HBM4EU project

HBM4EU

science and policy
for a healthy future

The application of extracellular vesicles
in malignant mesothelioma diagnosis

Metka Lenassi

1st HBM4EU Training School 2018
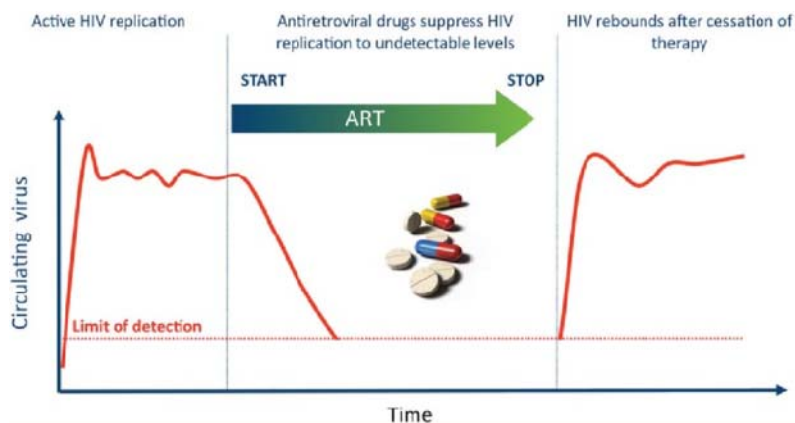

Presentation outline:

- Example 1: EV proteins as putative biomarkers of active HIV reservoir

- Example 2: EV miRNAs as putative biomarkers of response to treatment of malignant mesothelioma (MM)

Example 1:
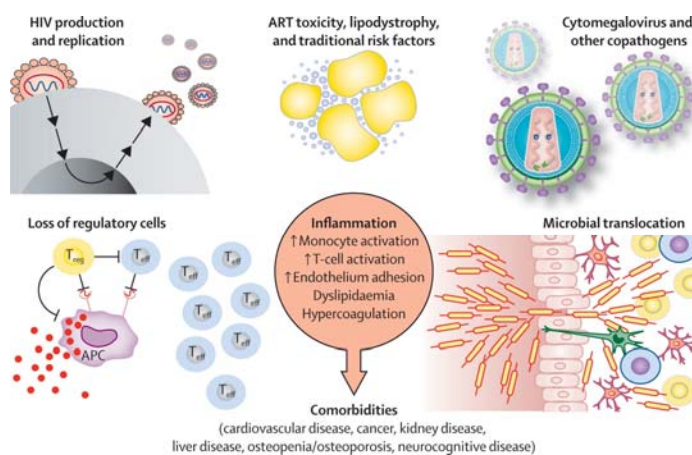*HIV infection as a chronic disease*

Kulpa and Chomont; Journal of Virus Eradication 2015; 1: 59–66

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Example 1:
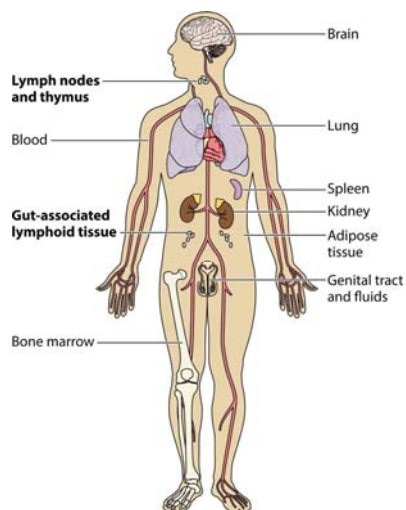*Aviremic individuals with non-AIDS-related disorders*

Deeks et al.; Lancet. 2013 Nov 2;382(9903):1525-33.

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Example 1:
What are HIV reservoirs?

Brain
Lymph nodes and thymus
Blood
Lung
Spleen
Kidney
Gut-associated lymphoid tissue
Adipose tissue
Genital tract and fluids
Bone marrow

Conservative definition:

CD4^{+} T-cells with silent HIV provirus, capable of replication if stimulated

Still relevant?

93% proviruses are defective, unable to produce infective viruses

but produce viral transcripts and proteins
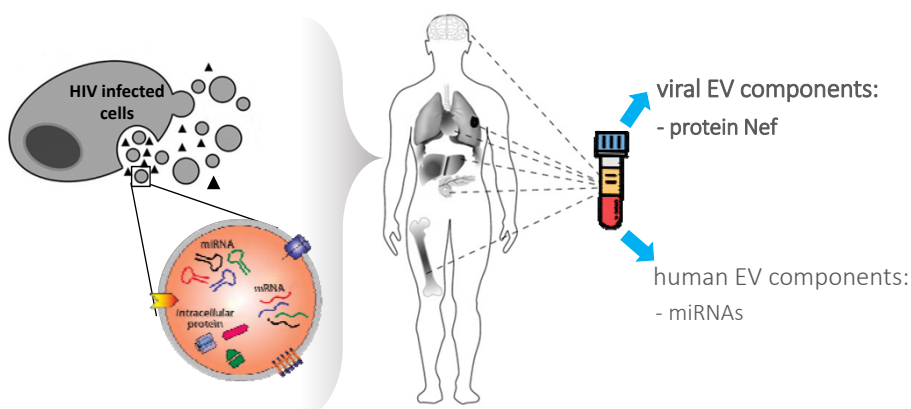
active HIV reservoirs

biomarkers ?

