

Aging and Inflammation

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Aging

Kidney disease



Cardiovascular disease



Liver disease



Sarcopenia



Frailty



Osteoporosis



Neurocognitive Impairment

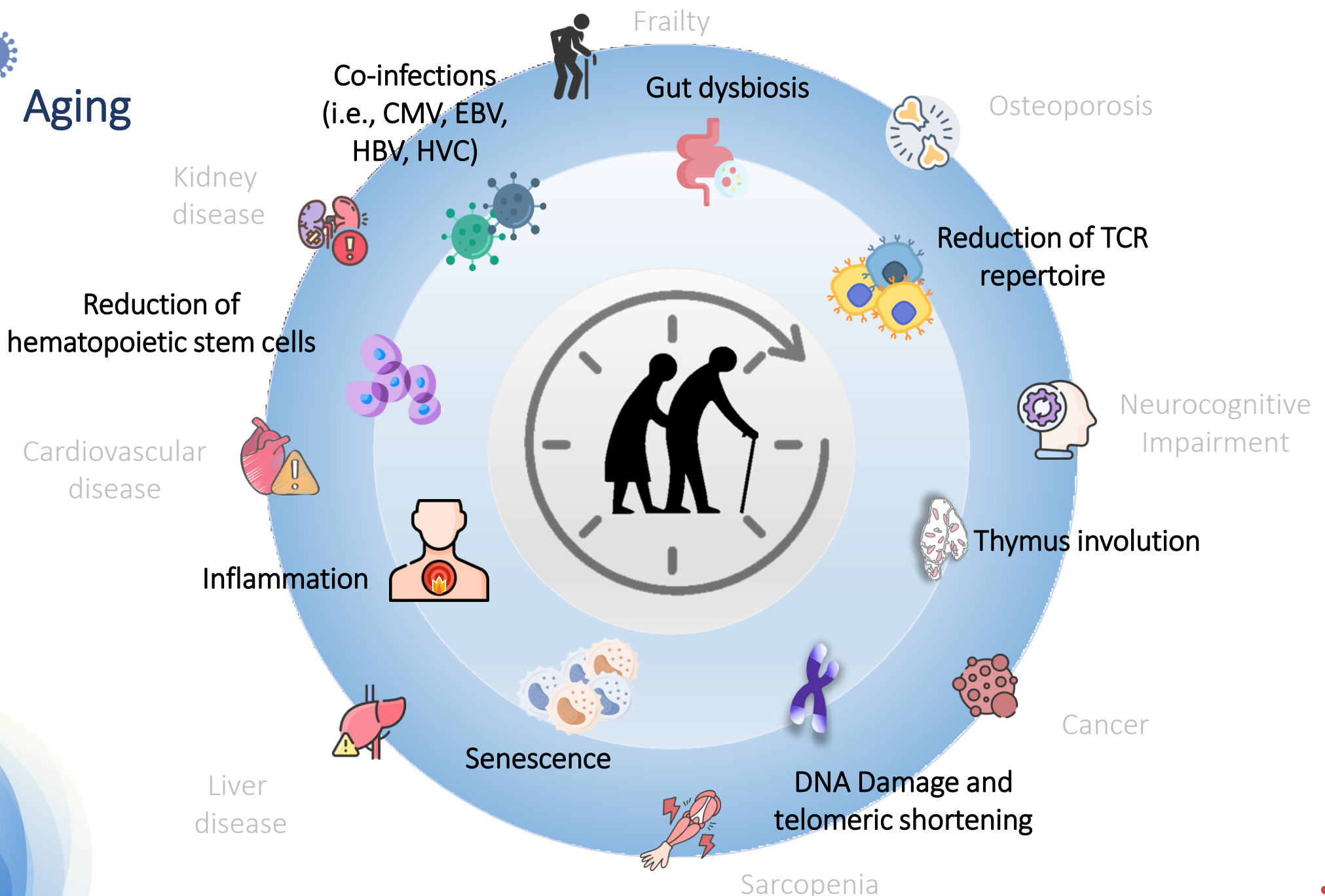


Cancer



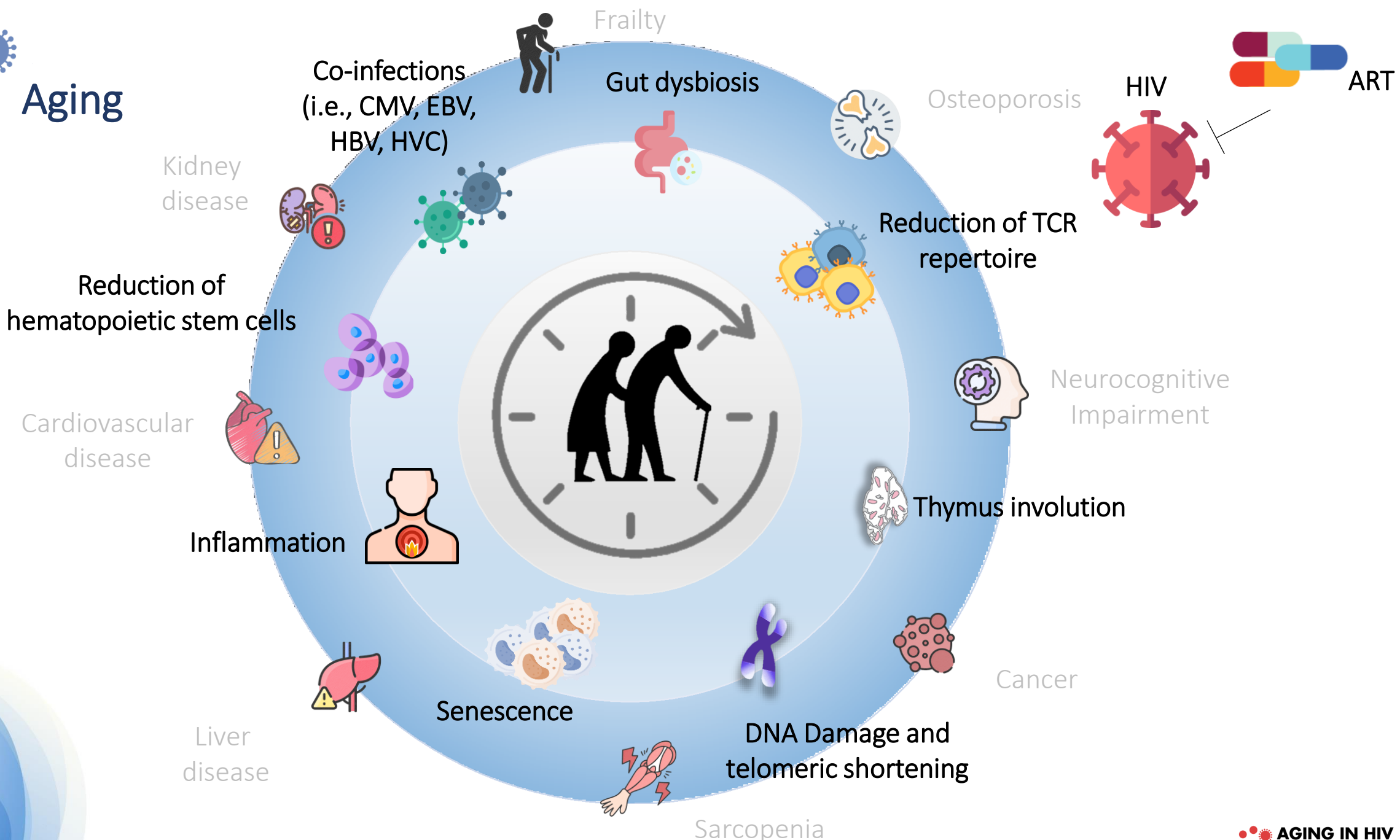
Based from the literature: Calcagno A et al, Infection 2015; Blanco JR et al, Exp Rev Anti-Effective Therapy 2021; Rodés et al, ebiomed 2022;

Aging



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Aging

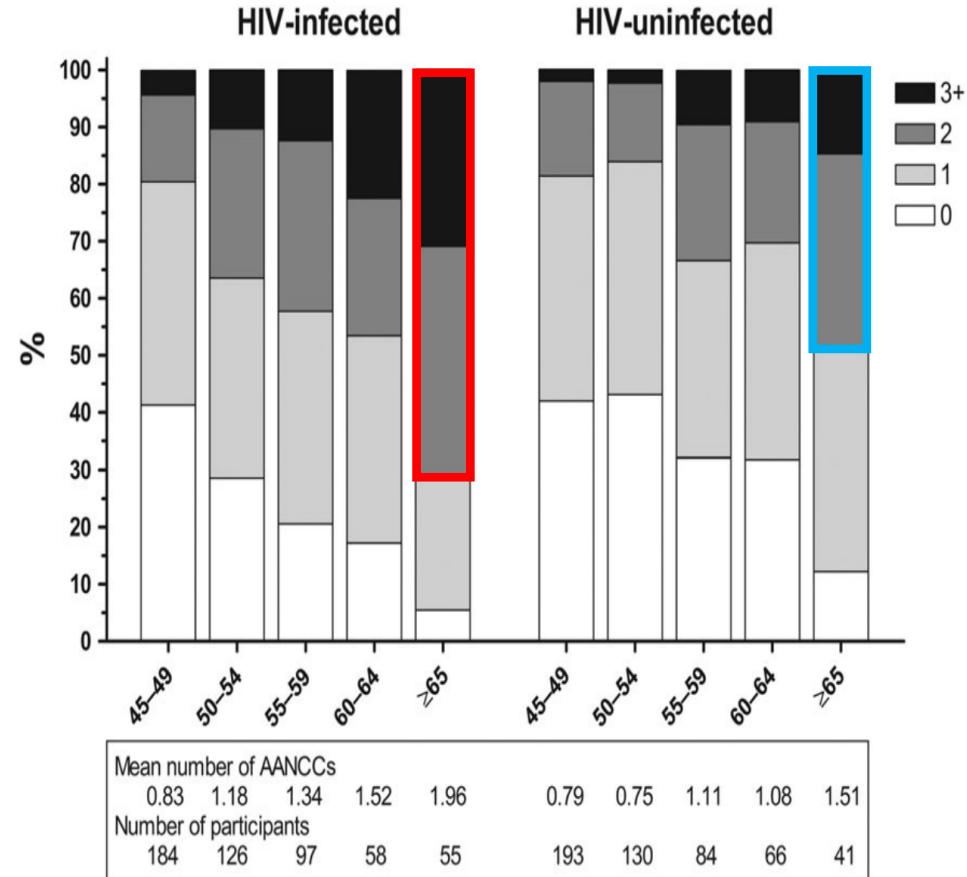


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Comorbidities

Serious non-AIDS defining events



PWH, N=540 (mean 12 years since diagnosis & 10 years on ART)

HIV-, N=524

> People with HIV (PWH) on ART show higher frequencies of **non-AIDS defining comorbidities associated with aging**

