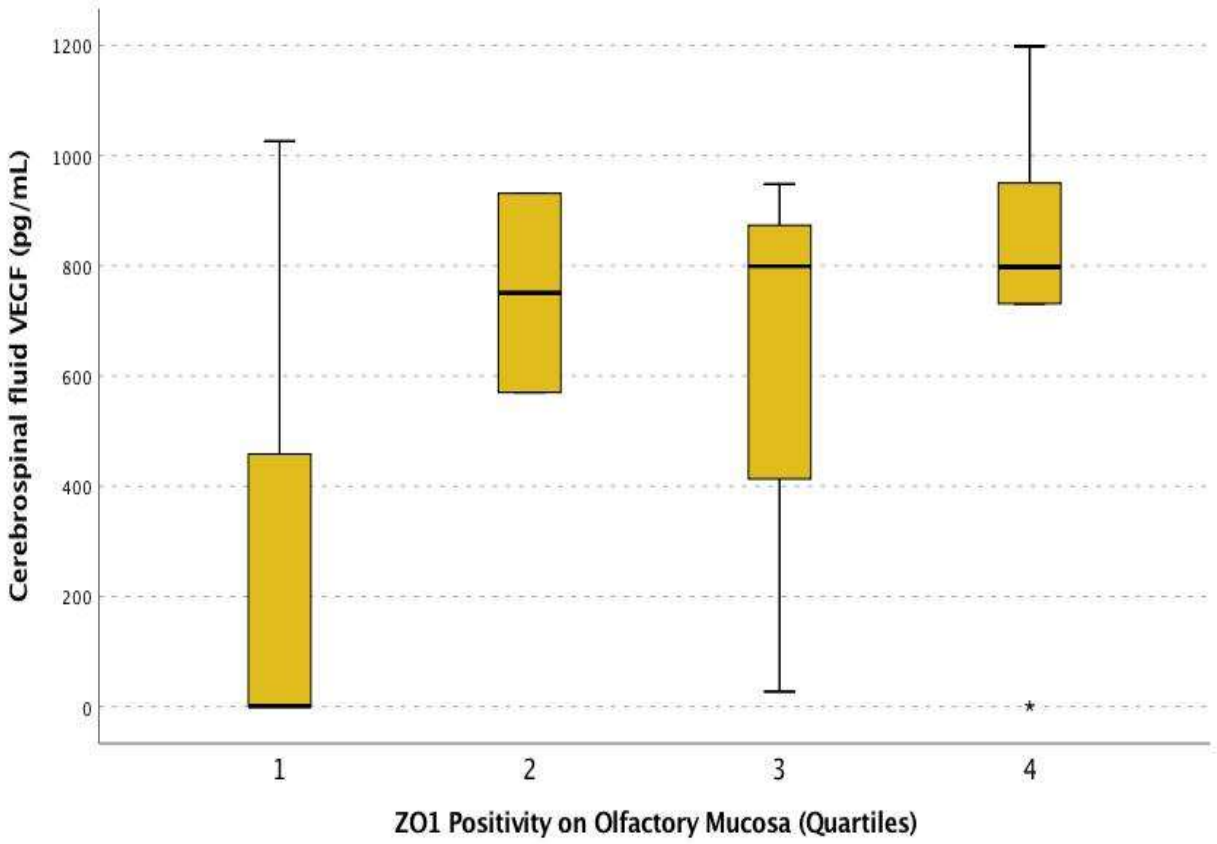
- **Olfactory mucosa HIV-DNA was detectable** in 3/5 samples in naïve and in 1/10 sample in treated patients
- Viral escape was observed in 5 patients in the CSF (10.6%) and in 4 in the OM (8.5%)
- CSF escape was more common in patients with OM escape: 50 vs. 7.9%, OR 12.7, p=0.01

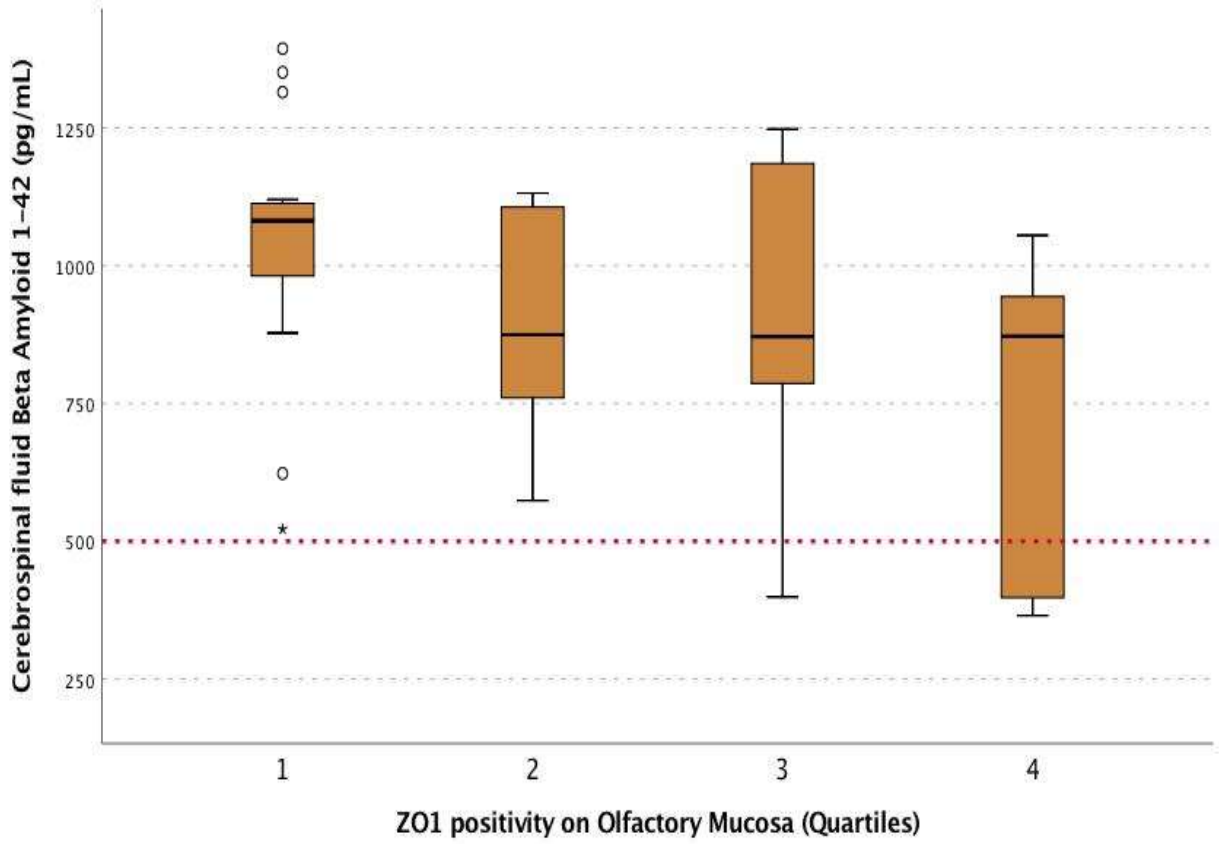




Bertero L et al, AIDS 2019

Blood Brain Integrity Biomarkers in Cerebrospinal Fluid and Olfactory Mucosa

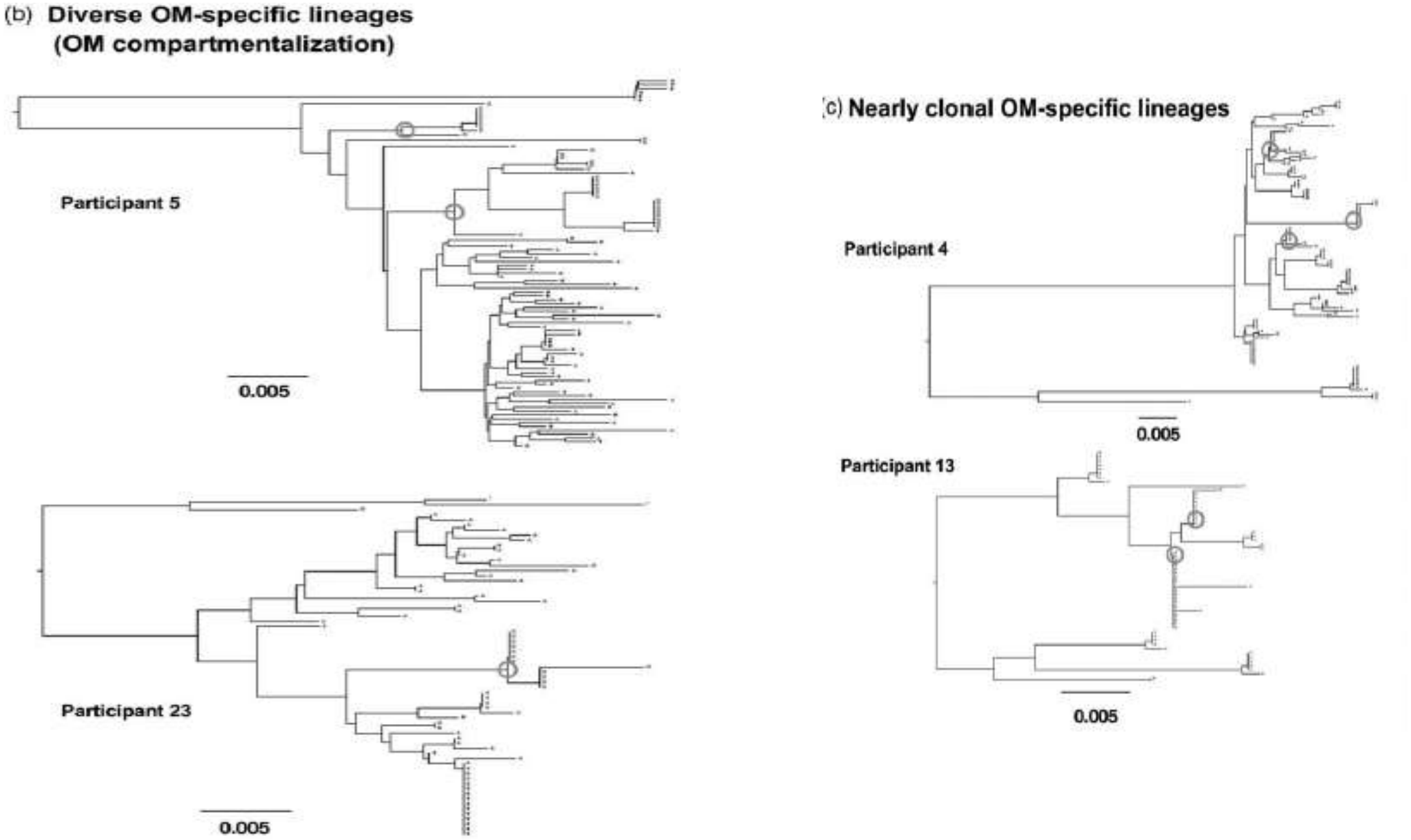




- An OM Zo-1 positivity above 20% was associated with lower CSF 1-42 Beta amyloid (p=0.025), higher CSF VEGF (p=0.020) and Zo-1 (p=0.038) and CSF cells (p=0.010)

