COMORBID DISEASE: HIV and INFLAMMATION
Dr Dave Spencer HIV Clinicians Society Conference October 2018

ACTG 384

Multinational RCT
Comparing:
2NRTI + EFV
vs 2NRTI + NLF

N = 978 total group
n* = 621 with immune subsets

Duration: 3 years

Baseline CD4+ cell strata
- CD4 ≤50 c/µL
- CD4 51-200 c/µL
- CD4 201-350 c/µL
- CD4 351-500 c/µL
- CD4 >500 c/µL

Immune cell subsets defined with flow cytometry
- Naive CD4+ cells: CD4+, CD45RA, CD62L+
- Memory CD4+ cells: CD4+, CD45RO+
- B cells: CD3-, CD19+
- Activated CD4+ and CD8+: CD38+, HLA-DR+
- NK cells: CD3-, CD56+, CD16+
Median CD4+ cell counts by strata from baseline at week 0 to week 144 on HAART. The shaded band represents CD4+ counts in aged matched HIV-ve control subjects.

Median naïve CD4+ cell counts by baseline CD4 strata followed from week 0 to week 144. Shaded background reflects the lowest and highest range of HIV-negative control subjects matched by age.

Median memory CD4+ cell counts by baseline CD4 count strata from 0 to 144 weeks on HAART. The shaded region represents the upper and lower ranges of HIV-ve control subjects according to age.

ACTG Protocols A5113/ ACTG384

RESULTS

Activated CD4+ cells

Patients in the lower CD4 strata had higher activated CD4+ cell %.... all the way up to stratum level 5 (i.e. >500CD4) up to wk 24

CD8+ cells, activated CD8+ cells, CD4:CD8 ratios

Except for stratum 1 (<50CD4) CD8+ were abnormally higher than in HIV-ve controls. Activated CD8+% was elevated for all strata at baseline and followed a biphasic decrease but never reached the (low) levels of HIV-ve controls. Improvements in CD4:CD8 ratios were lower in the lower strata and even in the highest strata ratios never matched HIV-ve controls.
RESULTS

**Natural killer cells**

NK cell counts increased slightly in CD4 strata 1-4 but counts for all strata reached ‘normal’ after baseline analysis.

**B cells**

Baseline B cell counts tended to be lower in the lower strata.
Differences in the CD4+ naïve and memory cell populations in the lowest strata suggest a profound immune deficit in these strata. Furthermore this deficit may never be fully corrected despite the apparent return of CD4 levels to normal on ART.

T-cell activation persisted in all strata of CD4+ groups despite ongoing ART. This may result from ongoing viral replication (even if low-grade) and bacterial translocation from the GUT. This activation is possibly associated with higher risks of heart disease and cancer in these patients.

Patients initiating ART with baseline CD4+ > 350c/µL appeared to achieve T cell subsets more similar to HIV-ve volunteers.

Absolute CD4+ cell counts alone are probably NOT an adequate measure of immune reconstitution and may be misleading in the long term.
Chronic Inflammation and Increased Risk for Comorbidities in HIV-Positive Pts

Untreated HIV Infection

- Loss of immunoregulatory cells
- HIV replication
- Loss of gut mucosal integrity and microbial translocation

ART

Decreased but persistent chronic inflammation, immune activation, elevated coagulation markers, microbial translocation, and increased risk of coinfection

Traditional comorbidity risk factors, such as dyslipidemia, smoking, lipodystrophy, HTN, obesity, substance use

Increased incidence of comorbidities and clinical disease

A cytokine “storm” contributes to immune activation and CD4 loss and clinical signs and symptoms.
PREMATURE AGEING IN HIV PERSONS

Factors significantly associated with poly-pathology in HIV patients were:

- **Age**: per year increase, OR 1.11, 95% CI, 1.10-1.12
- **Male sex**: OR 1.77, 95% CI, 1.44-2.17, P<.001
- **Nadir CD4 count <200**: OR 4.46, 95% CI, 3.73-5.34, P<.001
- **Exposure to ART, per mth of exposure**: OR, 1.01, 95% CI, 1.001-1.019, P=.001

Figure. The risk (probability) of poly-pathology (Pp) by age – as a continuous variable – for HIV+ve patients and uninfected controls in the cohort.

Case-control study  n=2854 patients, n=8562 control subjects
Modena University, Italy. 2002-2009. Age = 46yr (Mean)
Conclusion: Specific age-related non-infectious comorbidities and poly-pathologies were more common among HIV+ve group.


Expected age difference between patients (HIV+ve) and control subjects (HIV-ve) at which poly-pathology would be observed across a spectrum of odds ratios for Pp. An approximately 10-15 year “earlier aging” phenomenon!!