Non-AIDS defining events

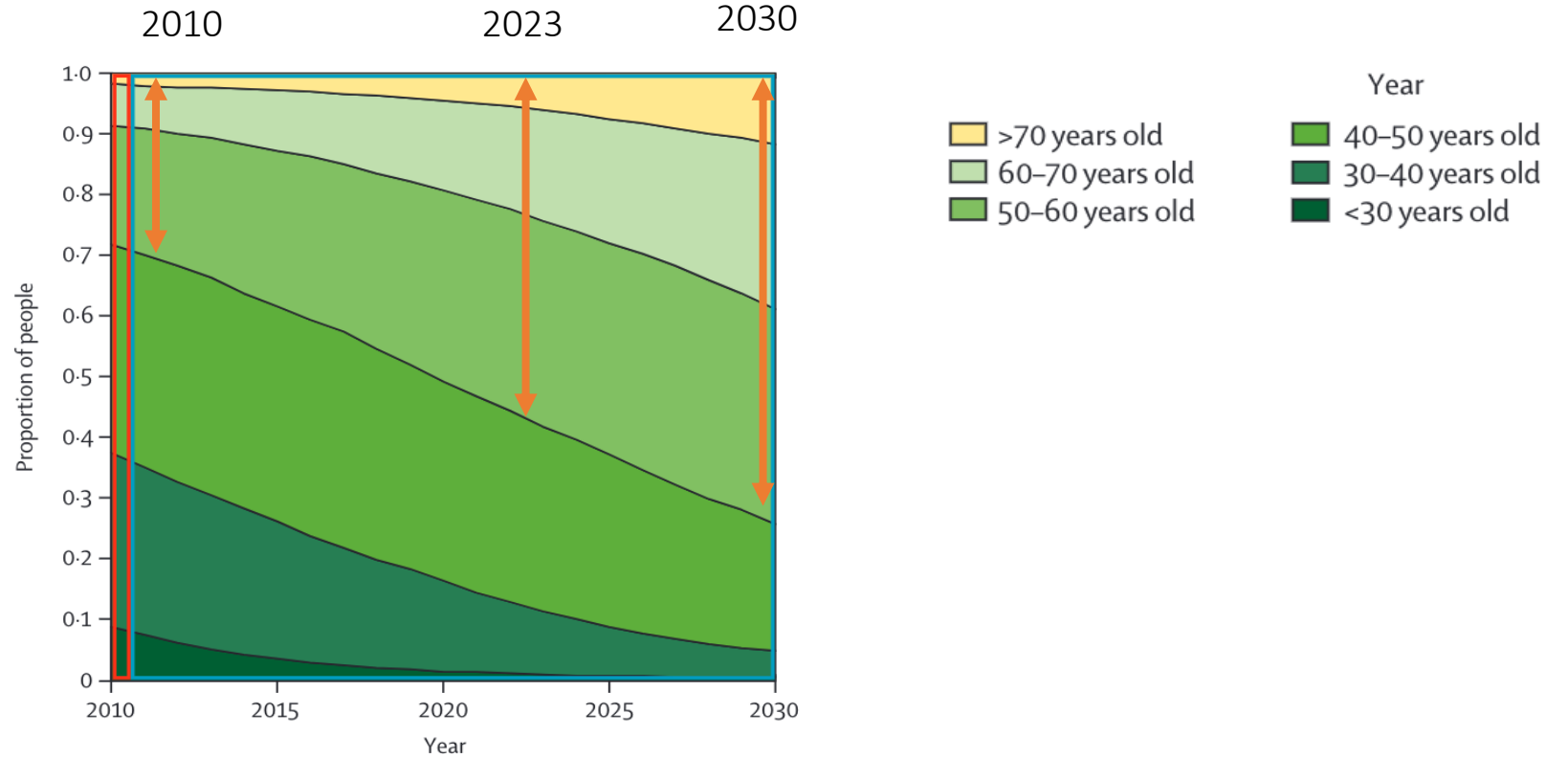
Comorbidities	HIV cohort n = 1,969 %	Matched non-HIV cohort n = 3,938 %	P value
→ Acute renal disease	0.5	0.2	0.045
Alcohol abuse	3.4	2.8	0.176
→ Bone fractures due to osteoporosis	6.4	2.1	<0.001
→ Cardiovascular disease	12.8	10.4	0.006
→ Chronic renal disease	4.3	2.4	<0.001
Diabetes mellitus (type II)	8.4	8.6	0.818
Dyslipidemia	23.9	24.0	0.914
→ HBV infection	5.9	0.3	<0.001
→ HCV infection	8.8	0.3	<0.001
→ Hypertension	29.3	32.6	0.010

HBV: hepatitis B virus, HCV: hepatitis C virus, P; p value for comparison between HIV and non-HIV matched control cohorts



New health problem issue

Prediction of the distribution of PWH by age



> It is expected that in 2030 than **>70%** of PWH will have more than **50 years old**

Normal process of
aging



**AGING WITH
HIV**

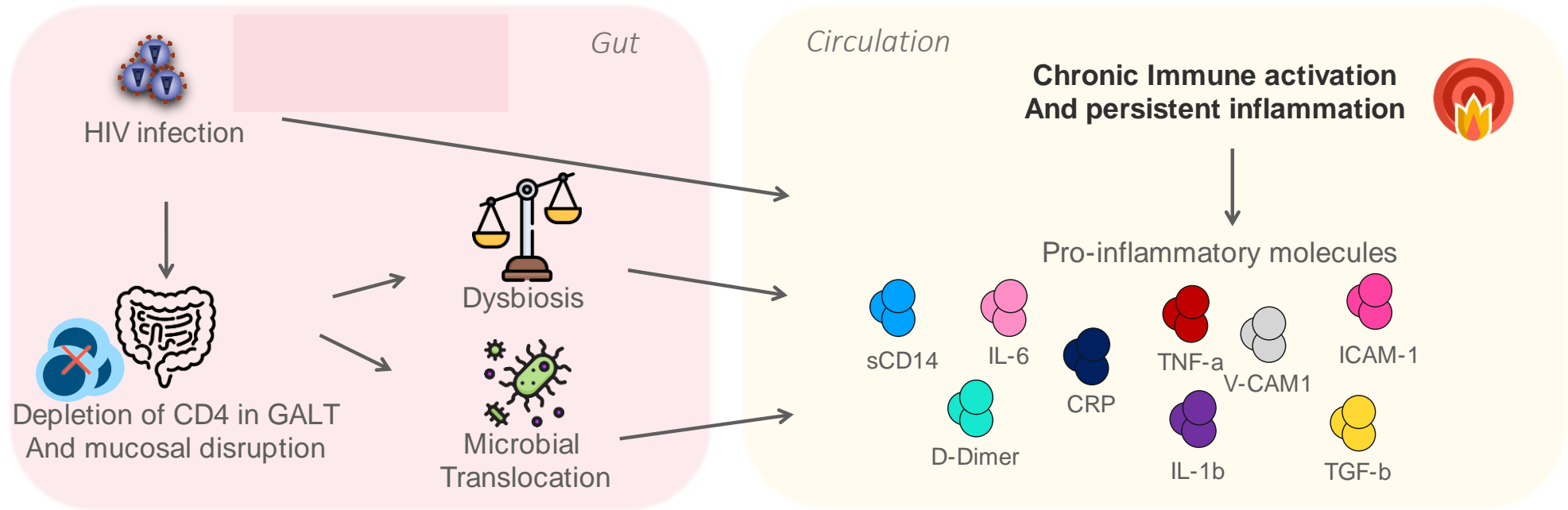
Persistent
inflammation and
immune dysfunction