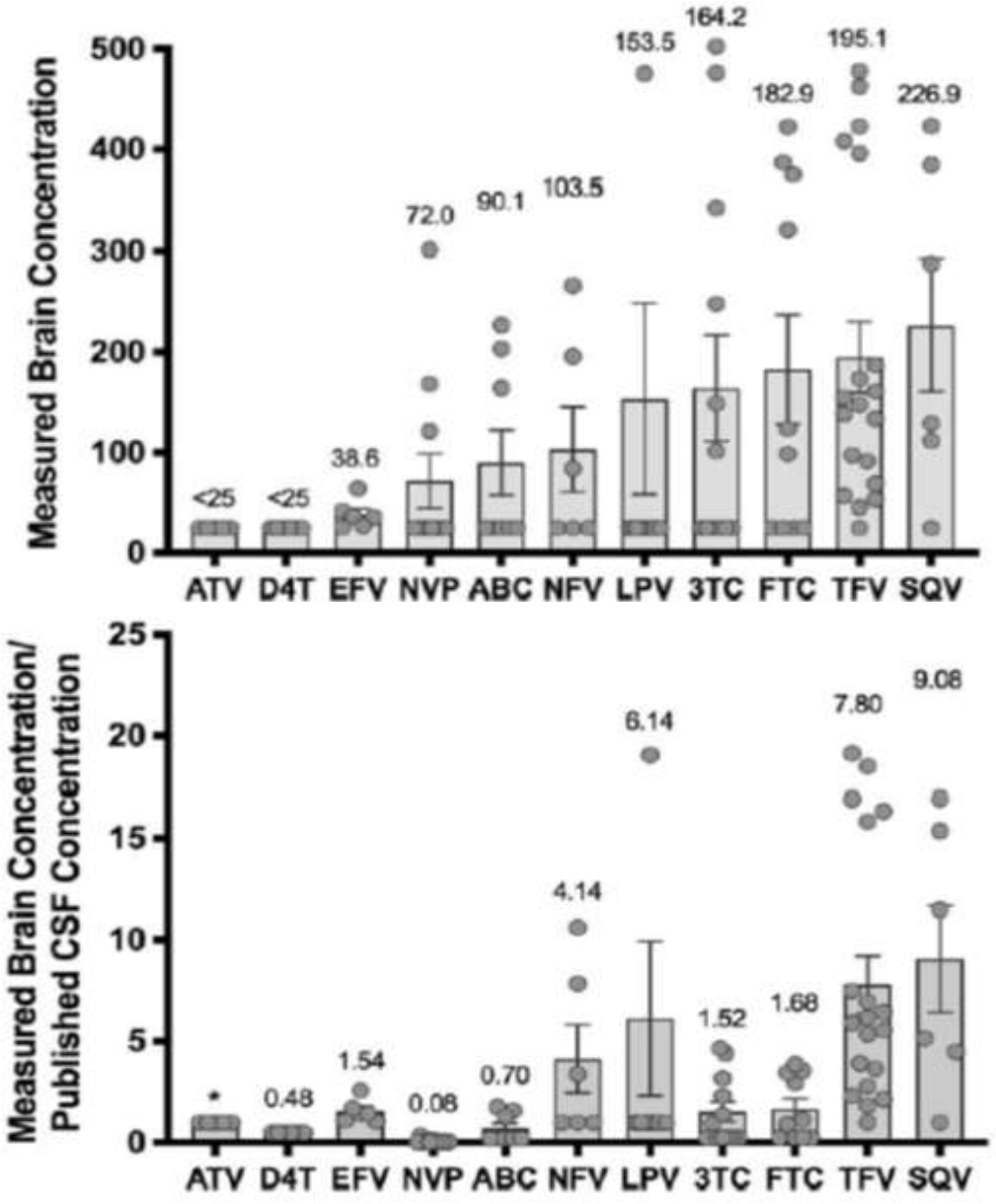
Unpublished data

HIV-1 env deep sequencing in OM vs CSF and Plasma



Bertero L et al, AIDS 2019

Antiretrovirals concentrations in Brain and Olfactory Mucosa



	Samples	OM concentrations (pg/mg)	OM to plasma ratio (‰)	OM to CSF ratio (‰)
Lamivudine	7	**15** (10-38)	**10** (7-51)	**175** (62-550)
Emtricitabine	14	**4** (1-12)	**10.4** (0-20)	**42** (0-54)
Abacavir	6	**1** (0-9.7)	**0** (0-72)	**0** (0-14)
Tenofovir (TDF)	10	**0**	**0**	**0**
Tenofovir (TAF)	2	**0**	**0**	**0**
Nevirapine	2	**10-12.5**	**2.3-2.6**	**5.7-33.2**
Rilpivirine	4	**5.5** (1.7-15.2)	**76** (25-76)	**6***
Etravirine	3	**9** (5-9)	**34** (6-34)	**1000** (136-1000)
Atazanavir	1	**160**	**356**	**26666**
Darunavir	11	**106** (22-286)	**58** (17-83)	**11047** (2650-16426)
Ritonavir	4	**133** (45-360)	**1190** (458-1190)	**0***
Cobicistat	11	**288** (119-662)	**1052** (731-7721)	**0***
Raltegravir	6	**12** (4.5-45.7)	**18** (6-93)	**70** (38-220)
Elvitegravir	3	**3** (0-3)	**4.4***	**0***
Dolutegravir	9	**8** (4.5-16)	**7** (2-20)	**1636** (775-2602)
Maraviroc	4	**57** (34-73)	**193** (47-193)	**5333***

Ferrara M et al, AIDS 2020;
Calcagno A et al, CROI 2019, n 476

1. Is OM an HIV reservoir and could it be a surrogate for CNS reservoir?

2. Do we need new reservoirs?

3. If yes, is OM a valuable candidate?

We are working on it….

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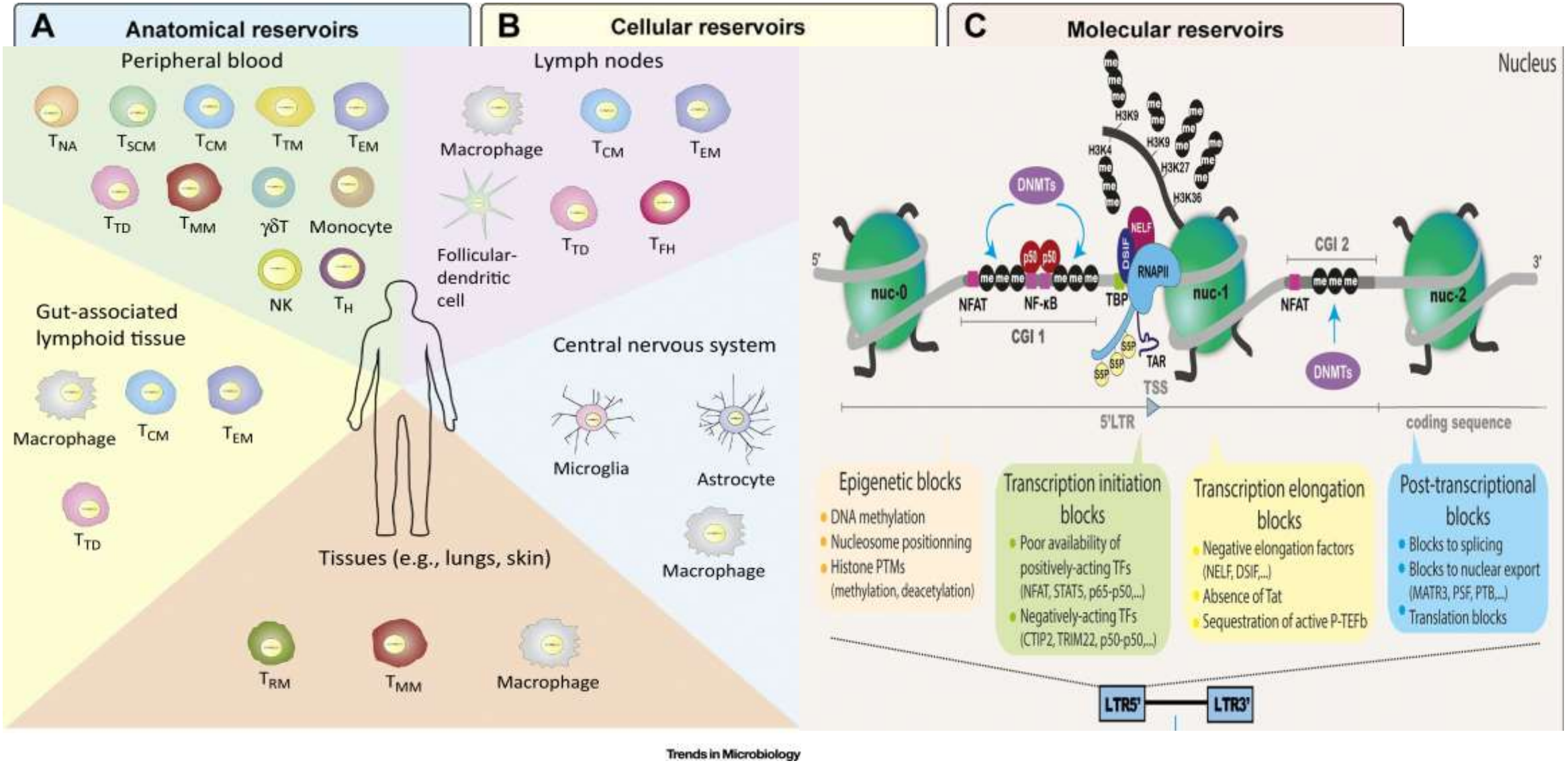
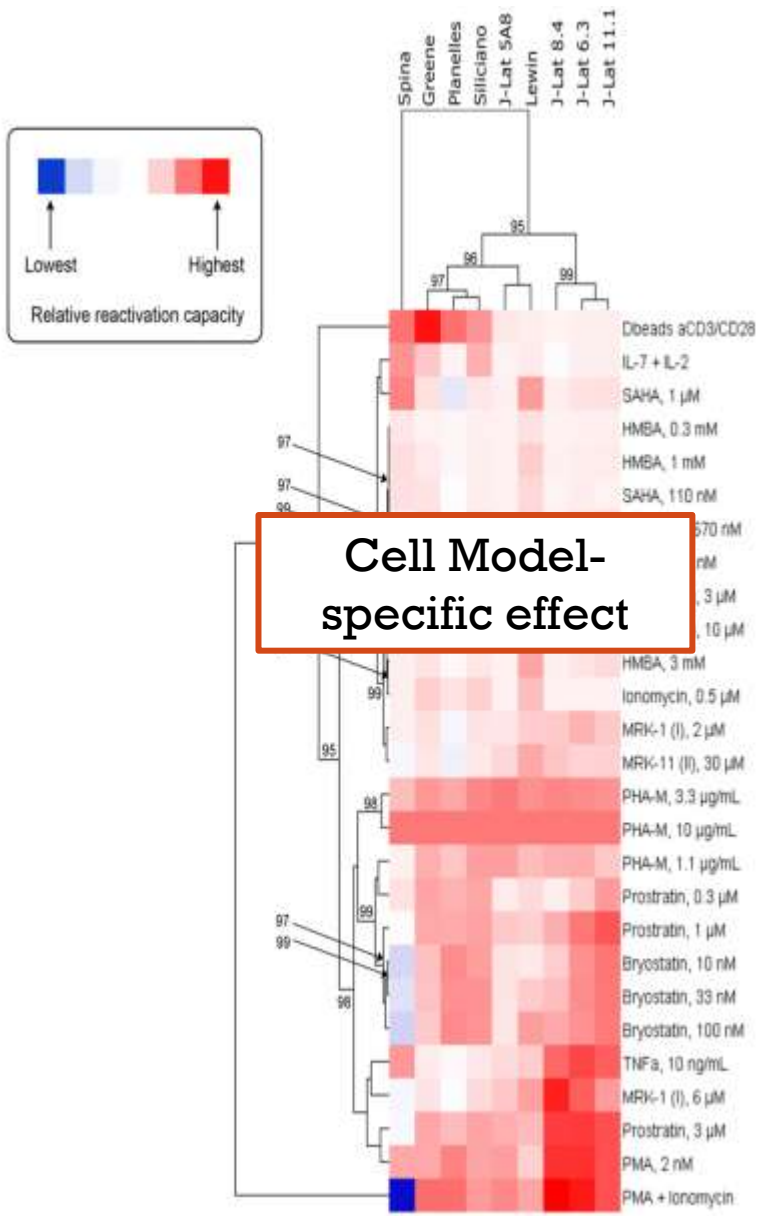