Véronique Avettand-Fènoël et al. Clin. Microbiol. Rev. 2016;29:859-880

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Example 1:
Study aim - active HIV reservoir biomarker discovery

HIV infected cells

miRNA
mRNA
Intracellular protein

viral EV components:
- protein Nef

human EV components:
- miRNAs

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Example 1:
Study subjects

- SCOPE cohort at UCSF: included 134 individuals
  - age
  - gender
  - ethnicity
  - blood CD4+ and CD8+ cell counts, CD4+/CD8+ ratio
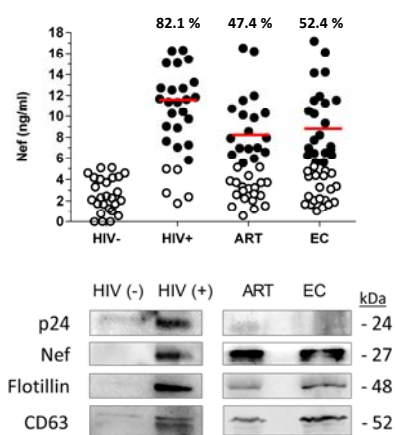 - plasma HIV RNA levels
  - ART regimen

- stratified into 4 groups:
  - uninfected (n = 26): HIV -
  - infected and ART-naïve (n = 28): HIV+
  - aviremic infected individuals treated with ART (n = 42): ART
  - aviremic infected elite controllers (n = 38): EC

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Example 1:
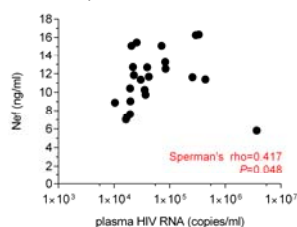*Nef-EVs are in plasma of half of aviremic individuals*

Individual's HIV status affects molecular composition of plasma EVs.

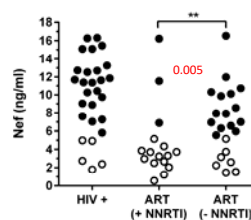1st HBM4EU Training School, Ljubljana, June 18-22, 2018   8

Example 1:
Nef is present in plasma of half of aviremic individuals

Plasma HIV RNA correlates
with plasma Nef in HIV+

Plasma Nef levels depend on
ART regimen in ART
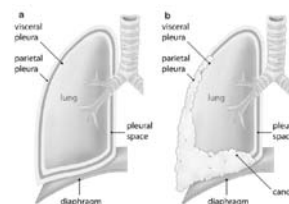

Plasma Nef levels were not consistently associated with
any of available clinical characteristics.

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Example 2:
EVs as biomarkers of response to treatment of MM

Genes (Basel). 2015
Jul 8;6(3):500-11

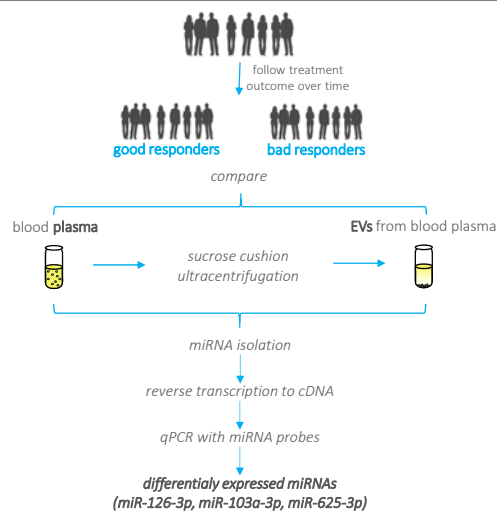Table 3: miRandola and miRNAExpress analysis of the most significant circulating miRNAs.

Circulating MM miRs	miR-126-3p ↓	miR-103a-3p ↓	miR-625-3p ↑	miR-29c-5p ↑	miR-92a-3p ↑
Samples	Plasma/serum	T cells/Dendritic cells	Plasma/serum	Serum/T cells	Plasma/serum/T cells/Dendritic cells
Specific carrier identified	Exosome	Ago2/Exosome	HDL (normal)/ Exosomes (prostate cancer)	Exosomes	Ago2/Exosome
Validated target and function	VCAM1 (vascular cell adhesion molecule 1)	ICOS (inducible T-cell co-stimulator), SERBP1 (SERPINE1 MRNA Binding Protein 1), FBXW11 (F-box and WD repeat domain containing 11)	Unknown	Unknown	Unknown
Potential biomarker in other diseases	Yes: metastatic colorectal cancer*, prostate cancer*, urothelial bladder cancer*, osteoarthritis, type 2 diabetes, acute myocardial infarction, endurance exercise, stable/ unstable angina	Unknown	Yes: low levels in NSCLC	Unknown	Yes: high level in gastric cancer, colorectal carcinomas, and hepatitis C infection
miRNAExpress output	Cells and circulation	Cells and circulation	Circulation	Cells	Cells and circulation

Oncotarget. 2016 Sep
6;7(36):58606-58637

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Example 2:
EVs as biomarkers of response to treatment of MM

follow treatment
outcome over time

good responders   **bad responders**

compare

blood **plasma**   **EVs** from blood plasma

sucrose cushion
ultracentrifugation

miRNA isolation

reverse transcription to cDNA

qPCR with miRNA probes

differentialy expressed miRNAs
(miR-126-3p, miR-103a-3p, miR-625-3p)

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