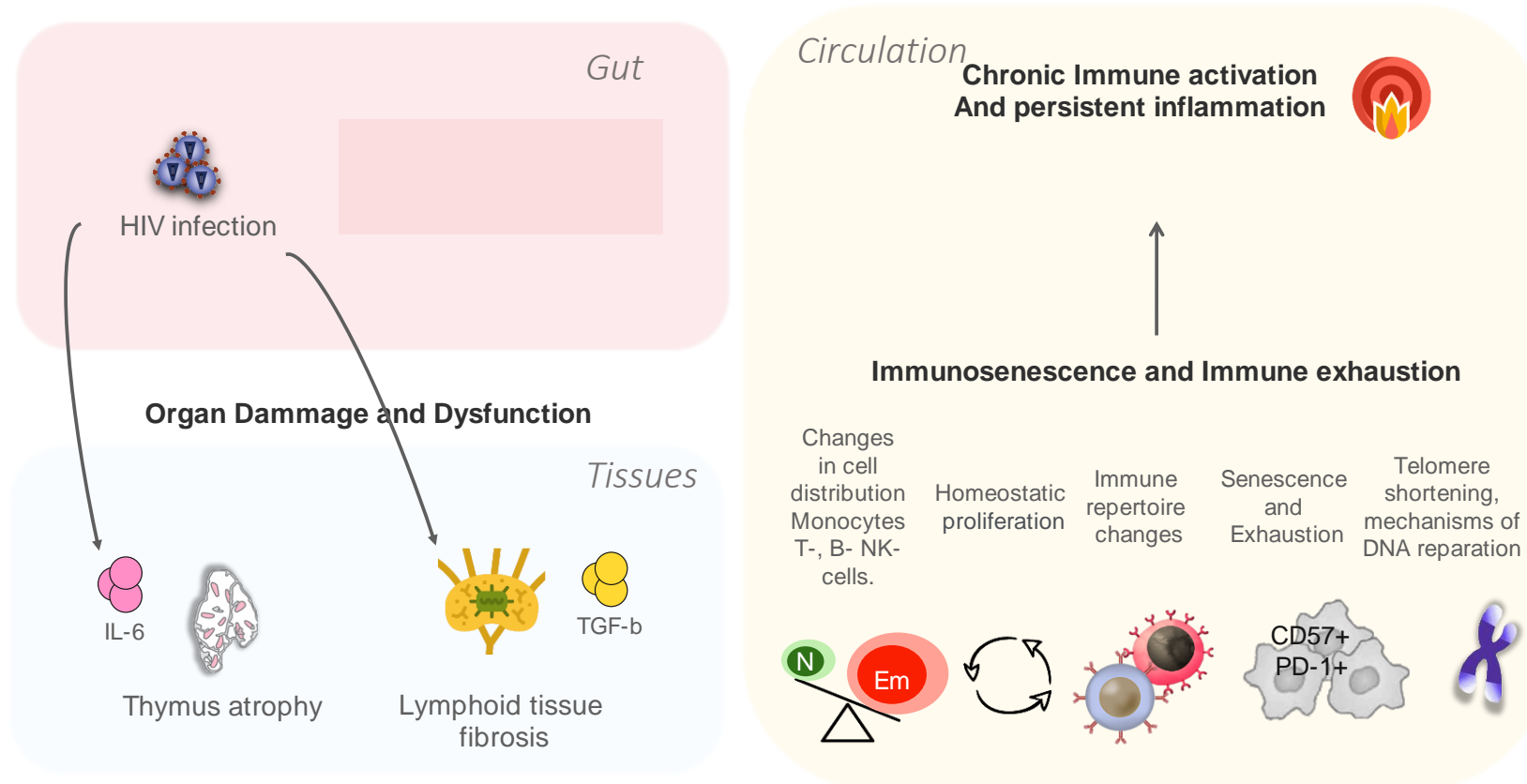
Bacterial translocation

Before treatment initiation



Thymic function and lymphoid tissue

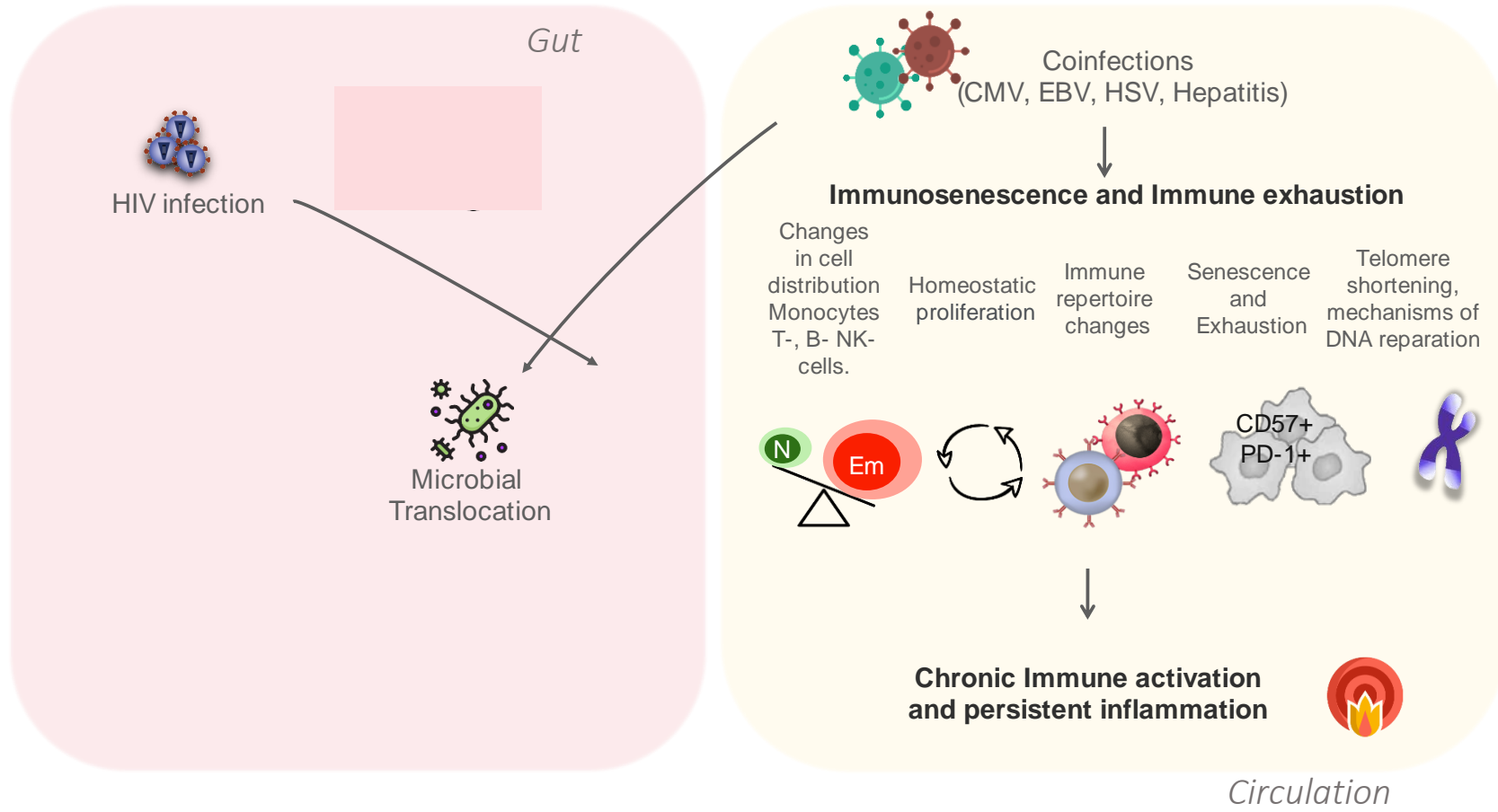
Before treatment initiation



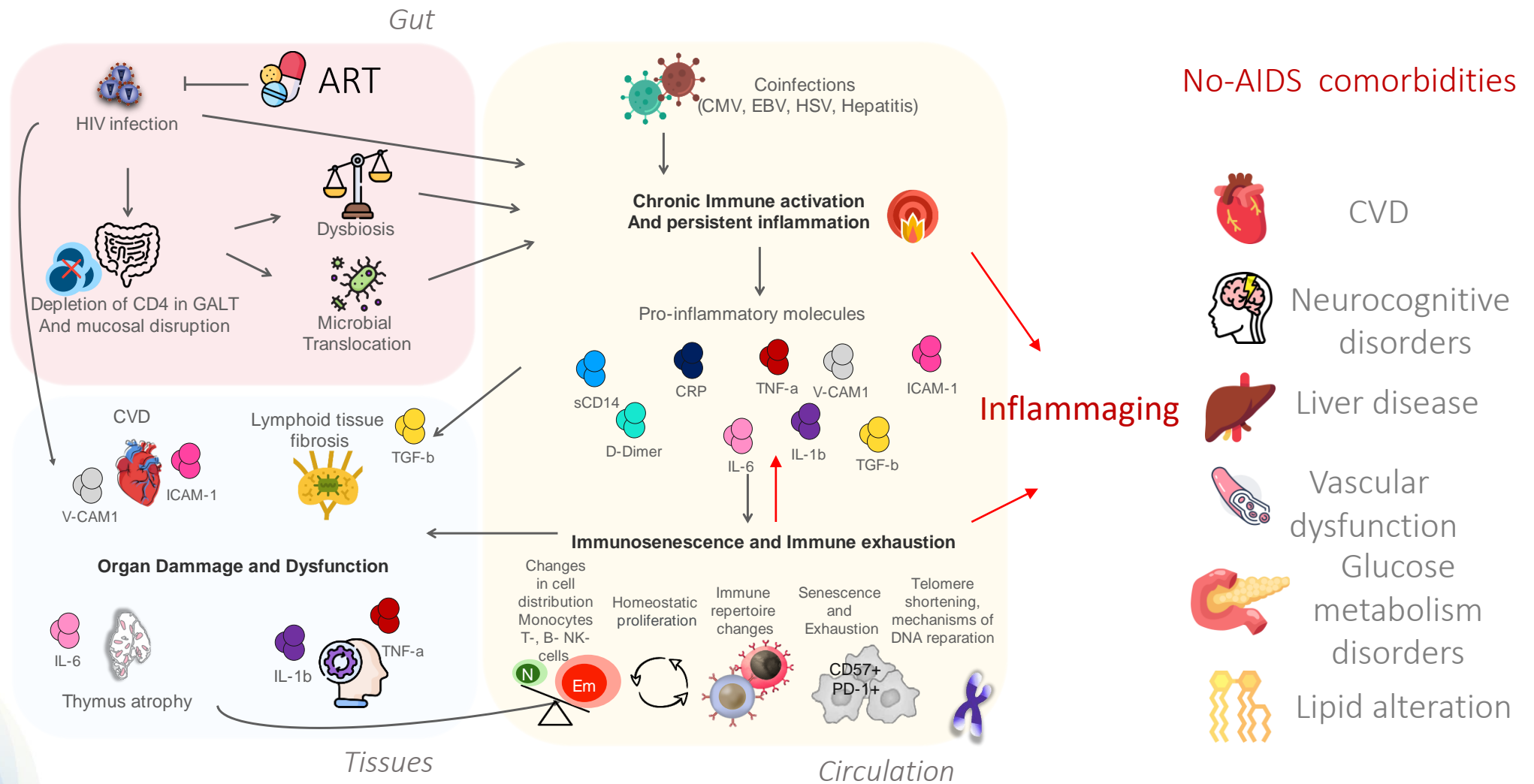


Co-infections

Before treatment initiation



No-AIDS defining comorbidities



No-AIDS defining comorbidities & inflammaging



Cardiovascular disease

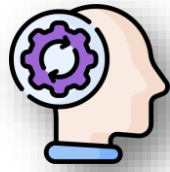
HIV / ART / **Inflammation** / Risk factors

HIV involved in:

- Activation of monocytes
- Thrombotic and fibrinolysis
- Increased oxidized lipids

• **Biomarkers** for atherosclerosis → CVD

- VCAM-1
- Fibrinogen
- D-Dimer
- CRP
- Selectins
- IL-6
- TNF
- Changes in Monocytes populations



Cognitive impairment

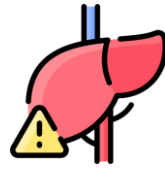
HIV / **Inflammation** / Stress

• HIV infection of microglia (HIV reservoir → immune activation and senescence)

• Inflammatory response → increase oxidative stress

• **Biomarkers** with neurocognitive decline → dementia

- IL-6, CRP (*plasma*)
- TNFa, B2macroglobulin, S100beta, neopterin (*CSF*)
- Brain imaging



Hepatic disease

Inflammation / HCV / ART

• Inflammation, coinfections (HCV) oxidative stress, mitochondrial injury, gut microbial translocation

• Recruitment of CTL, neutrophils, monocytes and NK cells to the liver → chronic hepatitis

• **Biomarkers**

- Inflammation
- Endothelial dysfunction
- Coagulopathy



Osteoporosis

HIV / **Inflammation** / **Activation** / ART (some drugs)

• TNFa increases NF-kb receptor, which stimulates bone resorption by osteoclasts

• TNFa and IL-1 inhibit osteoblast function

• Tenofovir or protease inhibitors → neg effects on bone mineral density.



Chronic Kidney disease

Activation / ART (some drugs) / **Polypharmacy** / Risk factors

• General immune activation

Cancer



Inflammation / coinfection (HPV) / Risk factors

• Oncogenic viruses with immunosuppression → expression of genes implicated in cell proliferation, apoptosis and carcinogenesis

Normal process of
aging



Toxicity of ART and
Polypharmacy

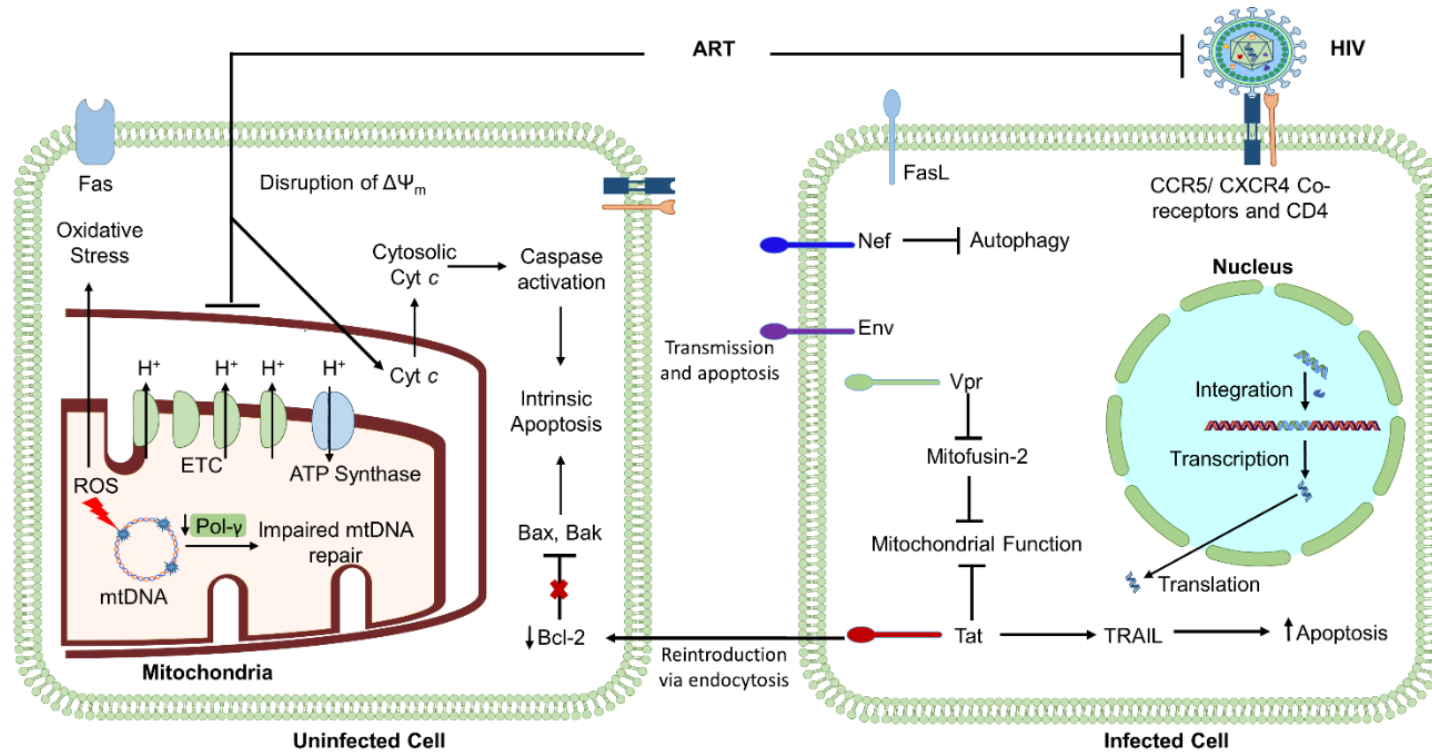


**AGING WITH
HIV**

Persistent
inflammation and
immune dysfunction



ART Toxicity and polypharmacy



> ART toxicity accumulates over time causing clinically meaningful **metabolic abnormalities (mitochondrial dysfunction)** and organ damage, independently of the virus

> Older PWH are at greater risk of **polypharmacy**

Normal process of
aging



Toxicity of ART and
Polypharmacy



AGING WITH
HIV

Life style (smoking,
alcohol & drugs)