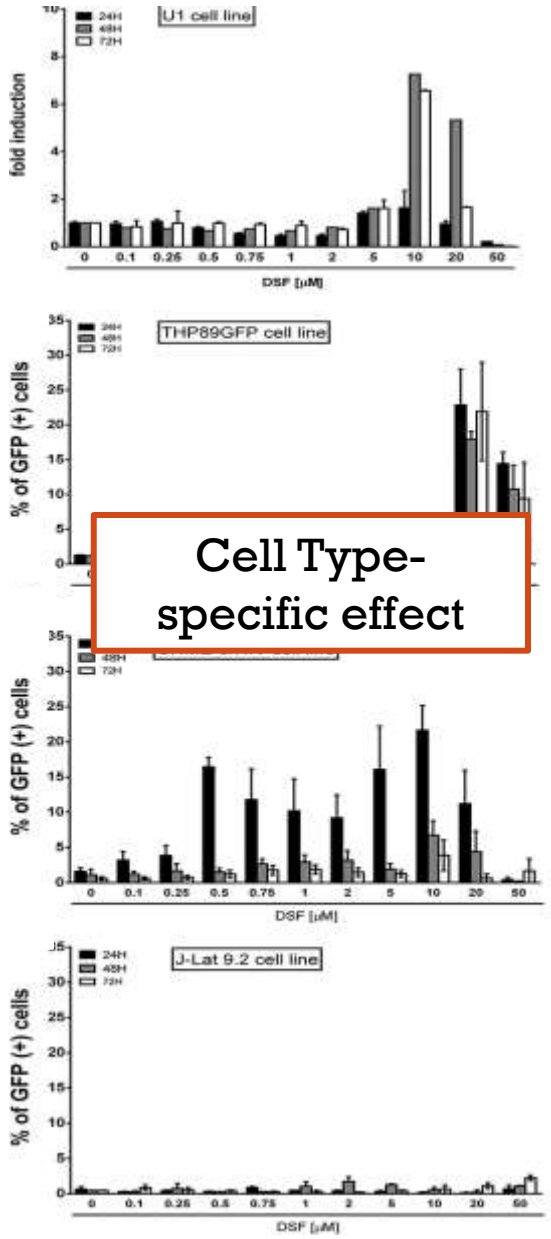
Trends in Microbiology

Henderson L J et al, J Virol 2020
Barton K et al, Trends Microbiol 2016
Ait-Ammar A et al, Front Microbiol 2020

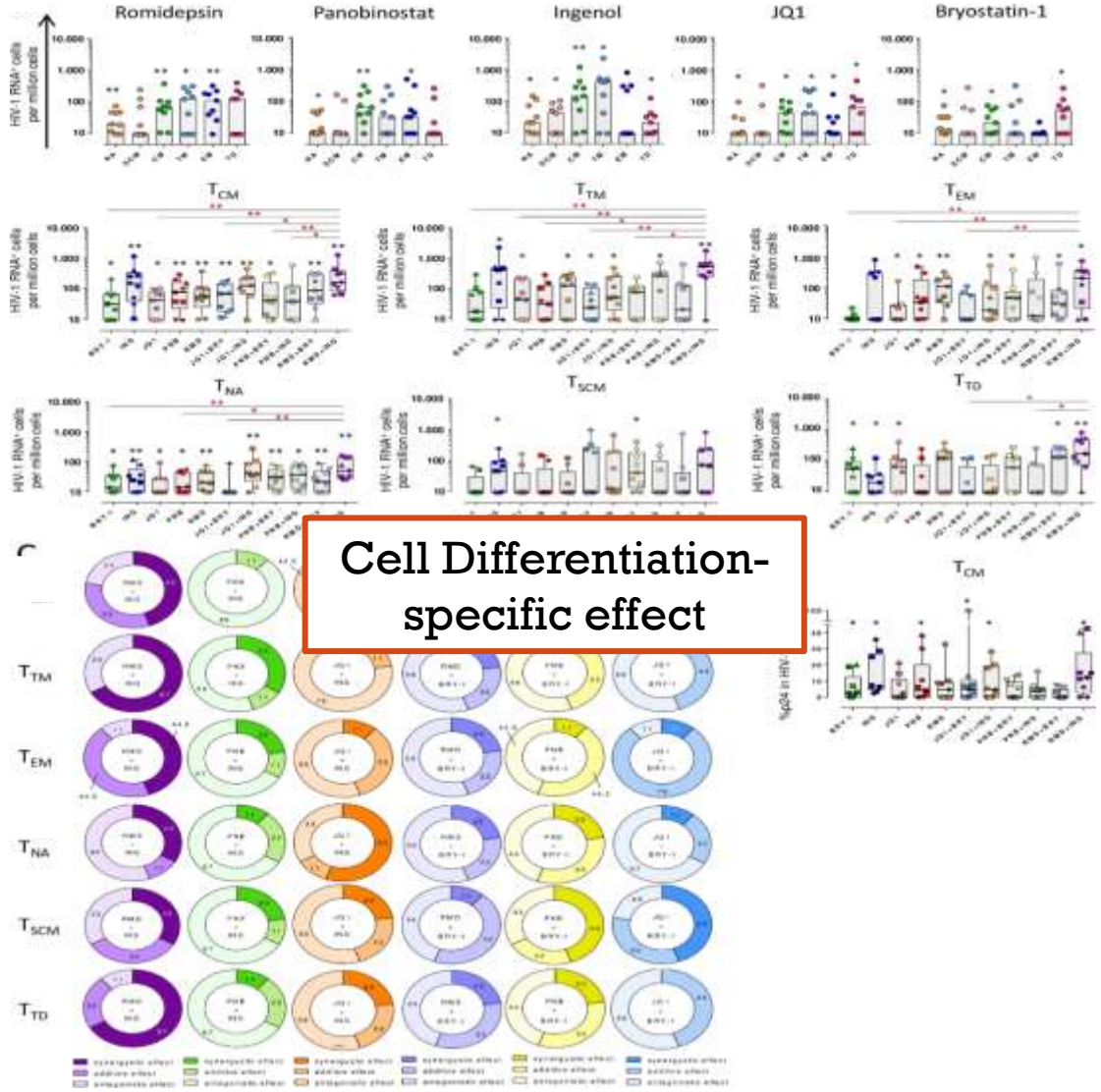
LRAs vs Heterogeneity of HIV-1 Cellular and Tissue Reservoirs



Cell Model-specific effect



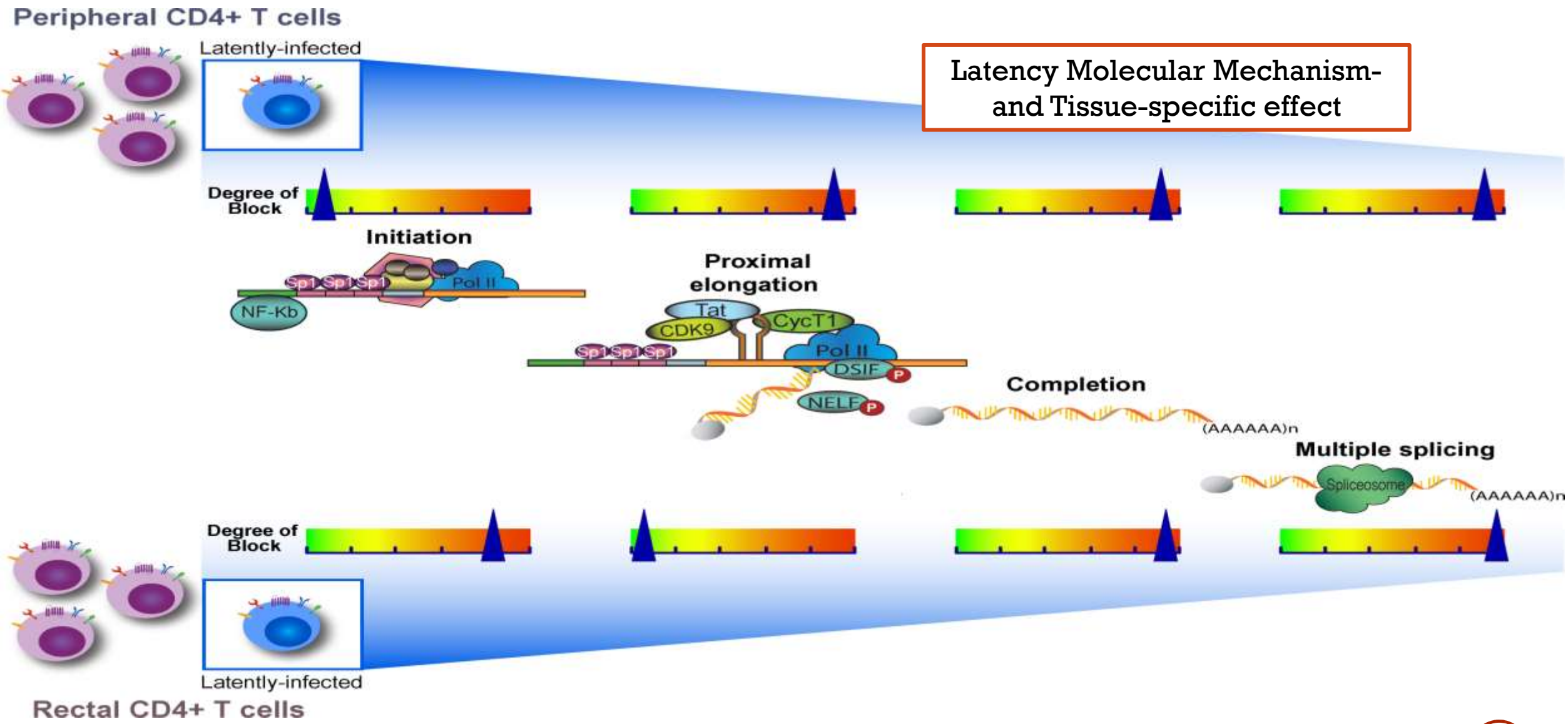
Cell Type-specific effect



Cell Differentiation-specific effect

Spina CA et al, Plos Pathog 2013
Kula A et al, J Acquir Immune Defic Syndr 2019
Grau-Exposito J et al, Plos Pathog 2019

LRAs vs Heterogeneity of HIV-1 Cellular and Tissue Reservoirs



Elliott J et al, PLoS Pathog 2014
Telwatte S et al, PLoS Pathog 2018

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An effective "Shock and Kill" strategy must take into account all these determinants but, due to the lack of knowledge of all the cellular factors and pathways impacting HIV gene expression and leading to productive viral replication, it is not possible at the moment