Across all age groups by strata, poly-pathology prevalence was significantly higher among patients (HIV+ve), compared with uninfected controls, *P*<.001.

Legend
- No age-related comorbidity
- One comorbidity
- Two comorbidities
- Three comorbidities
- Four comorbidities

Comorbidities assessed in this Study
- Cardiovascular
- Hypertension
- Renal disease
- Bone fractures
- Diabetes Mellitus Type II
MORBIDITY and MORTALITY AMONG THE HIV-INFECTED: IS IT INFLAMMATORY?

**OBJECTIVE:** Describe the rate of Grade 4 conditions in a RCT Cohort Study – NOT attributable to AIDS, CVD, non-AIDS cancer and the association of the Grade 4 conditions with IL-6 and D-dimer.

**METHODS:** N = 3568 HIV+ve participants
VL ≤ 500 cp/mL
Follow-up x 4.3 yr

**RESULTS:**
- n =339 subjects w. Grade 4 events: rate 22/1000 person years
- N = 165 chronic-inflammatoy disease, rate 10.7/1000 person years
  - These events were more frequent than AIDS-events (n=54 persons), CVD (n=132), non-AIDS cancer (n=80)
  - Higher IL-6 and D-dimer levels were associated with a Grade 4 event and with chronic-inflammatoy disease (HR = 1.38, p<0.001).

GRADE 4: any serious condition that requires hospitalisation or is potentially life-threatening: liver, renal, CNS, GIT, Rheumatological, etc.
By week 2-3 following infection, the intestinal CD4 cells in the GIT are profoundly depleted although at this time there is no measurable depletion of CD4 cells in lymph nodes or peripheral blood.

Centlivre M, Sommer P et al. The HIV-1 clade C promoter is particularly well adapted to replication in the gut in primary infection. *AIDS* 2006;20:657-66
The afferent arm of the GIT immune system incorporates specialized surface epithelial tissue including the microfold or M cells.

Below these cells and in close proximity to them, are discrete lymphoid follicles buried in the intestinal sub-mucosa.

This system permits the recognition and trapping of antigen and its subsequent processing.

A. Plasma 16S rDNA levels correlate directly with the frequency of CD38 and HLA-DR expression on CD8+ T cells.

B. Plasma 16S rDNA levels correlate inversely with the magnitude of CD4+ T lymphocyte restoration after ART.

C. Increases in CD4+ T lymphocyte counts after ART correlate inversely with the proportions of CD8+ T cells expressing CD38 and HLA-DR. $P$ values were calculated using Spearman’s correlation test.
**Method:** Observational Longitudinal Study
[Longitudinal Study of the Ocular Complications of AIDS]
N = 64 subjects who died within 12m of ART-mediated viral suppression + matched controls (n = 128)

**Results:**

1. **GIT epithelial-barrier integrity markers** (intestinal fatty acid binding protein + zonulin-1) + soluble CD14, kynurenine/tryptophan ratio, soluble TNF receptor-1, HS-CRP and D-dimer = all increased and predicted mortality (P ≤ 0.001) even after adjustment for baseline CD4 level.

   Levels of senescent cells (CD28(-) HLA DR(+), exhausted cells viz. PD1(+), naïve and CMV-specific T cells did **NOT** predict mortality

2. **CD38+ HLA-DR+ CD8 T-cells** (‘generalised inflammation’) did not predict mortality when adjusted for CD4 T cell count.

   **Interpretation:** Not all inflammatory markers carry equal significance with regard to mortality.

   **Those related to GIT-integrity appear to be important in this regard.**

---

SYSTEMIC IMMUNE ACTIVATION / SENESCENCE

1. HIV ITSELF: TAT / NEF gp120

2. CONCURRENT INFECTION + INFLAMMATION

EBV CMV HBV HCV HPV HSV VZV

3. LOSS OF GALT and ANTIGEN TRANSLOCATION

BACTERIAL PROTOZOAL FUNGAL HELMINTHS PERSISTENT/RECURRENT

4. PROTHROMBOTIC STATE: ENDOTHELIAL DYSFUNCTION

5. THYMIC “COLLAPSE”: FAILURE OF CELLULAR PROGRAMMING

6. ACTIVATION OF LOCALLY ACTIVE PROTEINS, ENZYMES, ANTIGENS WITH SYSTEMIC AND LOCAL CONSEQUENCES

EBV CMV HBV HCV HPV HSV VZV

7. ACTIVATION OF ALL IMMUNE PARTICIPANTS: CELLULAR, HUMORAL, INNATE BARRIERS

EBV CMV HBV HCV HPV HSV VZV

8. EVOLUTION OF A CYTOKINE / AND CHEMOKINE MILEAU

Type I and II IFNs

TGF-β1: FIBROSIS

EBV CMV HBV HCV HPV HSV VZV

PRO-INFLAMMATORY [IL-1β, IL-2, IL-6, IL-8 + TNFα] VS ANTI-INFLAMMATORY [IL-4, IL-10, IL-13]