Persistent
inflammation and
immune dysfunction

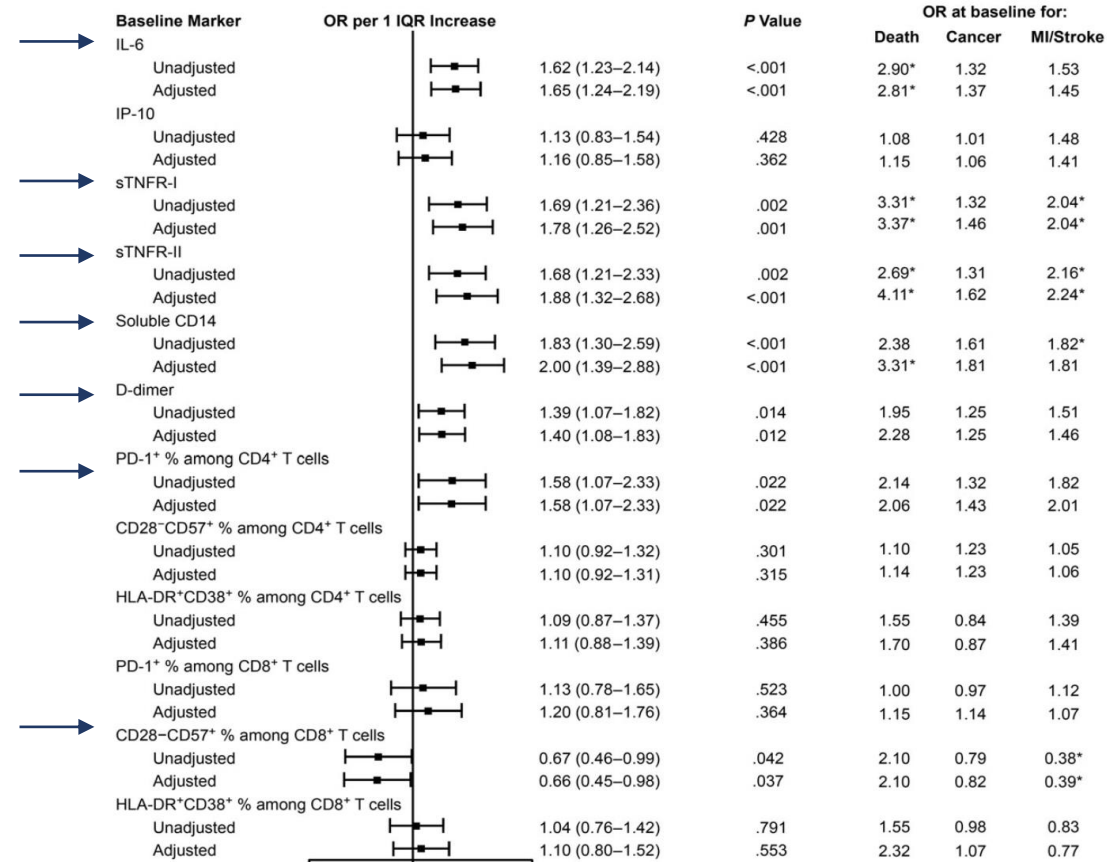


Effect of HIV infection on immunoaging

Pre-ART



Effect of time to ART initiation in immunoaging



PWH with non-AIDS events within 3 years of ART initiation, N=143

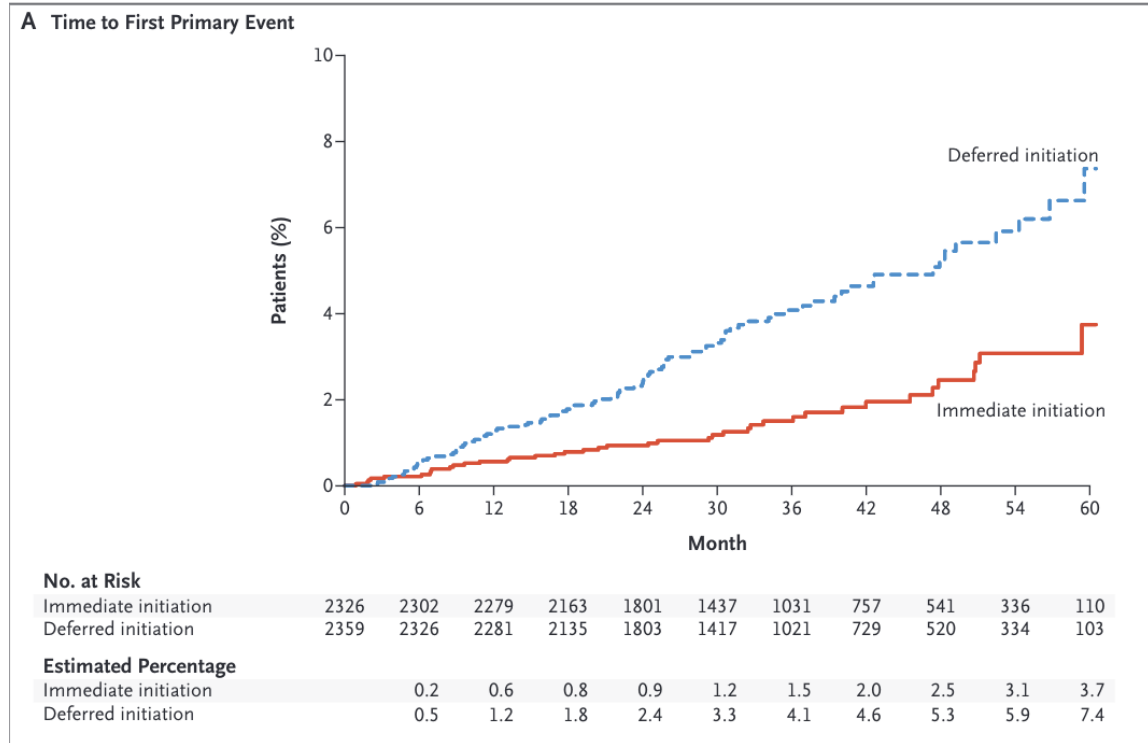
PWH without comorbidities N=345

Median Age: 45 years

> High levels of IL-6, sTNRF-I and sTNRF-II, sCD14 and D-dimer, as well as senescence of CD8 T cells and exhaustion of CD4 T cells before ART are predictors of development of comorbidities and death after ART initiation.



Immediate ART reduces non-AIDS events



Deferred ART, N=2359

Immediate ART, N=2326

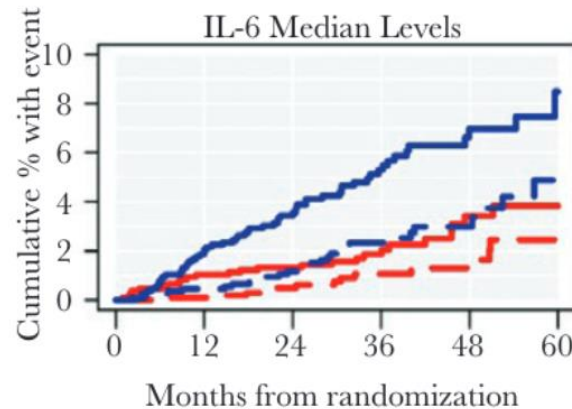
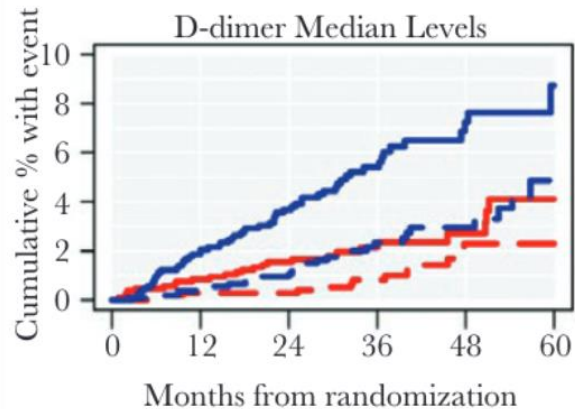
Median age: 36 Years

ART initiation: Median 1 year after diagnosis and 3.2 years of follow up

> START study showed that **immediate ART provide net benefits**, independently of immunologic state, to prevent AIDS and non-AIDS events.

Immediate ART reduces non-AIDS events

Primary End Point, AIDS, Serious Non-AIDS or Death



No. at risk					
Immediate, above median:					
1063	1043	839	470	246	69
Immediate, below median:					
1053	1036	926	587	319	85
Deferred, above median:					
1072	1033	839	471	246	73
Deferred, below median:					
1086	1058	923	580	294	77

No. at risk					
Immediate, above median:					
1074	1052	891	544	287	80
Immediate, below median:					
1048	1033	878	515	279	74
Deferred, above median:					
1076	1036	887	531	274	83
Deferred, below median:					
1098	1071	891	530	271	68

— Immediate, above median — Deferred, above median
- - Immediate, below median - - Deferred, below median

Deferred ART, N=2359

Immediate ART, N=2326

Median age: 36 Years

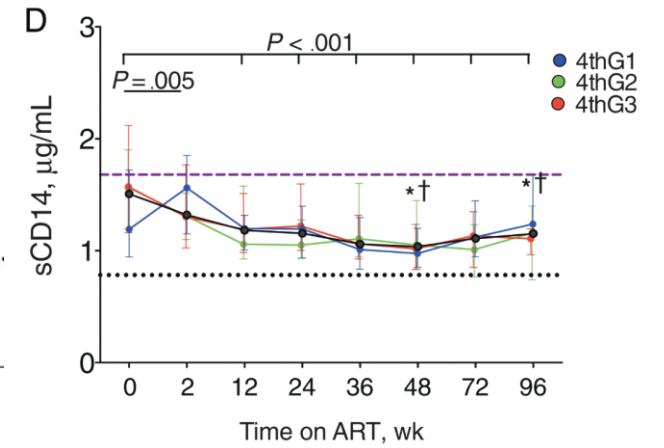
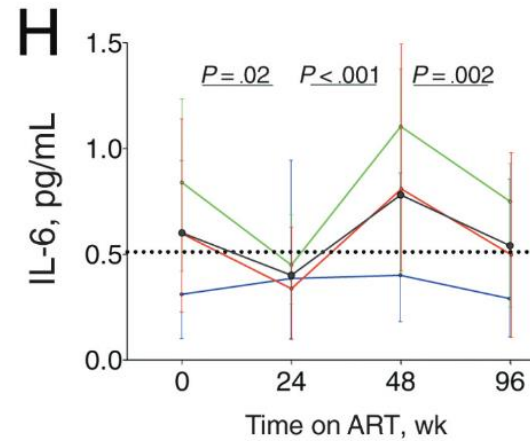
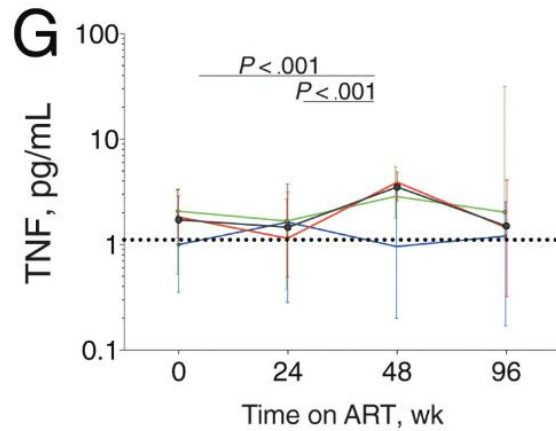
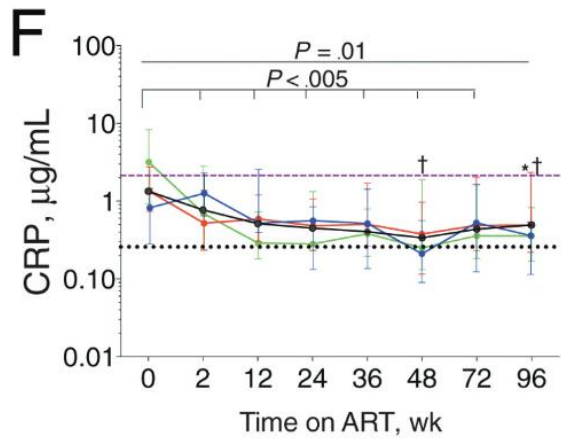
ART initiation:

Median 1 year after diagnosis and
3.2 years of follow up

> D-Dimer and IL-6 levels before ART predict the apparition of AIDS, severe non-AIDS events or death after ART initiation

Very early ART reduces inflammation to almost normal levels

--- Median Levels from PWH treated in the chronic phase
..... Median levels in uninfected



> ART initiation during the acute phase reduce significantly most inflammation markers

Aging with HIV, before and after 2015



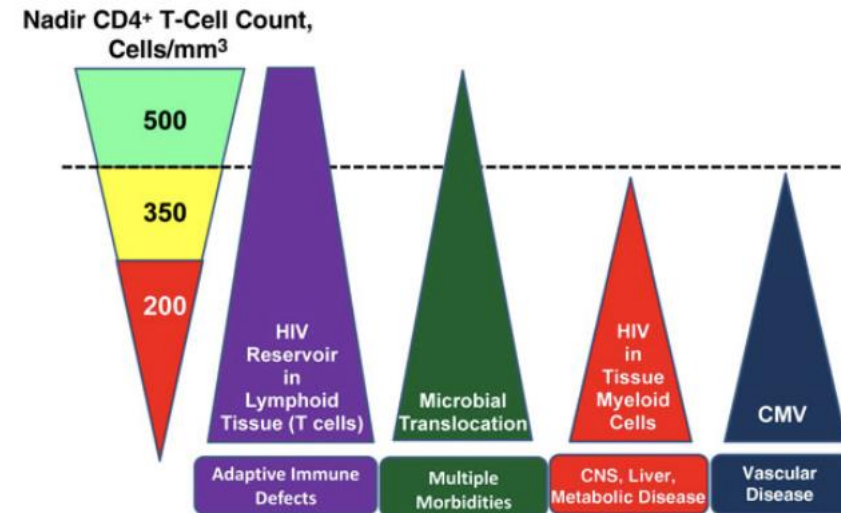
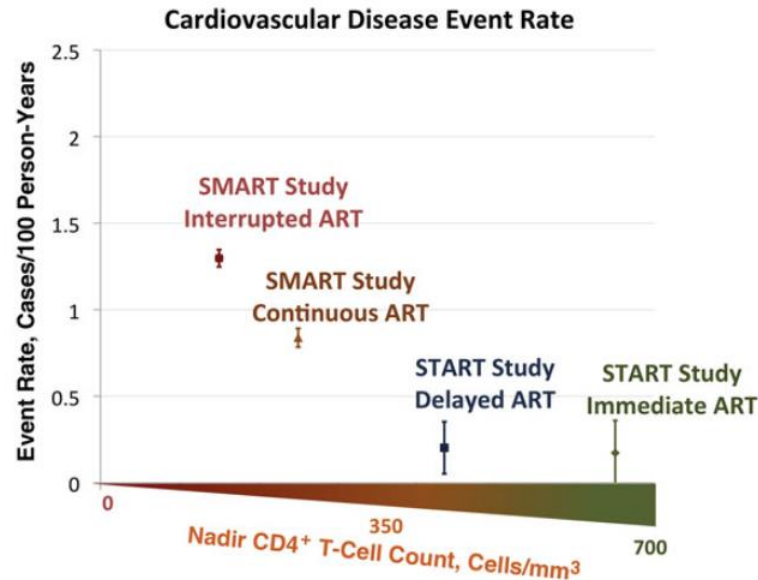
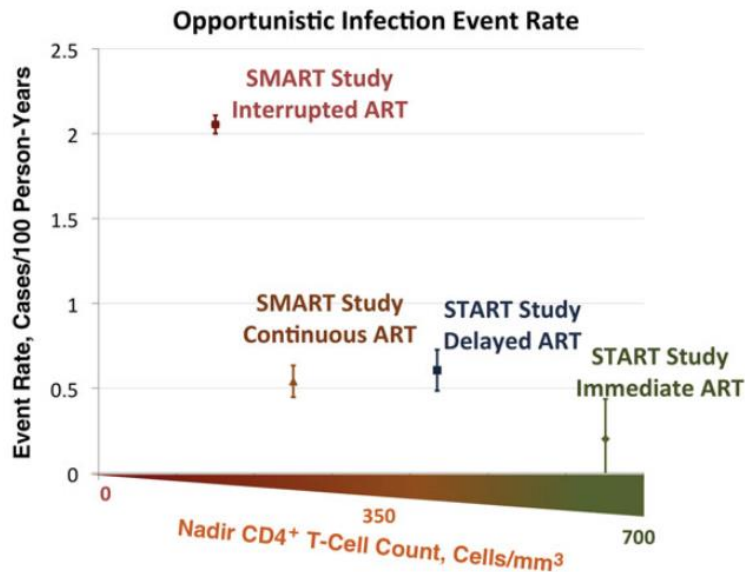
INFLECTION POINT