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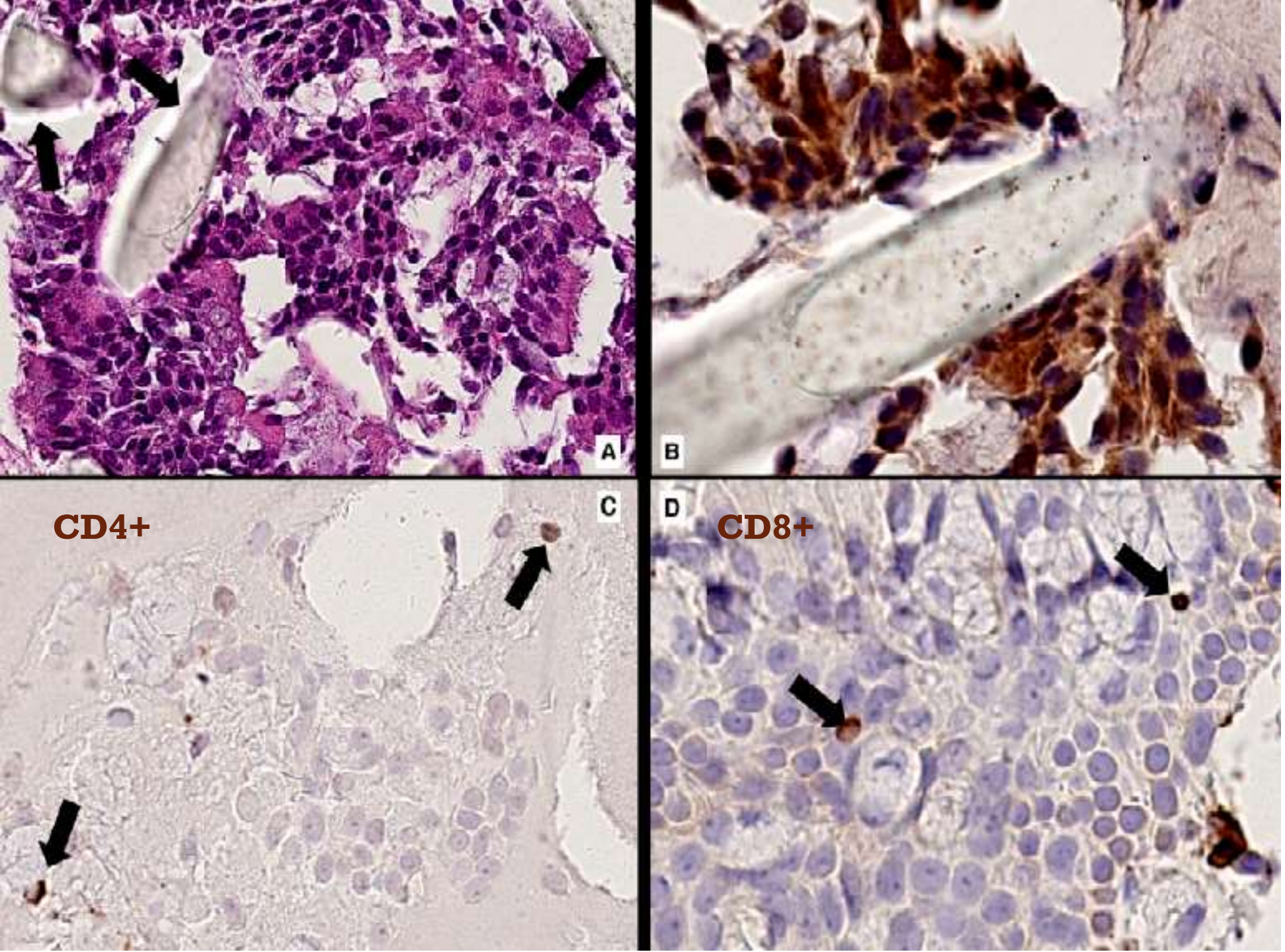
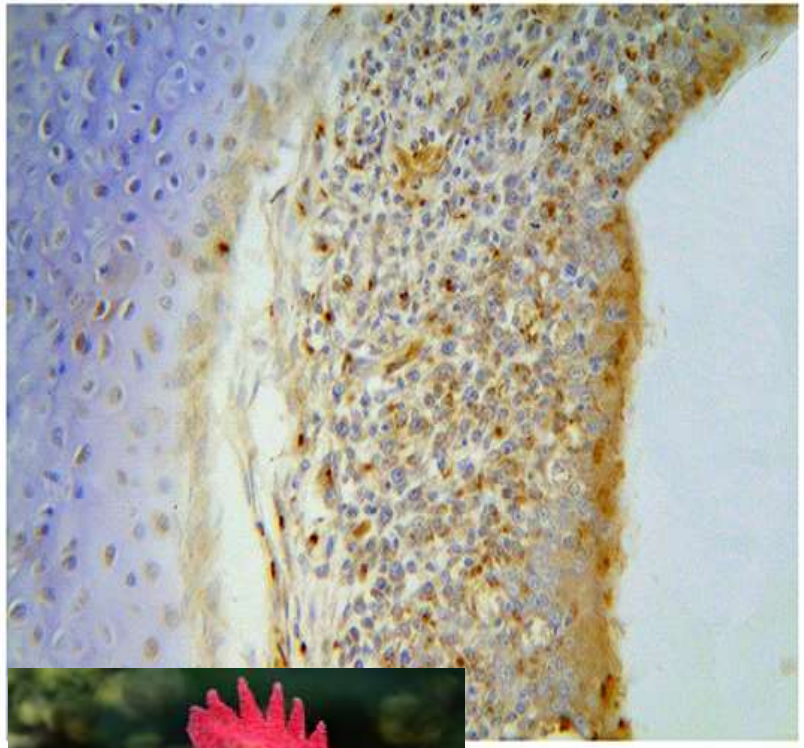


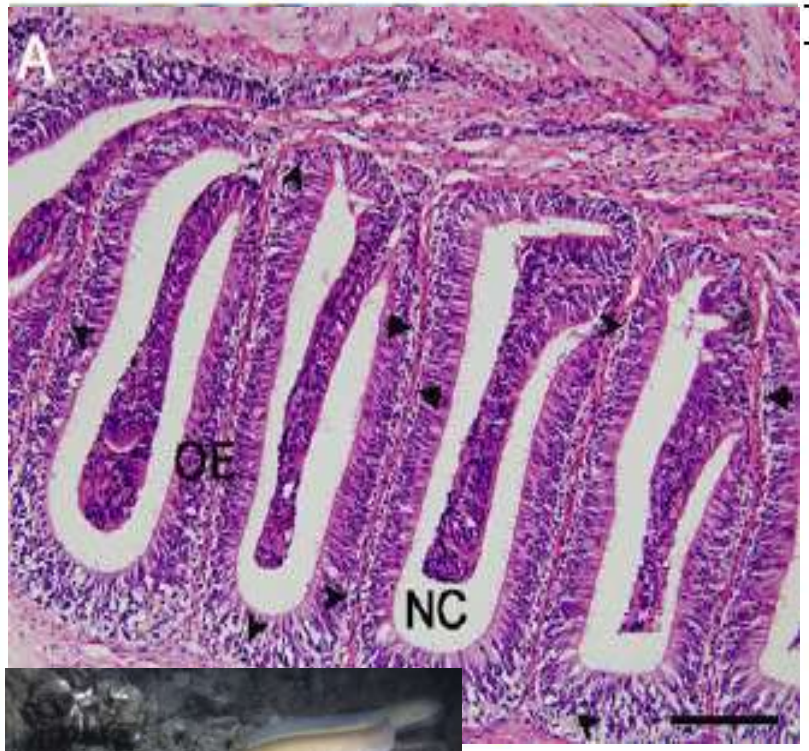
Nasal Brushing of Olfactory Epithelium

Anti-OMP IHC showing a strong and diffuse staining

Bertero L et al, AIDS 2019

Diffuse Nasal Associated Lymphoid tissue (NALT)



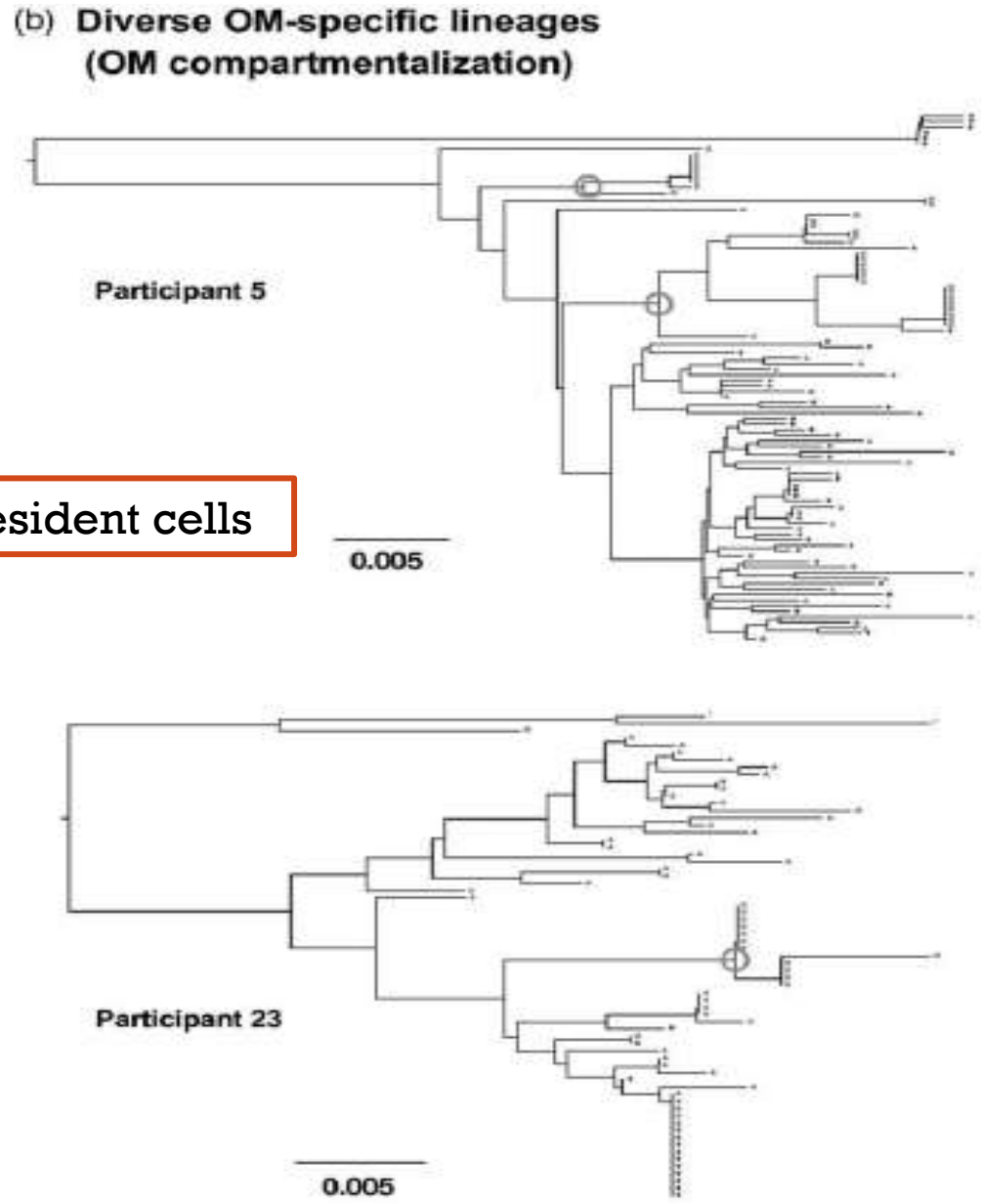


	O-NALT	D-NALT
Number of lymphocytes	+	++
T/B cells ratio	0.76–1.2	0.33–1.0
Percentage of B cells[a]	47–79%	55–74%
Plasma cells	+	++
IgM+ B cells	47 or 85%	0.5–9%
IgA+ B cells	1–1.6%	7.7–10.8%
$B220^{+hi}$ B cells	Present	Present
$B220^{+hi}$ $B220^{+low}$ B cells	Absent	Present
IgM/IgG/IgA secreting cells ratio (Uninfected)	10/3/3	10/3/3
IgM/IgG/IgA secreting cells ratio (infected with influenza)[b]	4/50/40	1/25/10
IgM/IgG/IgA secreting cells ratio(immunized with Cry1Ac protoxin)[c]	1/80/125	1/125/350
IgM/IgG/IgA secreting cells ratio(immunized with cholera toxin)	1/30/65	1/90/160
IgA isotype class switching	Present	Absent
Class switch recombination-associated molecules	Present	Absent
Long-lasting, specific effector antibody response[e]	Absent	Present
Frequency of AFCs[e]	+	++
Generation of virus-specific antibody forming cells (AFCs)[e]	+	+
Infected/uninfected IFN-γ production ratio[d]	~1000	~700
Infected/uninfected IL4 production ratio	1	2
$CD3^+$ T cells	30–40%	13–20%
$CD4^+/CD8^+$ T cells ratio	3–4.4	1.5–6.4
αβ /γδ of $CD3^+$ T cells ratio	49–100	2.5–3
αβ /γδ of $CD4^+$ $CD8^-$ T cells ratio	49–100	49–100
αβ /γδ of$CD4^-$ $CD8^+$ T cells ratio	49–100	9–19
αβ /γδ of$CD4^-$ $CD8^-$ T cells ratio	0–1	0–1
Type of $CD4^+$ T cells	Th_0	Th_2