2015

GUIDELINES

GUIDELINE ON WHEN TO START ANTIRETROVIRAL THERAPY AND ON PRE-EXPOSURE PROPHYLAXIS FOR HIV

SEPTEMBER 2015



> Lower incidence of opportunistic infections and cardiovascular events when ART is started a higher median pretherapy CD4+T-cell counts (according to data reported SMART and START Trials)

Effect of HIV infection on immunoaging

Post-ART





Long-term effect of ART on inflammation

Biomarker	Event type	No. of pts with event	HR per 2x higher biomarker(s) with 95% CI	P-value
IL-6	SNA or death	260	1.45	<0.001
	CVD	84	1.33	0.009
	Cancer	99	1.39	<0.001
	Renal or hepatic	31	2.35	<0.001
	All-cause death	144	1.70	<0.001
D-dimer	SNA or death	260	1.28	<0.001
	CVD	84	1.26	0.03
	Cancer	99	1.20	0.06
	Renal or hepatic	31	1.71	<0.001
	All-cause death	144	1.43	<0.001

PWH, N=3766,

Mean 42 years of age

Mean 7 years since diagnosis

Mean 4.9 years on ART

> Persistent high levels of IL-6 and D-Dimer is independently associated to comorbidities and mortality in PWH on ART.

Long-term effect of ART on Activation, senescence and exhaustion

Table 2. Relationship of baseline immune scores and immune markers to comorbidities.

Characteristics ^a	Total (N=828)	Less than three comorbidities (N=678)	At least three comorbidities (N=150)	p ^b
Cellular CIADIS score	-0.1 (-1.5;1.6)	-0.4 (-1.7;1.3)	0.9 (-0.5;2.5)	≤0.01
Soluble CIADIS score	-0.1 (-1.2;1.2)	-0.2 (-1.3;1.1)	0.3 (-0.7;1.4)	≤0.01
VACS	18 (10;28)	16 (6;27)	23 (12 ; 37)	≤0.01
IRP	2 (1;4)	2 (1;4)	3 (2;5)	≤0.01
→ CD4 ⁺ /CD8 ⁺ ratio	0.9 (0.6;1.2)	0.9 (0.6;1.2)	0.7 (0.5;1.1)	0.12
Activation and senescence of T cells				
→ T Lymphocytes: CD4 ⁺ DR+ (%)	14 (10;19)	13 (10;18)	15 (11;21)	≤0.01
→ CD4 ⁺ CD57 ⁺ CD28 ⁻ (%)	3 (1;7)	2 (0;7)	4 (1;10)	≤0.01
→ CD4 ⁺ TN (%)	40 (28;51)	41 (30; 52)	33 (22;47)	≤0.01
→ CD4 ⁺ TEMRA (%)	1 (0;4)	1 (0;3)	2 (1;5)	≤0.01
→ T Lymphocytes: CD8 ⁺ DR+ (%)	35 (26;47)	34 (25;46)	39 (30;51)	≤0.01
→ CD8 ⁺ CD57 ⁺ CD28 ⁻ (%)	27 (17;36)	26 (17;35)	28 (20;37)	0.02
→ CD8 ⁺ TN (%)	38 (29;48)	40 (30;50)	33 (24;41)	≤0.01
→ CD8 ⁺ TEMRA (%)	26 (16;36)	25 (15;35)	30 (18;39)	≤0.01
Coagulation				
→ Coagulation markers				
→ D-Dimer (ng/ml)	215 (144;320)	210 (142;307)	256 (160;379)	≤0.01
Monocytes activation				
→ B Lymphocyte marker				
→ BAFF BLYSS (pg/ml)	951 (749;1214)	944 (739;1202)	975 (779;1256)	0.15
→ Monocyte/macrophage markers				
→ sCD163 (ng/ml)	451 (370;547)	446 (367;534)	483 (393;608)	≤0.01
→ sCD14 (ng/ml)	59 (40;112)	58 (39;109)	64 (48;133)	0.04
→ Neutrophil marker				
→ MPO (ng/ml)	31 (17;67)	31 (18;68)	31 (16;55)	0.52
→ T Lymphocyte and platelet marker				
→ sCD40L (pg/ml)	601 (306;1198)	632 (320;1218)	486 (292;966)	≤0.01
Inflammation				
→ Pro-inflammatory cytokines				
→ CRP (ng/ml)	1753 (673;4857)	1598 (641;4587)	2218 (922;5491)	0.02
→ IL-18 (pg/ml)	116 (61;177)	117 (58;175)	113 (70;193)	0.28
→ IP10 (pg/ml)	230 (148;367)	227 (148;362)	249 (151;373)	0.65
→ IL-6 (pg/ml)	13 (9;20)	13 (8;19)	13 (9;21)	0.22
→ TNFR1 (pg/ml)	208 (142;288)	204 (138;273)	249 (166;350)	≤0.01
→ CD54 (ng/ml)	39 (27;54)	38 (27;54)	39 (27;55)	0.73

PWH, N=828

Mean age: 51 years

Mean 5 years of suppressive ART

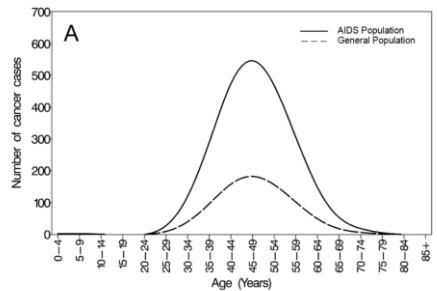
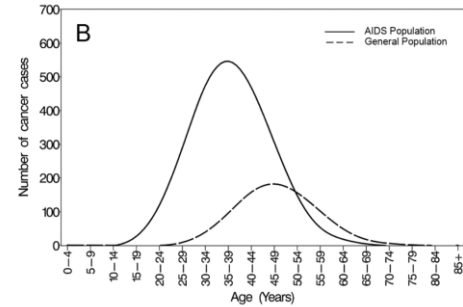
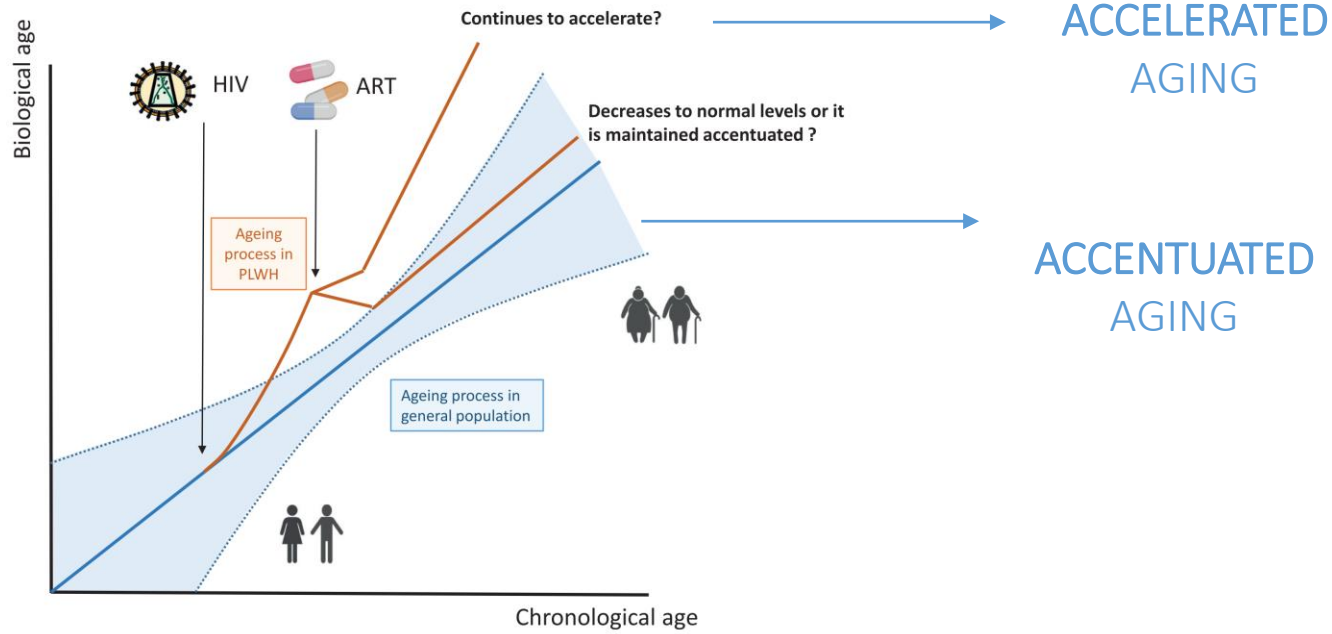
BAFF, B-cell activating factor; CRP, C-reactive protein; IL, interleukin; IP10, interferon gamma-induced protein 10; MPO, myeloperoxidase; sCD40L, sCD40 ligand; TEMRA, terminally differentiated T cells; TN: T-naïve; TNFR1, tumor necrosis factor receptor-1.

^aValues are medians (IQR) unless stated otherwise.

^bStudent's t-test or Mann-Whitney U-test was used for quantitative variables/chi-square test or Fisher's exact test was used for qualitative variables.

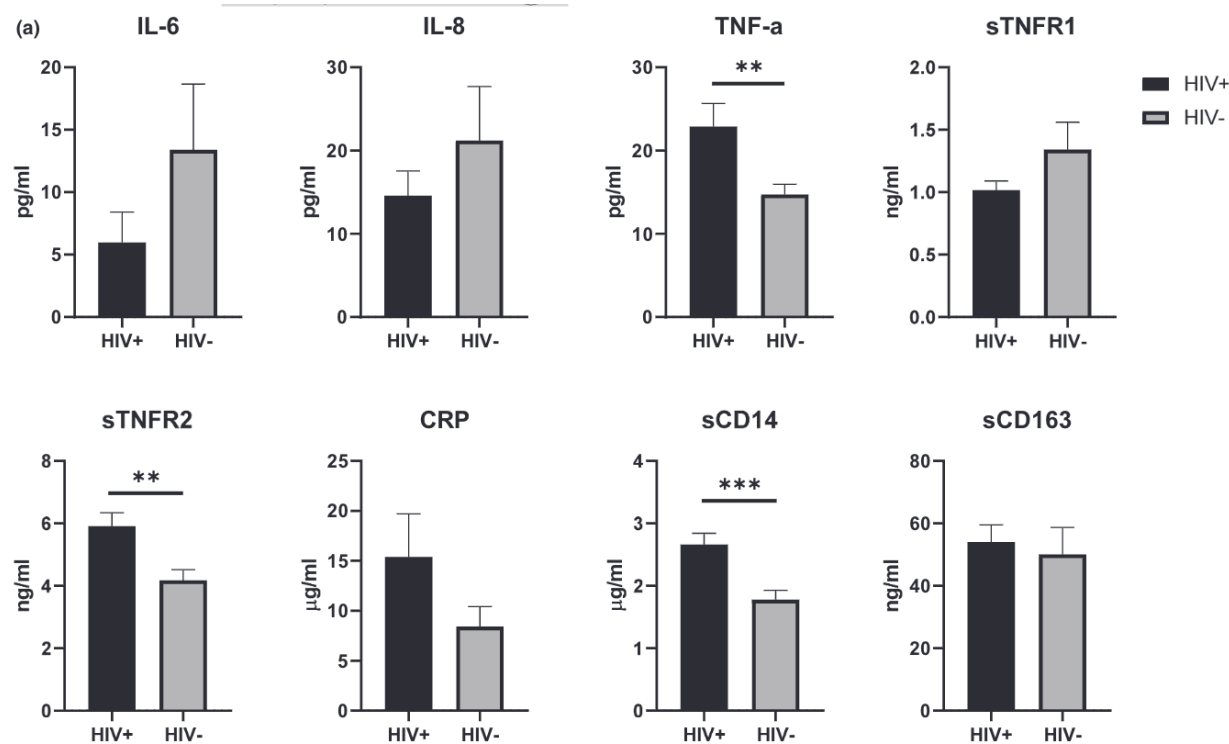
> There is a higher frequency of activated and senescent T cells, and increased inflammation (IL-6, CRP), coagulation factors (D-Dimer) and activation of monocytes in PWH with >3 comorbidities

ImmunoAging: accentuated or accelerated?





Long-term effect of ART - Inflammation



PWH, N=42, 20 years since diagnosis

Uninfected N=46

Median age: 59 years (>50 years)

Comorbidities: PWH more CVD and liver disease

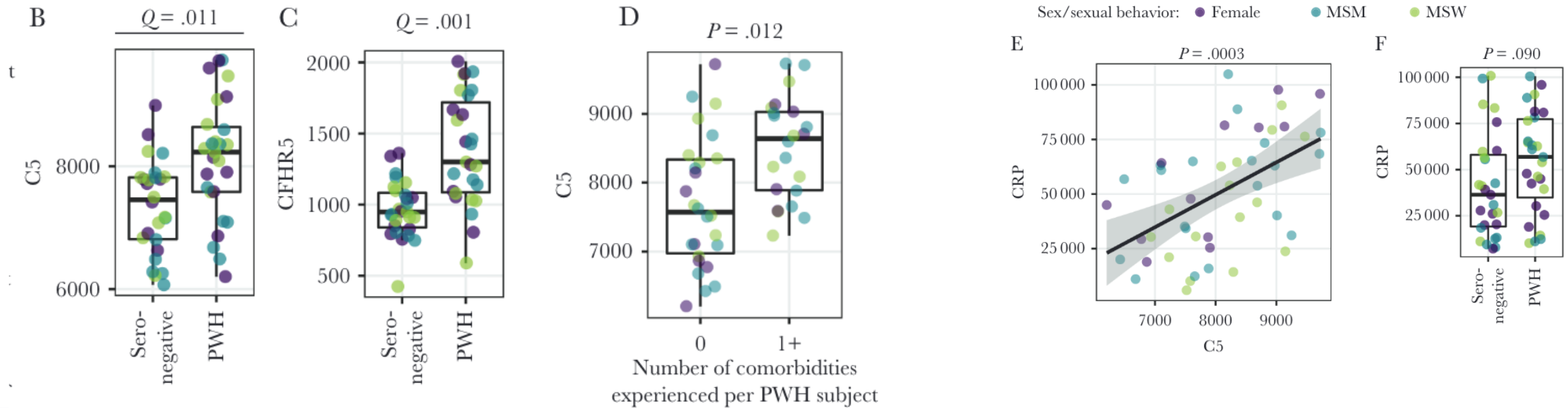
> Higher levels of sCD14, TNFa and sTNFR2 in PWH compared to uninfected individuals

Long-term effect of ART - Inflammation

PWH, N=27
Uninfected N=27

Median age: 55 years

Complement

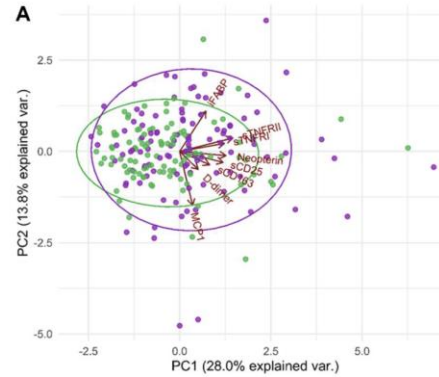


- > The Complement pathway is activated in PWH on ART, and is associated with no-AIDS events
- > C5 correlates with CRP, a traditional altered marker

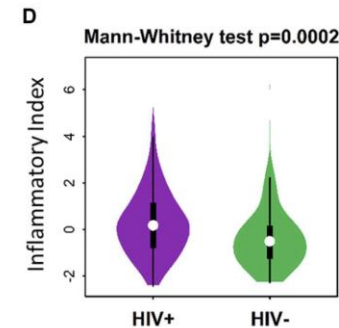
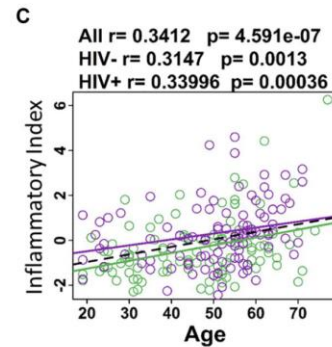


Immunologic age - Inflammation

Marker Name
BNP
CMV IgG
CRP
CXCL13
D-Dimer
Intestinal fatty acid binding protein (iFABP)
Interferon (IFN)-a
Interferon-g
Interleukin (IL)-10
Interleukin-12p70
Interleukin-17A
Interleukin-1B
Interleukin-2
Interleukin-21
Interleukin-6
Interleukin-8
LPS
MCP1
Neopterin
Soluble CD14
Soluble CD163
Soluble CD25
Soluble ICAM
Soluble TNFRI
Soluble TNFRII
Soluble VCAM
TNF



	PC1
sTNFR2	0.51
Neopterin	0.44
sTNFR1	0.43
sCD25	0.42
sCD163	0.29
iFABP	0.25
D-dimer	0.18
MCP1	0.12

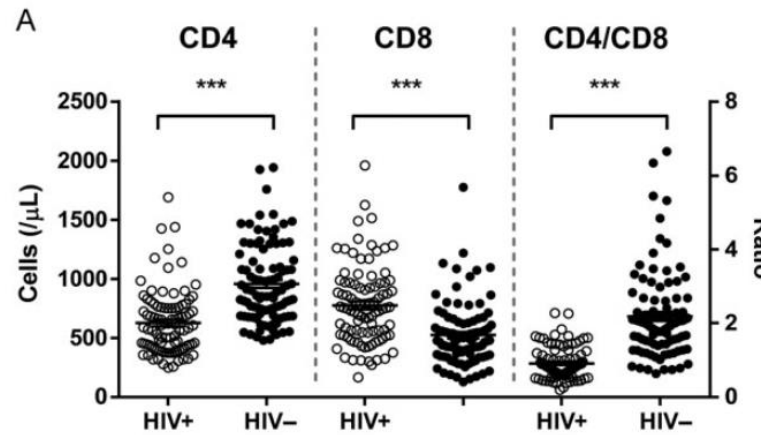


PWH N=106,
 Uninfected N=103
 Individuals between 19 and 79 years
 From 5 to 13 years on suppressive ART, depending on age group

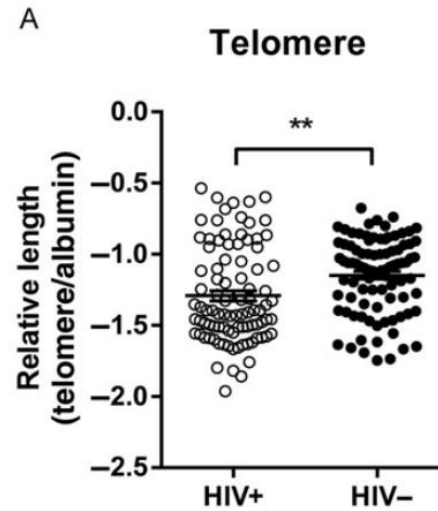
> The **inflammatory Index** is higher in PWH than uninfected individuals, and correlates with age.

Long-term effect of ART – Senescence and exhaustion

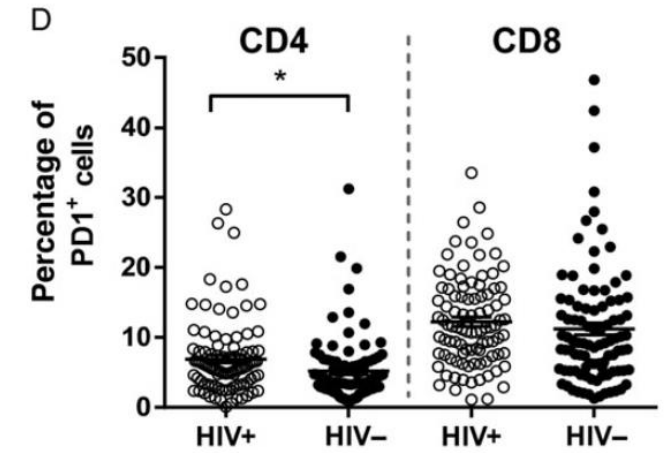
Low CD4/CD8 ratio



Reduction of telomere length



Increase immune exhaustion



PWH, N=94, 13 years since diagnosis and 10 years on ART
 Uninfected N=95
Median age: 56 years

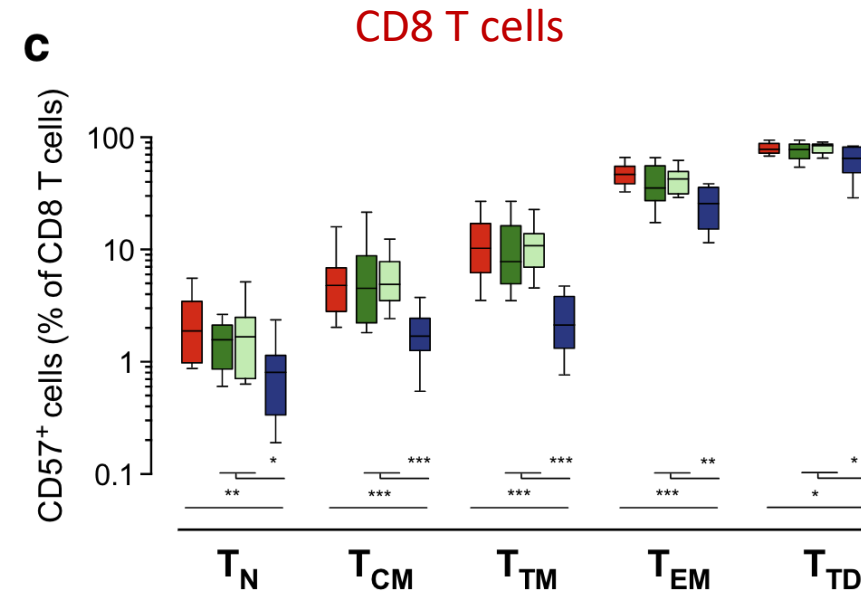
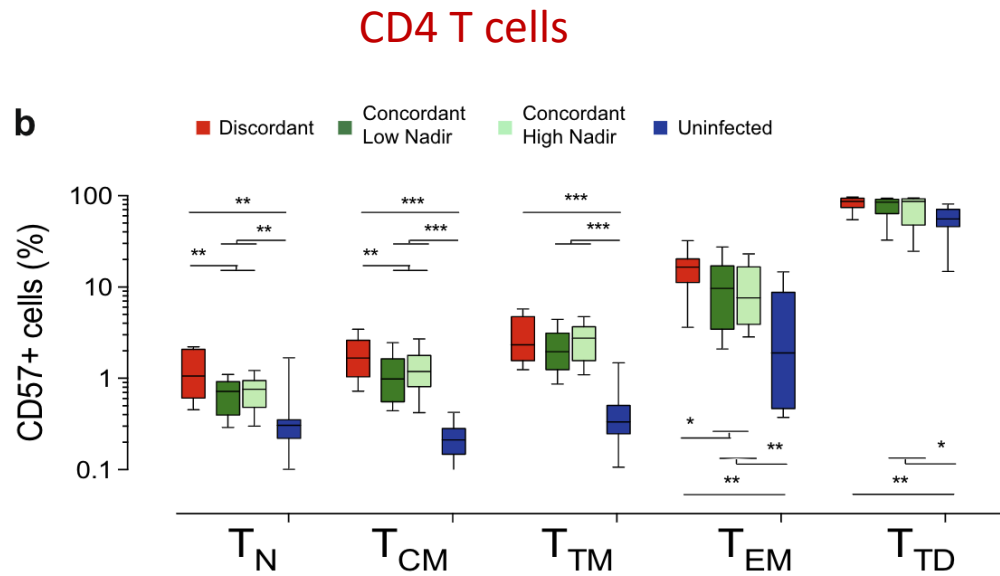
> Despite virologic suppression, PWH show higher levels of senescent hallmarks and exhaustion maker (PD-1), causing a negative impact in health and aging



Long-term effect of ART - Senescence

PWH, N=56, 12 years since infection
Uninfected N=11

Median age: 46 years



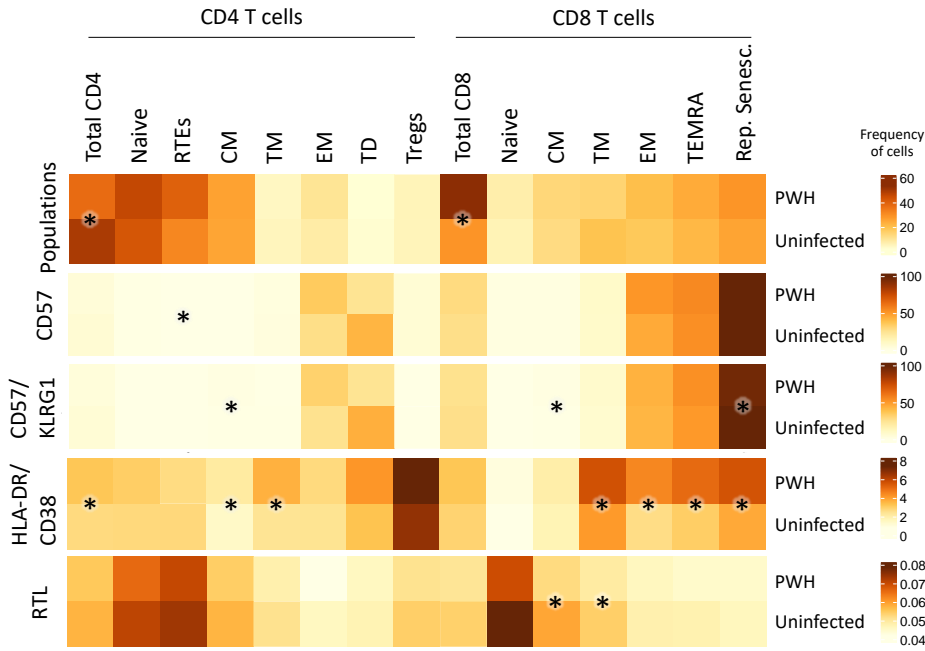
> Despite effective suppressive ART, PWH show higher levels of senescence in CD4 and CD8 T cell subsets (CD57+), especially those with low CD4 T-cell counts.