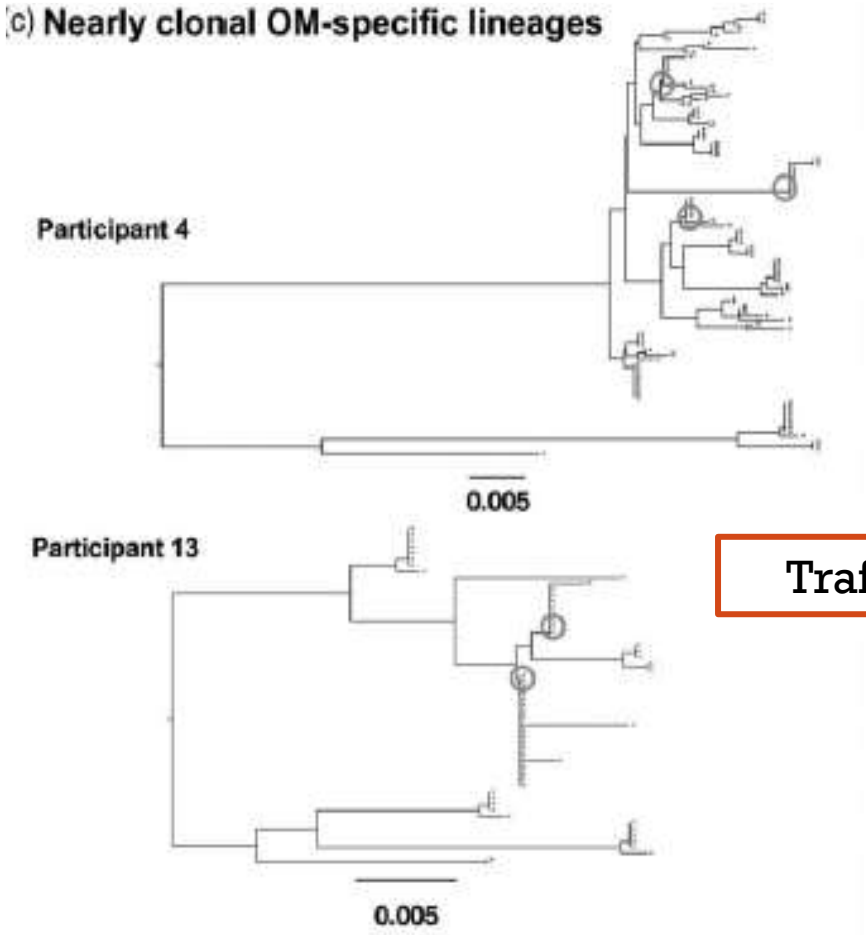
Scattered number of CD4+ and CD8+ T cells and three different types of Ig-containing cells in the lamina propria and occasionally in the epithelium

Ohshima K et al, Histol Histopathol 2000
Sepahi A et al, Mol Immunol 2016

HIV-1 env deep sequencing in OM vs CSF and Plasma



Resident cells

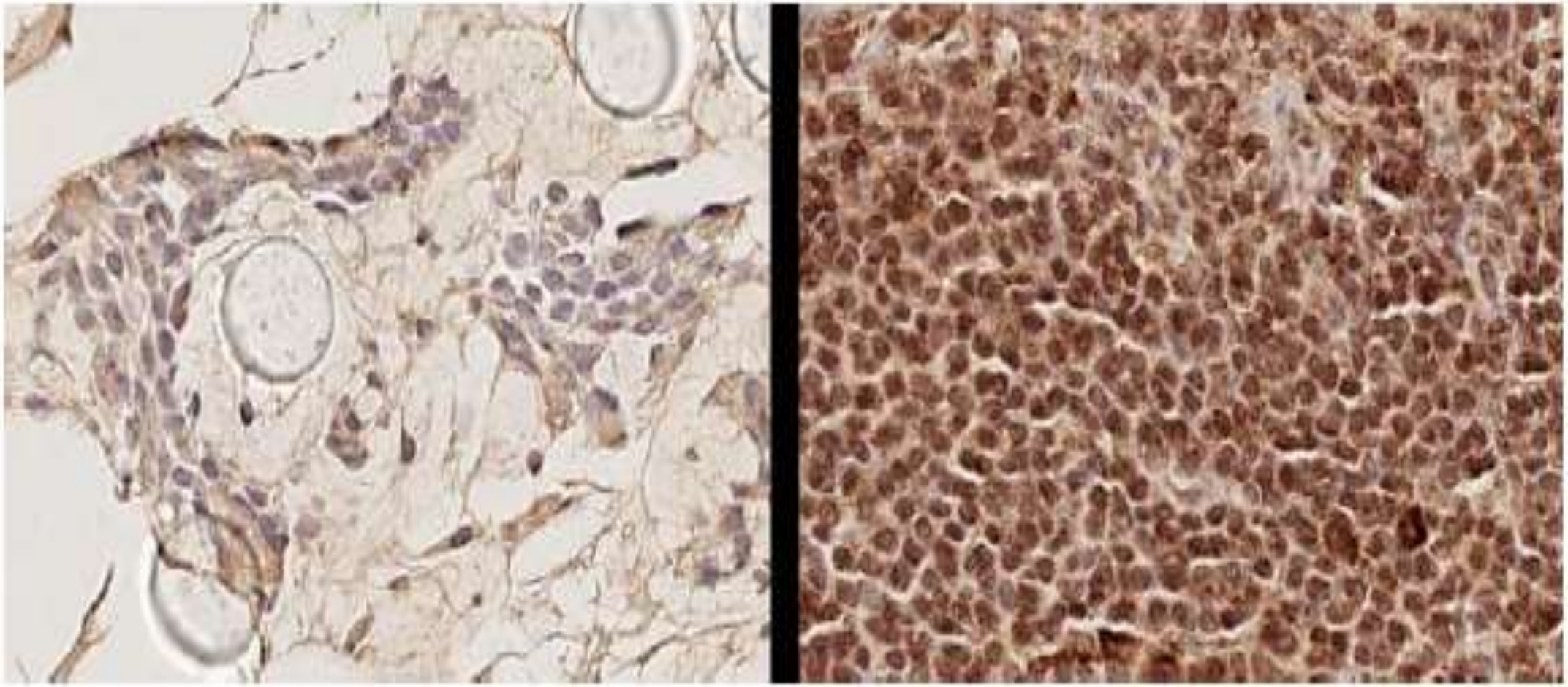


Trafficking cells?

Bertero L et al, AIDS 2019

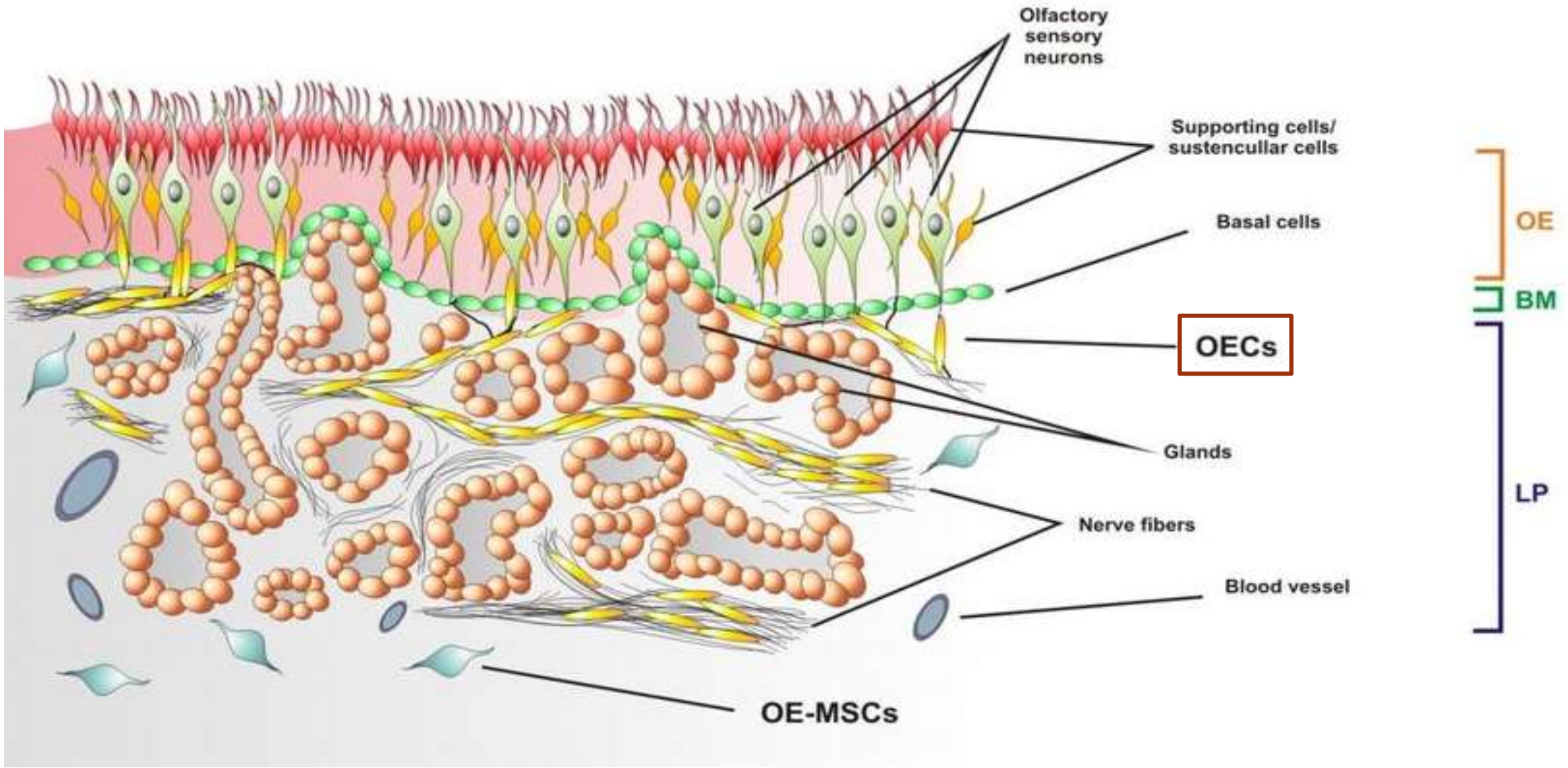
Anti-HIV-1 TAT staining of OE

Cells isolated from the OM were negative for HIV Tat (left); lymph node with HIV lymphadenopathy (positive control, right)