Long-term effect of ART – OVER50 STUDY



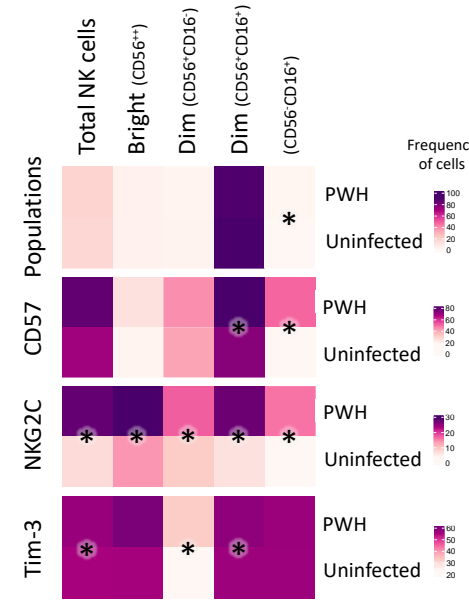
E. Negredo M. Trigueros

T-cells



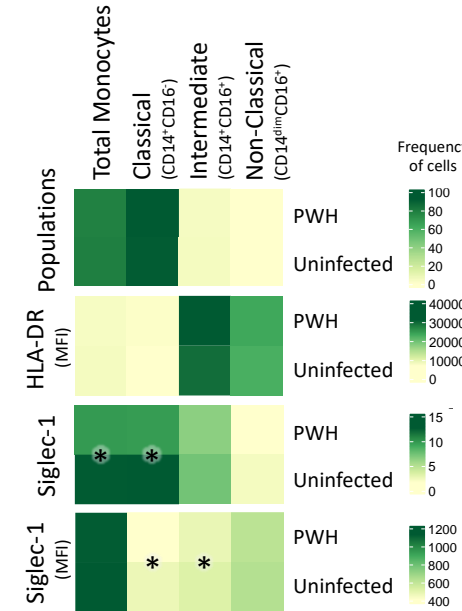
= senescence / ▲ Activation

NK-cells



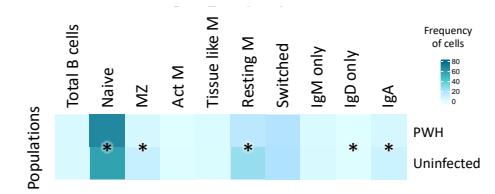
▲ Activation

Monocytes



= pro-inflammatory profile

B cells



PWH, N=27

Median 16 years on ART

Current CD4 T cells ≥ 350 cells/ μ l

Uninfected, N=24

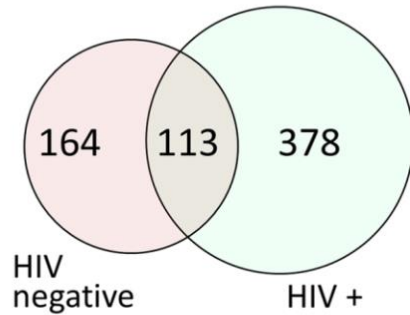
Median age, 72 years

Similar number of comorbidities

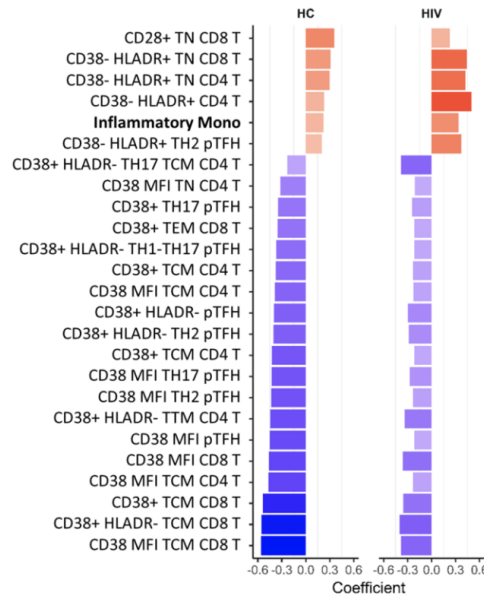
> Some immune dysfunctions were associated to HIV replication (pre-ART), which do not totally resolve after ART initiation (**HIV effect**). In contrast, other well-described immune differences between PLW and uninfected individuals are not found significant in our study groups, suggesting that some dysfunctions equalize over time (**Age effect**).

Immunologic age – Activation, Senescence and Exhaustion

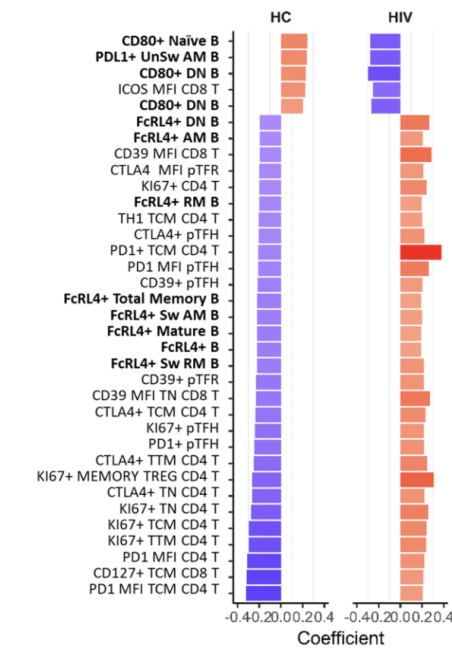
A Number of Parameters Correlating with Age ($p < 0.05$)



A Same Direction Parameters (Top 25)



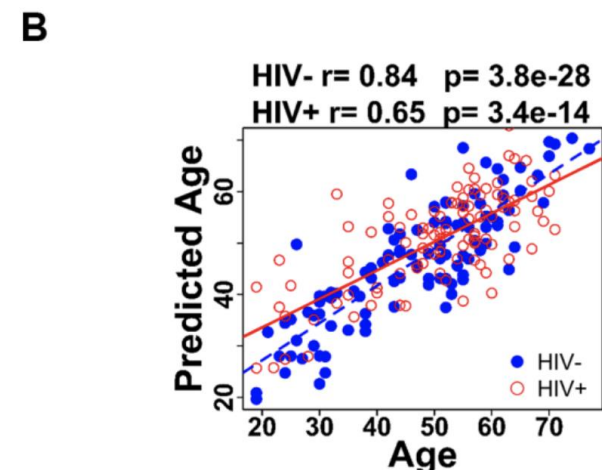
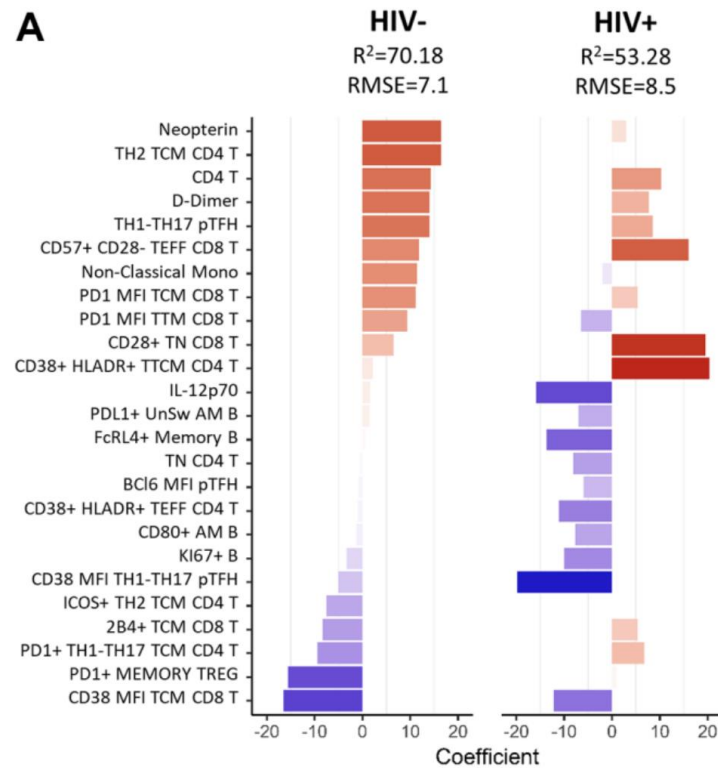
B Opposite Direction Parameters (35)



PWH N=106,
Uninfected N=103
Individuals between 19 and 79
years
From 5 to 13 years on
suppressive ART, depending on
age group

> Cellular markers associated with age are different between PWH and uninfected controls.

Immunologic age – Inflammation + cellular markers



PWH N=106,
Uninfected N=103
Individuals between 19 and 79 years
From 5 to 13 years on suppressive ART, depending on age group

- > Predicted age with the “Immunological Age prediction” with 25 parameters (IMAP25) correlated better with real age in the uninfected population.
- > Only PWH younger group (<40 years) show a premature aging rate compare with their uninfected counterparts



Immunologic age - HIV DNA repair study



Eugenia
Negredo

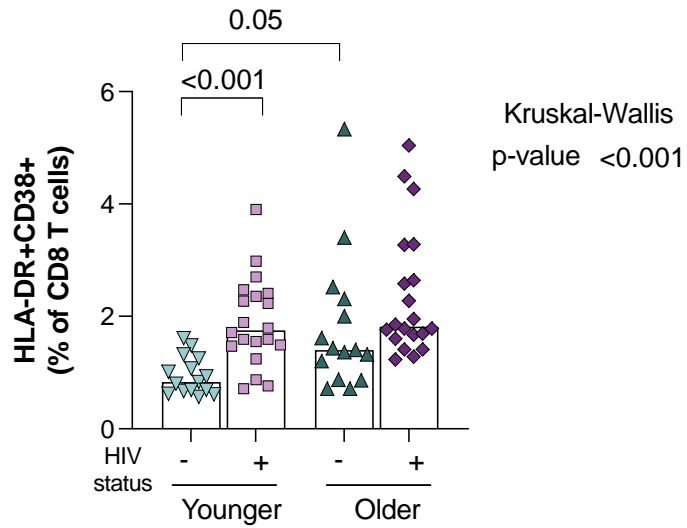


Cora
Loste

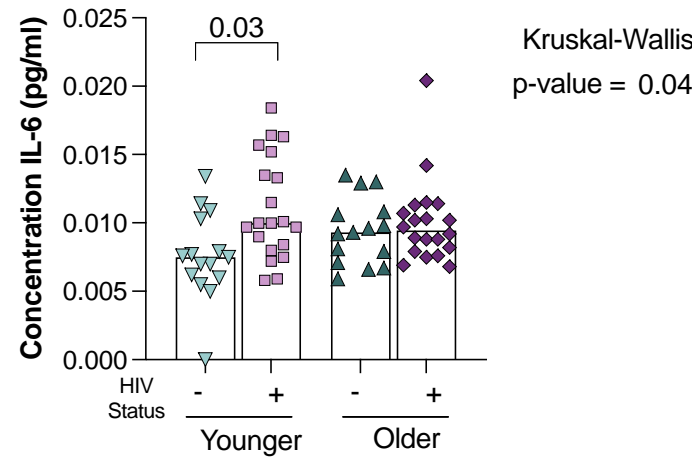


Macedonia
Trigueros

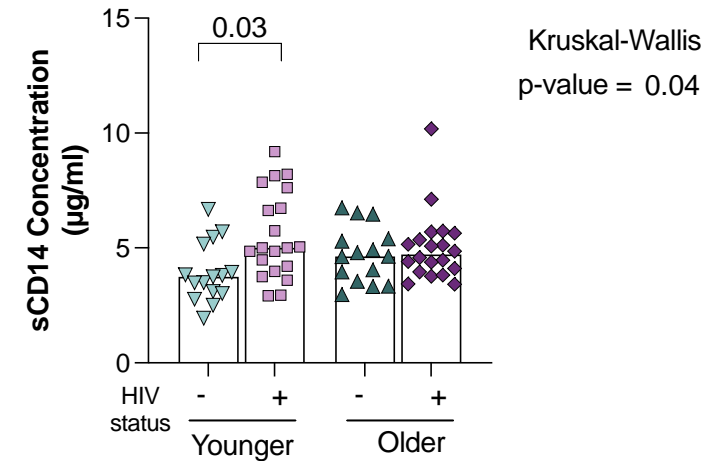
CD8 T-cell Activation



IL-6



Soluble CD14



PWH young vs old, N=20+20

Chronic HIV infection >10 years and suppressive ART >5 years

Nadir CD4 T lymphocytes ≥ 300 cells/ μ l

Uninfected young vs old, N=15+15

> Major differences in activation and inflammation are observed between younger PWH and uninfected, while no differences are found between the older group