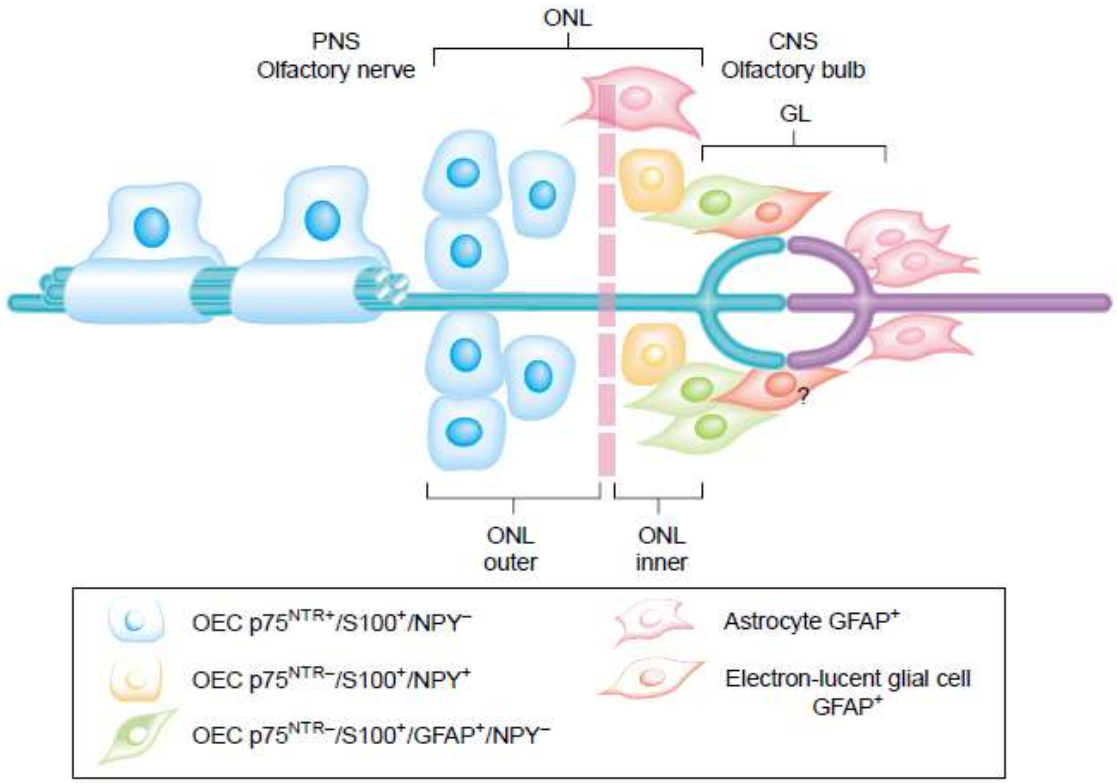
Bertero L et al, AIDS 2019

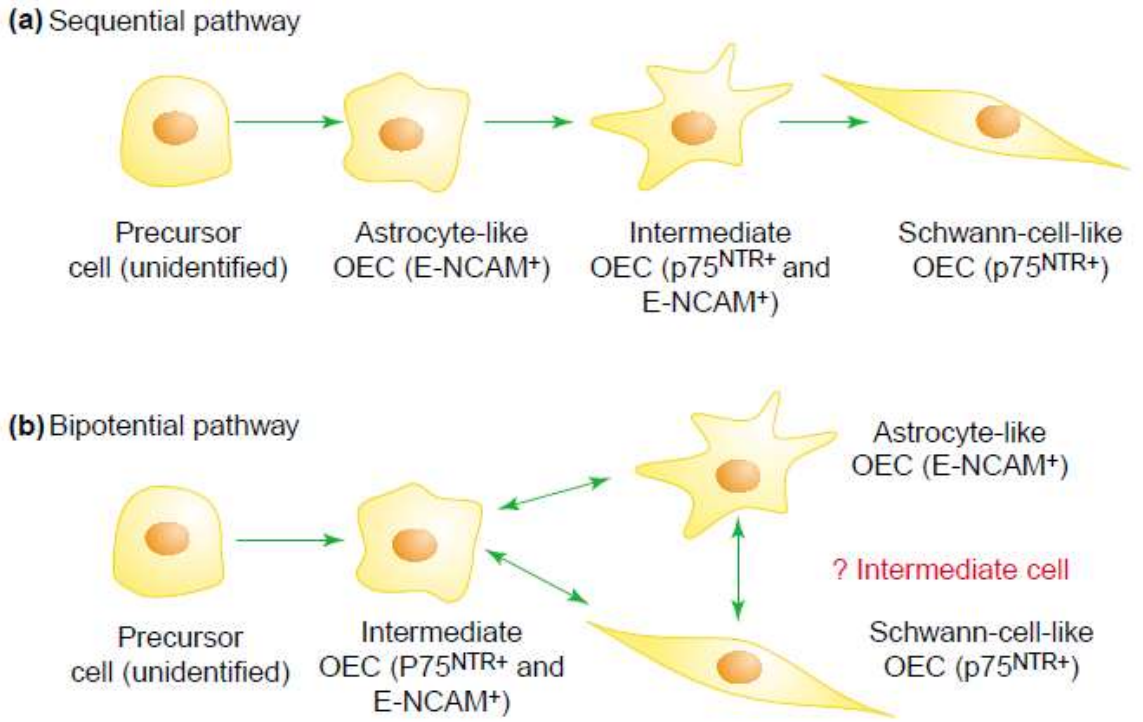
Alternative Candidates



Kaltschmidt B et al, Stem Cell Rev 2011

Olfactory Ensheathing Cells: the primary innate immunocytes



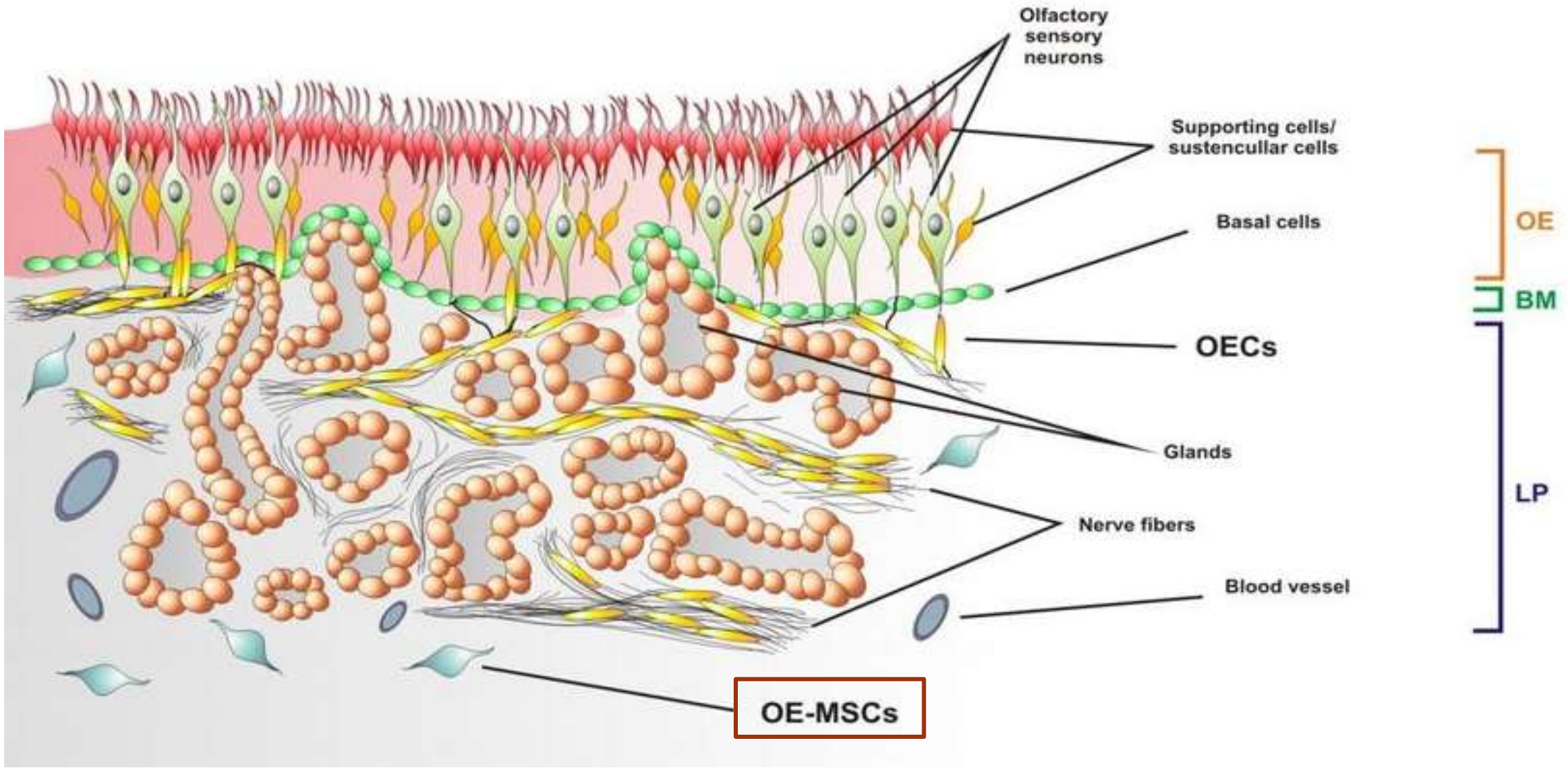


Sample	HIV status	Age	Sex	ART	CD4 counts (cells/mm)	Plasma Viral Load (copies/ml)	CSF Viral Load (copies/ml)	LCM of GFAP+ cells for Alu-Nef HIV DNA/10^6	% of GFAP+ cells for HIV gag mRNA	% of GFAP + cells for HIV p24
1	-	69	M	N.A.	N.A.	N.A.	N.A.	-	U.D.	U.D.
2	-	38	M	N.A.	N.A.	N.A.	N.A.	-	U.D.	U.D.
3	-	51	F	N.A.	N.A.	N.A.	N.A.	-	U.D.	U.D.
4	-	54	M	N.A.	N.A.	N.A.	N.A.	-	U.D.	U.D.
5	+	56	M	ATV, EFV, RTV, TFV	421	U.D.	U.D.	$1.6x10^4$	2.36±1.89	2.99±1.54
6	+	52	M	N.R.	630	40	<20	$3.75x10^3$	3.69±3.05	4.56±2.68
7	+	42	M	3TC, D4T, FTV, SQV	389	U.D.	<20	$5.25x10^4$	6.91±2.68	4.25±1.25
8	+	44	M	D4T, DDI, NFV	436	U.D.	U.D.	$2.22x10^4$	2.89±1.66	1.22±0.99

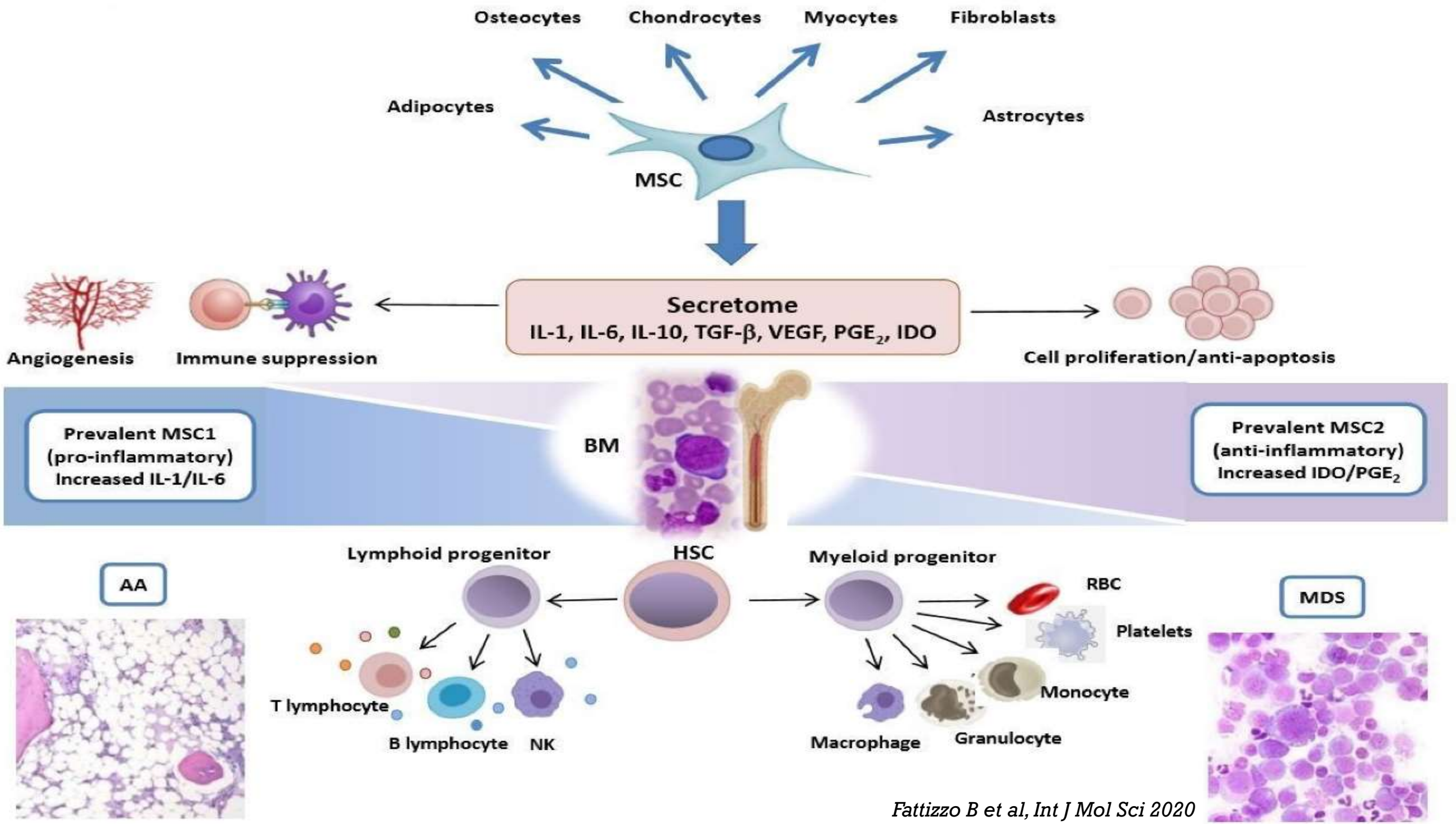
https://doi.org/10.1371/journal.ppat.1008381.t001