Immunologic Age

PWH Young vs Middle vs Old, N=28+67+45

Time on ART: 6y vs 11y vs 13y

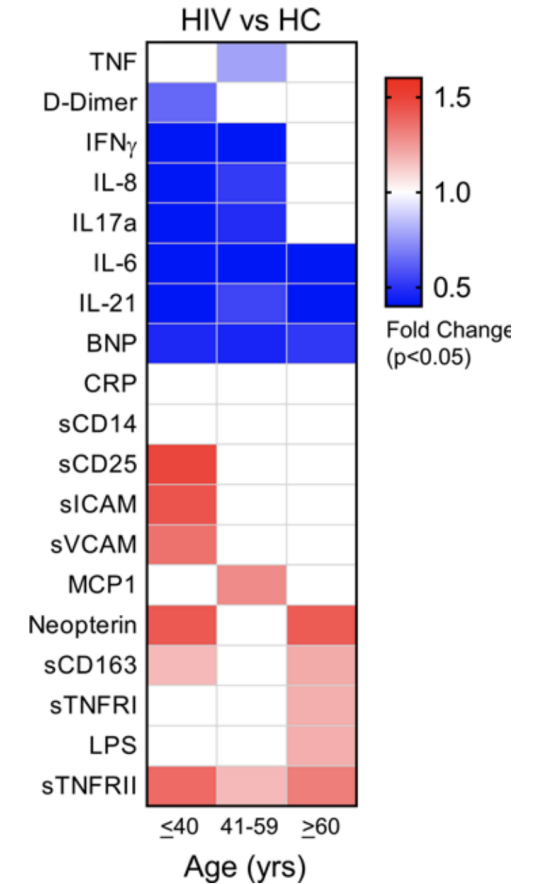
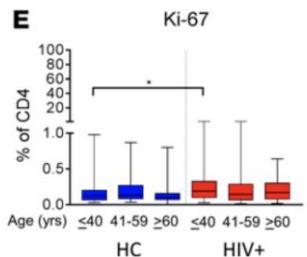
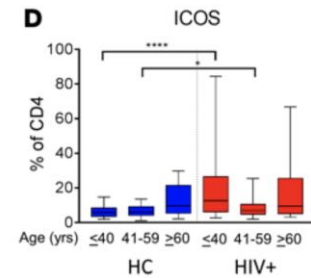
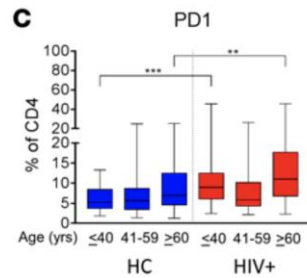
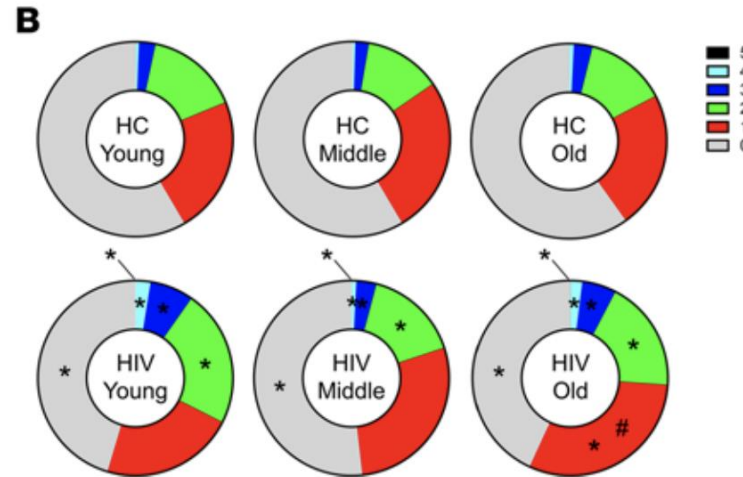
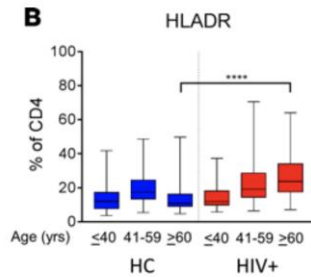
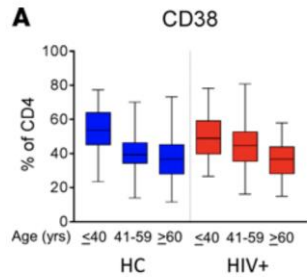
Uninfected Young vs Middle vs Old, N=42+57+42

Median ages:

Y:30 years vs M: 50 years vs O:65 years

Comorbidities: non-specified

CD4 T cell activation
(CD38, HLA-DR, PD-1, ICOS, Ki-67)

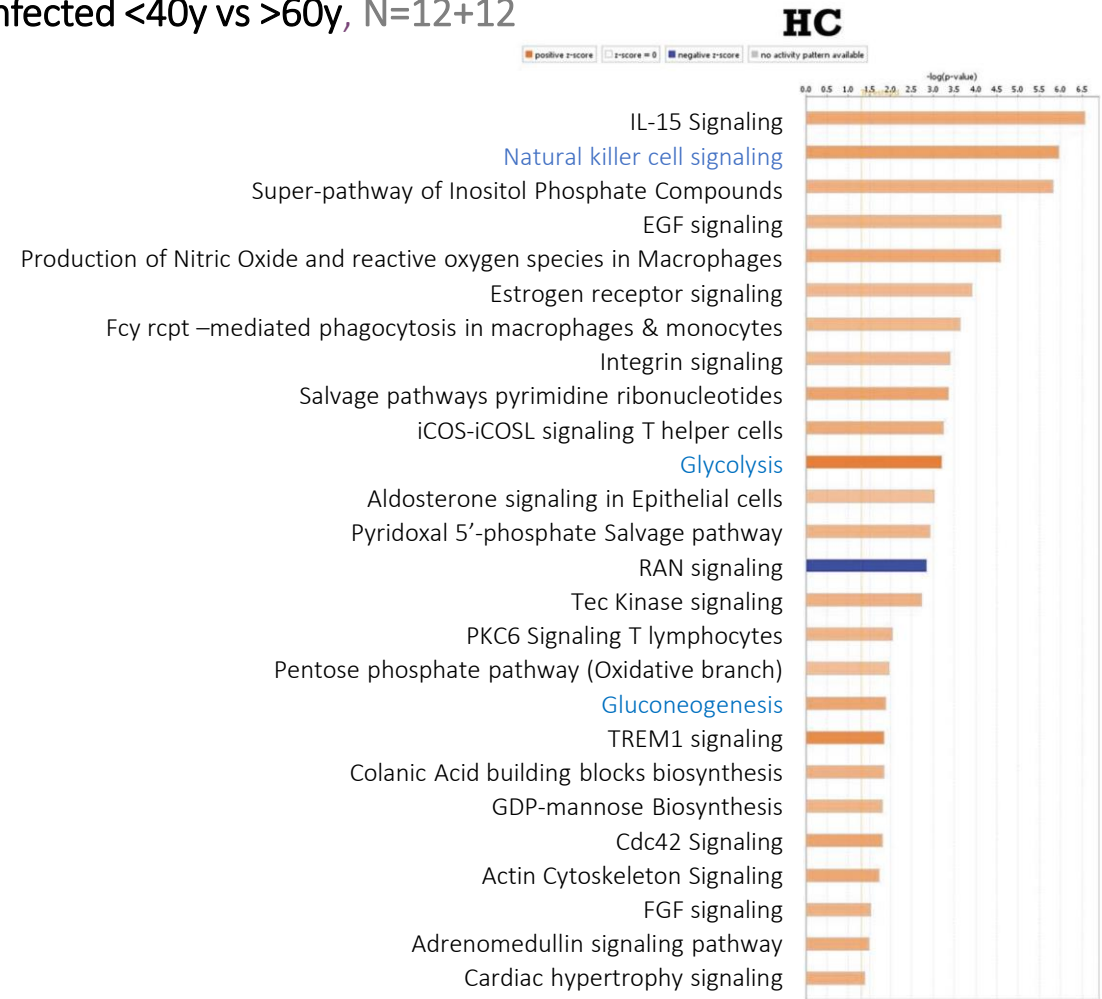
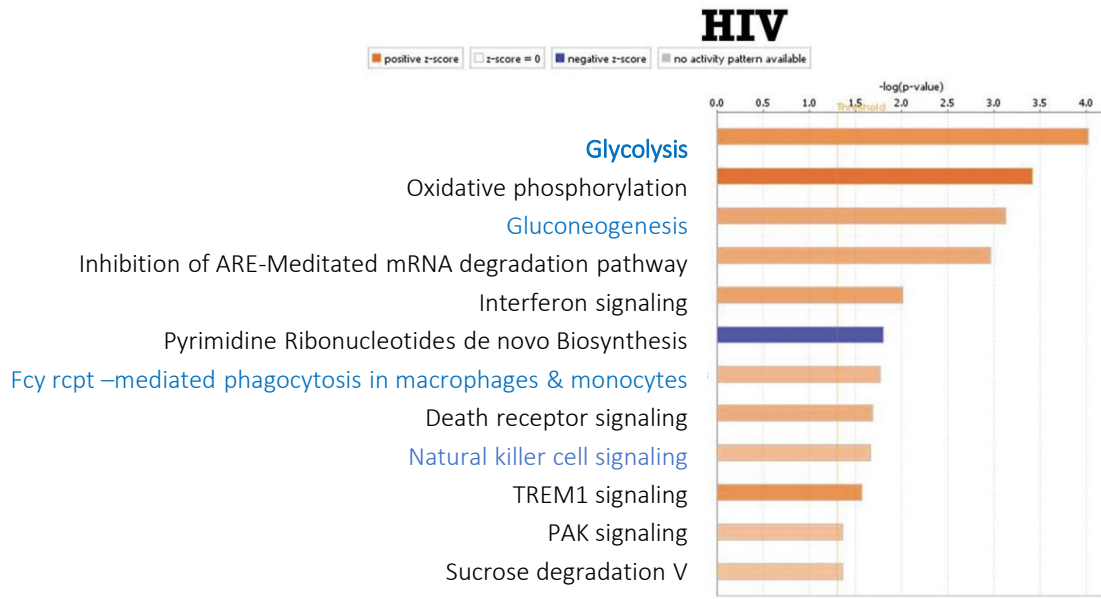


- > Higher differences are observed between PWH and uninfected control, especially in the younger group:
 - Higher individual expression of activation markers.
 - Higher number of co-expression of activation makers (at all age groups)
 - Higher differences in inflammation



Metabolic alterations

PWH <40y vs >60y, N=12+12
 Uninfected <40y vs >60y, N=12+12



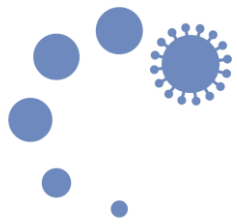
- > Age-related changes in metabolic pathways and innate immune activation are conserved in both PWH and uninfected
- > HIV showed weaker activation of age-related molecular differences compared with HC as a reference group
- > Age is also associated with an increase in T-cell immune activation in HC



CONCLUSIONS

- > Untreated PWH are markedly aging, particularly those with low CD4 T-cell counts and high VL.
- > Early ART-initiation may limit inflammation as well as cell activation, senescence and cell exhaustion, preventing the development of premature non-AIDS events.
- > Soluble markers of inflammation, and cell activation, senescence and exhaustion remain elevated in PWH who initiated ART during the chronic phase of infection, and are associated with the development of co-morbidities.
- > The most marked differences are identified in the younger population.
- > The process of aging (and inflammatory processes) might be different between PWH and uninfected individuals

Immunological studies are required on the new “generation” of People Aging with HIV



Aknowlegements



Macedonia Trigueros

Maria Nevot

Fran Muñoz-López

Marina Martínez-Velasco

Marissa Flores

Gooya Banaei

Julià Blanco

Montse Jiménez

Eugènia Negrodo

Cora Loste

Diana Hernández

Patricia Echevarría

Anna Bonjoch

Ana Martínez

Jordi Puig

Marco Fernández

Hammad Alzayat

Yacine Kharraz

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