Barnett SC et al, TRENDS Neurosci 2004
Lutgen V et al, PLoS Pathog 2020

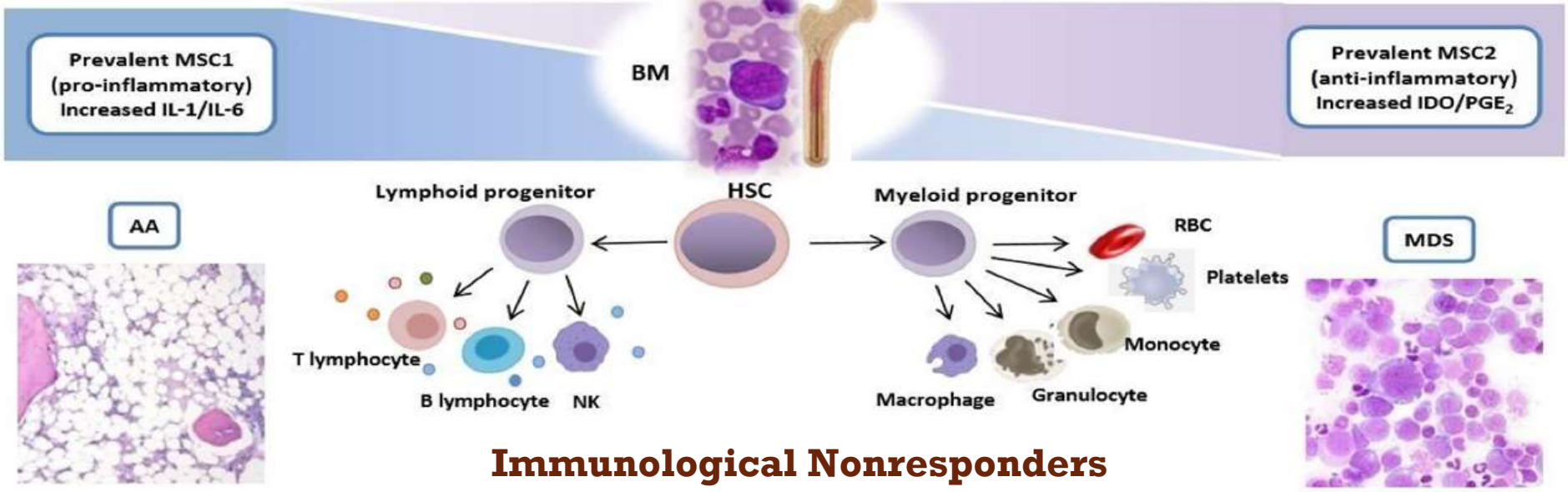
Alternative Targets



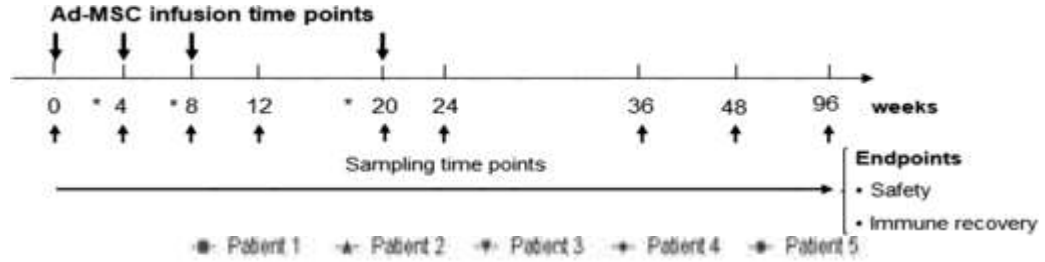
Kaltschmidt B et al, Stem Cell Rev 2011

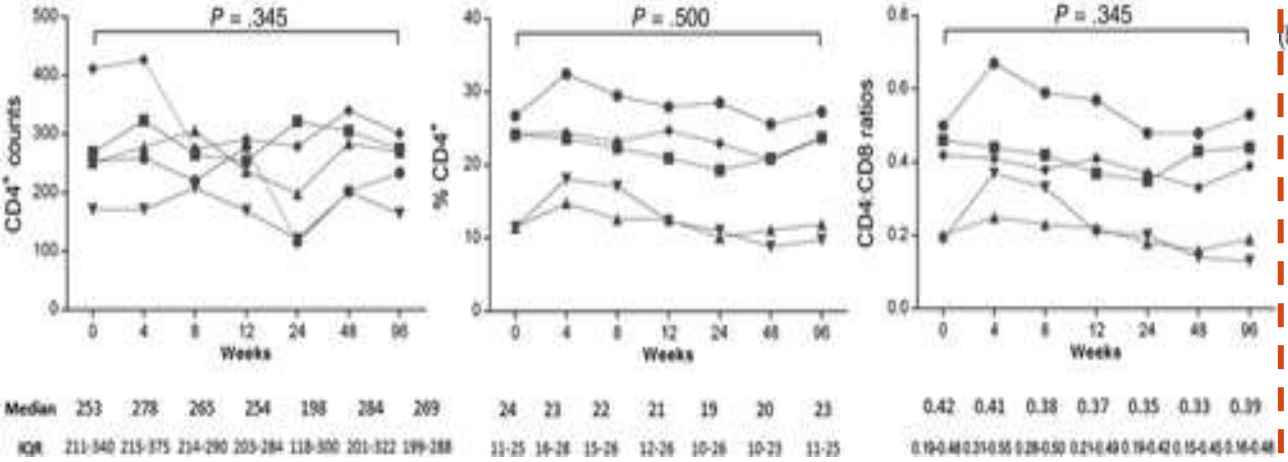


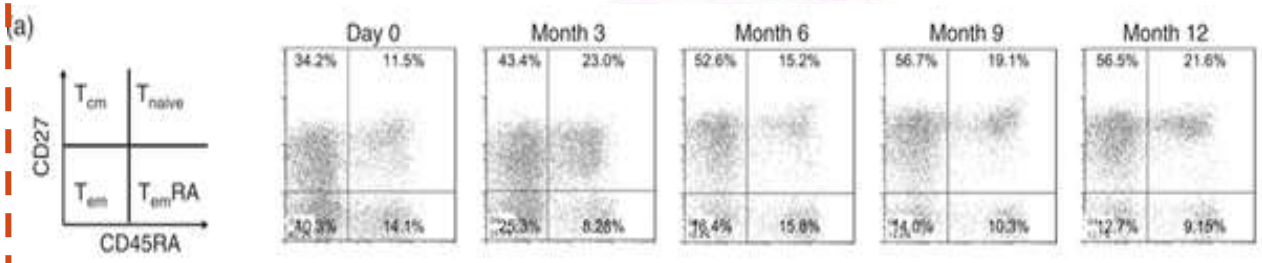
Fattizzo B et al, Int J Mol Sci 2020

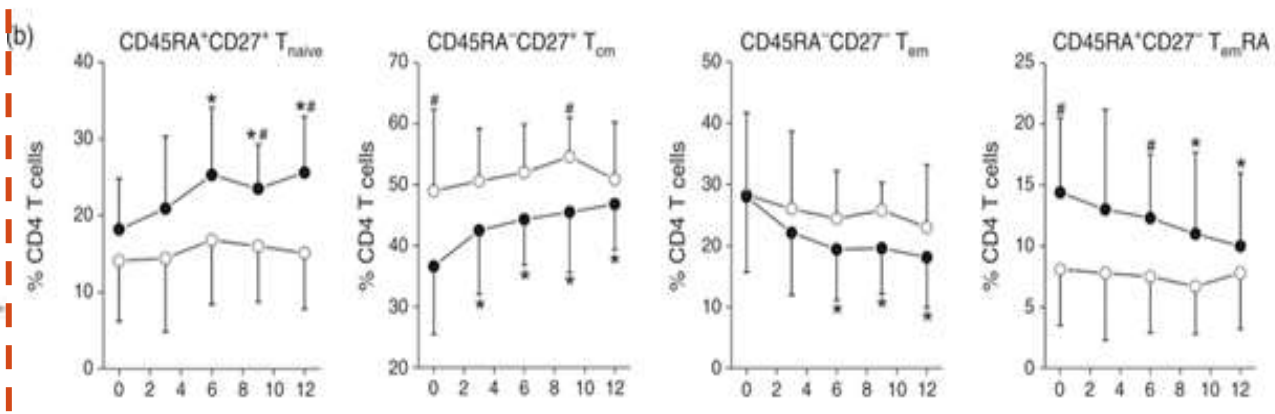


Immunological Nonresponders



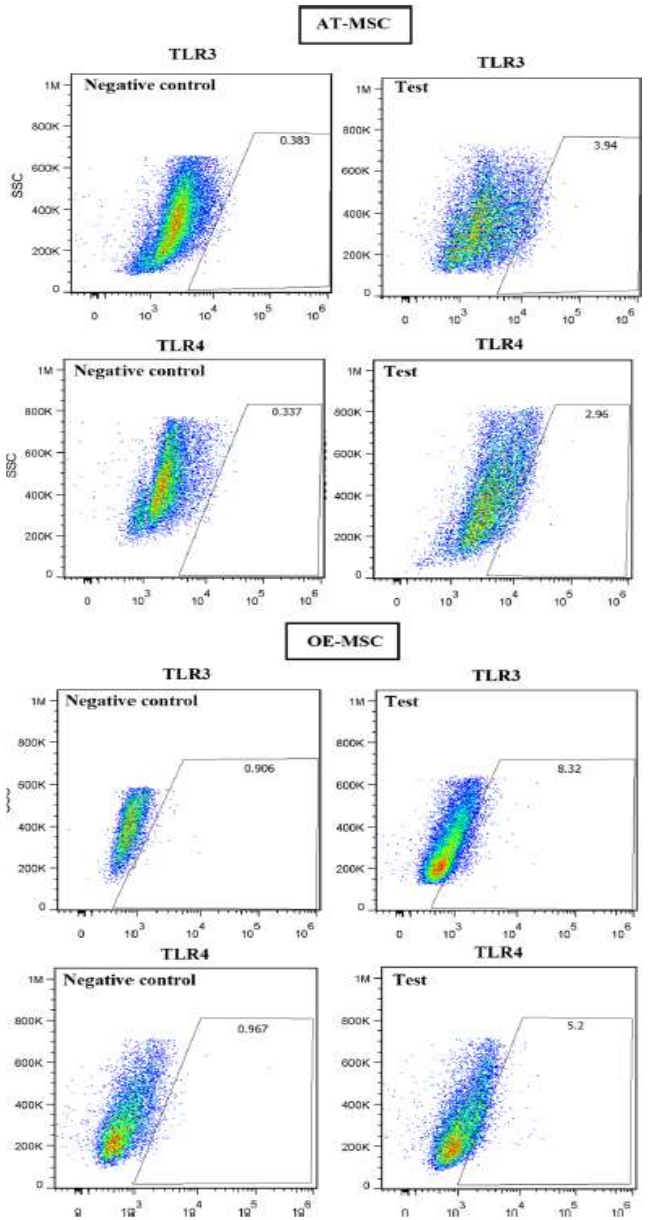


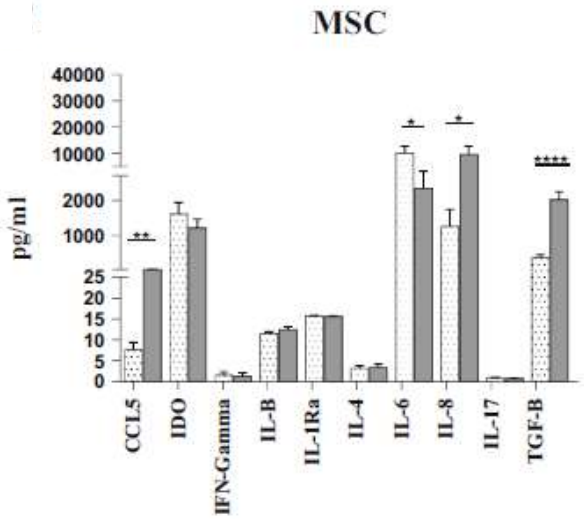


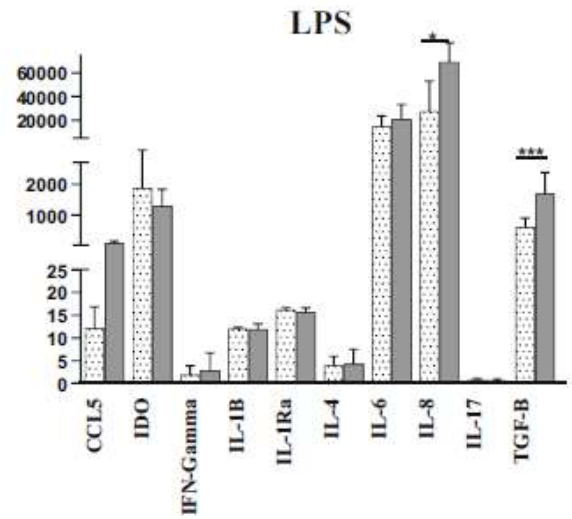


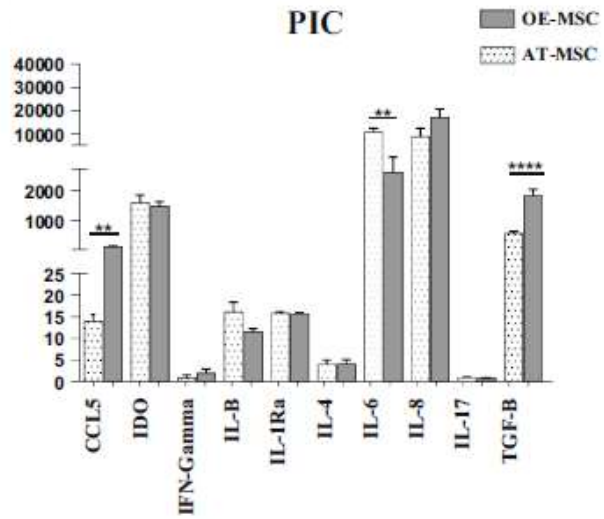
Trujillo-Rodriguez M et al, STEM CELLS Transl Med 2020
Zhang Z et al, AIDS 2013

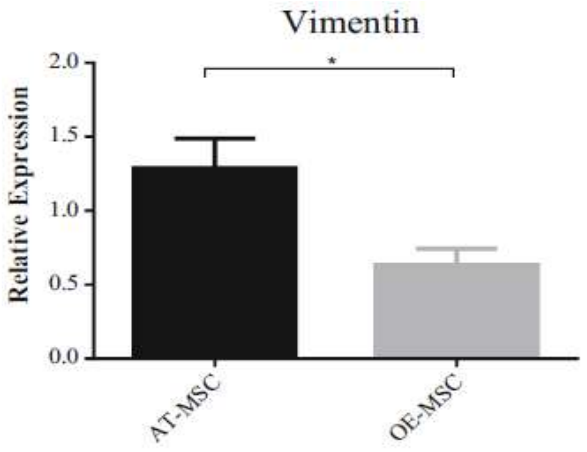
Olfactory Ecto-Mesenchymal Stem cells differ from other tissue Stem cells

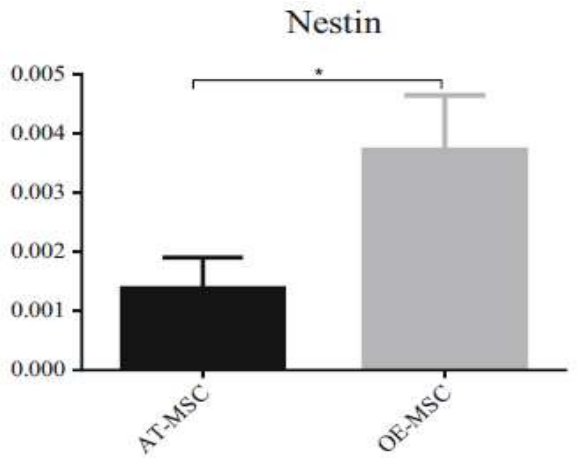


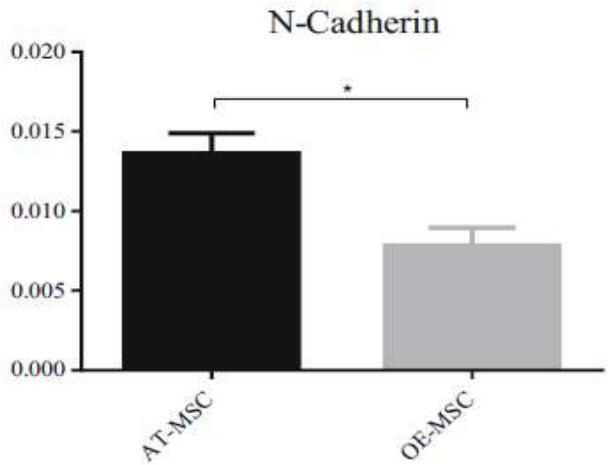






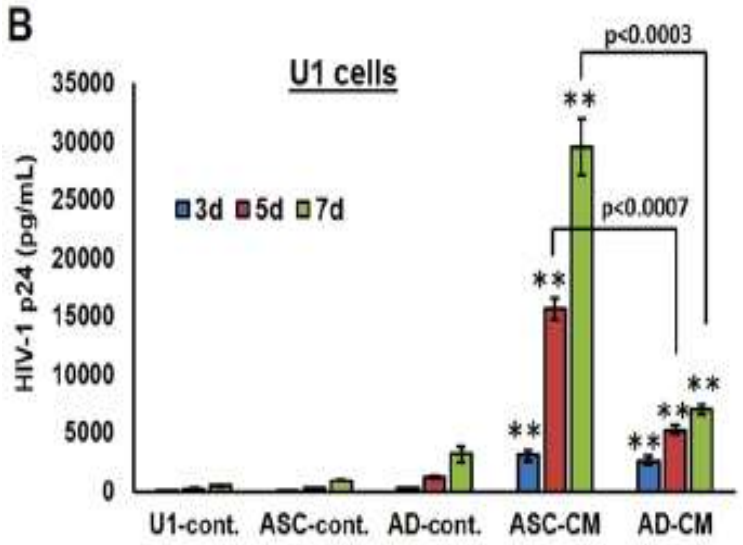


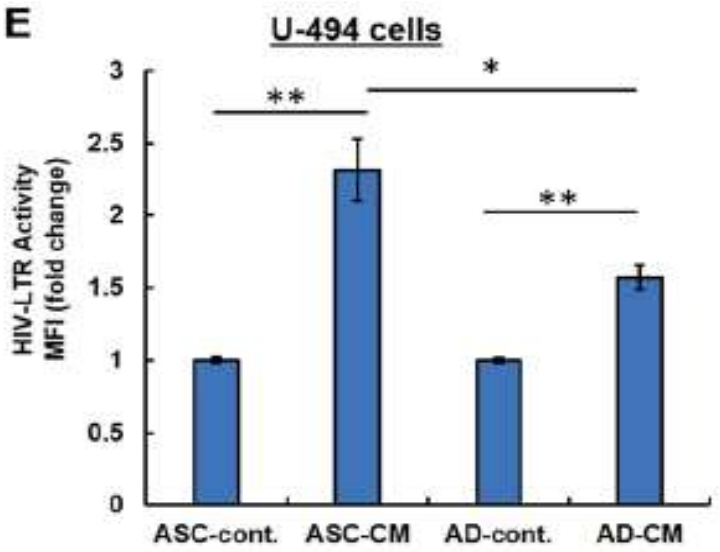


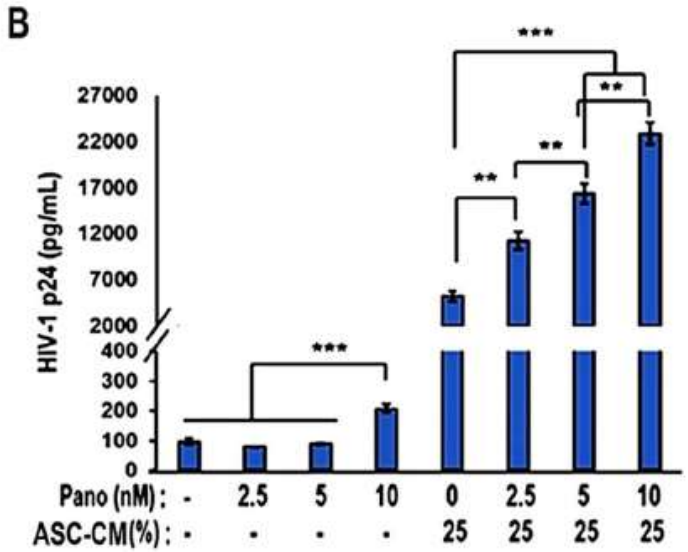


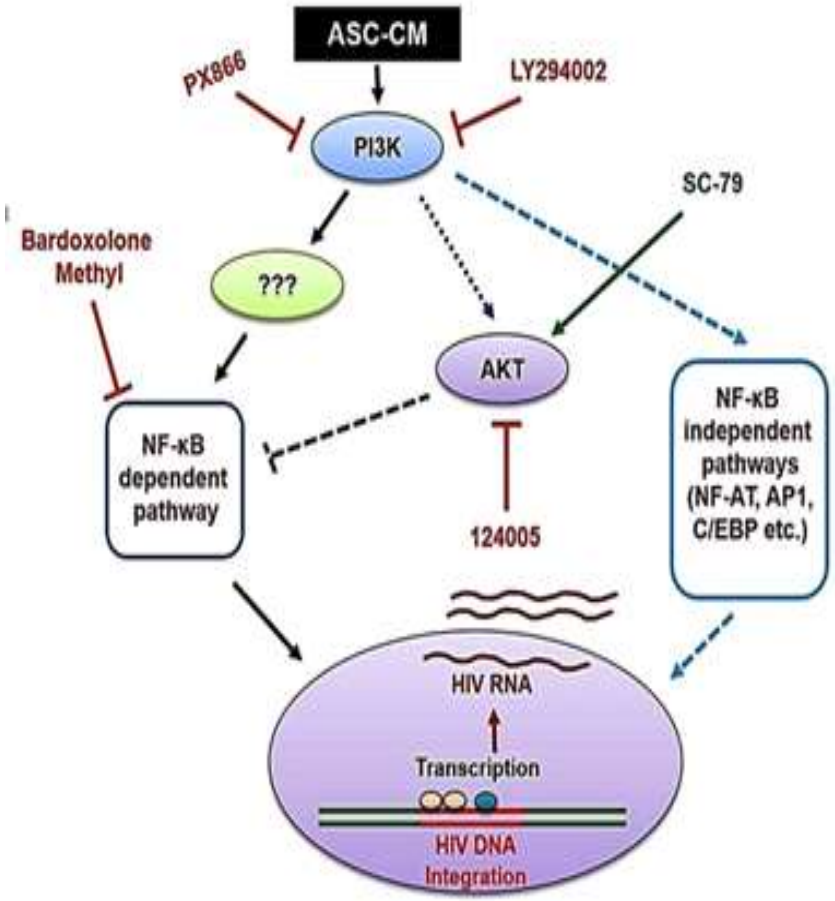
Jafari M et al, Cytotech 2020

Mesenchymal stem cells are attracted to latent HIV-1-infected cells and enable virus reactivation via a non-canonical PI3K-NFκB signaling pathway









Chandra PK et al, Scientific Rep 2018

1. Is OM an HIV reservoir and could it be a surrogate for CNS reservoir?

2. Do we need new reservoirs?

3. If yes, is OM a valuable candidate?

OM possess several cell types that may serve as HIV reservoir as well as specific cell lines that could affect tissue-specific responses to LRAs

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