EBV CMV HBV HCV HPV HSV VZV

COGNITIVE IMPAIRMENT:
- Cytokines in brain and CSF
- CCL20, IL-6, IL-8 CHEMOKINES UPREGULATED: CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8 (IL-8)

BONE and FRAILTY:
- Accelerated ageing, HIV itself, ARVs
- TNF-α promotes osteoclastic bone resorption via RANKL

CVD and STROKE:
- Vascular wall “stickiness”
- Increase of ICAM and VCAM
- Increase of E and P-selectins
- Lipid-loading of ‘foam cells’ (macrophages) in intimal wall

MALIGNANCY:
- HIV-immune deficiency
- Activation of proto-oncogenes
- Oxidative stress= DNA damage
- Increased IL-17 = abnormal B-cell development and apoptosis

IRIS and BACKGROUND IMMUNE ACTIVATION:
- Cytokine mediated
- Recovery of CD4 cells
- Suppression of HIV
- Early ART: increased mortality

RENAL DISEASE: ATN, HIVAN, AGEING
- Immune cell infiltrate
- Cytokines and chemokines in renal epithelial cells: CCL20, IL-6, IL-8 CHEMOKINES UPREGULATED: CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8 (IL-8)

CLINICAL EXPRESSION OF IMMUNE ACTIVATION and DYSREGULATION

SYSTEMIC IMMUNE ACTIVATION / SENESCEENCE

- **ANTIRETROVIRAL THERAPY (ART)**
- **GIT “REPAIR”:** prebiotics and probiotics – no reliable RCT data in HIV. Observational data in animal models.
- **TREAT CO-INFECTION and COMORBID DISEASE:** HBV and HCV
- **CONTROL OF INFLAMMATION:** STEROIDS and METHOTREXATE. Harm from Steroid use in CCM. RCT data for steroid use in PTB, TBM and disseminated TB = some support esp. in HIV-ve cohorts. Methotrexate = risk of harm. Use of steroids in disseminated KS and in KS IRIS = harm.
- **IMMUNE MODULATORS:** no convincing data in HIV+ve populations
  - Statins
  - Selective Cox-2 inhibitors
  - Ceflunomide
  - Rapamycin
  - Mycophenolate
- **SENOLYTICS:** promote apoptosis of senescent cells. In development.

**CANAKINUMAB:**
a monoclonal antibody that targets IL-1β

Ridker PM, Everett BM et al
Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease.
NEJM 2017 Sept 21; 377; 12: 1119-31

SMART: Subgroup Analysis in Patients Not Receiving ART at Study Entry

Deferred Arm
Intermittent ART
(n = 2720; 228 not receiving ART at trial start)

Immediate Arm
Continuous ART
(n = 2752; 249 not receiving ART at trial start)

Study halted prematurely; mean follow-up: 18 mos

HIV-infected patients with CD4+ cell count > 350 cells/mm³
(N = 5472)

• Treatment definitions for subanalysis
  • Deferred: ART initiated when CD4+ cell count < 250 cells/mm³, CD4+ cell percentage < 15%, or HIV symptoms
  • Immediate: ART initiated immediately after randomization

• Primary endpoints
  • OD or death from any cause
  • Fatal or nonfatal OD
  • Serious non-AIDS events
  • Fatal and nonfatal OD plus serious non-AIDS events

**SMART:** Immediate ART Reduces Risk of Clinical Events

- Immediate group experienced substantially fewer events (opportunistic disease or serious non-AIDS events)
- Excess risk associated with deferring therapy: 5.4 events/100 person-yrs

<table>
<thead>
<tr>
<th>Event, n (Rate per 100 Person-Yrs)</th>
<th>Deferred Arm (n = 228)</th>
<th>Immediate Arm (n = 249)</th>
<th>HR (DC/VS)</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD/death</td>
<td>15 (4.8)</td>
<td>5 (1.3)</td>
<td>3.5</td>
<td>1.3-9.6</td>
<td>.02</td>
</tr>
<tr>
<td>OD only</td>
<td>11 (3.5)</td>
<td>4 (1.1)</td>
<td>3.3</td>
<td>1.0-10.3</td>
<td>.04</td>
</tr>
<tr>
<td>Serious non-AIDS events</td>
<td>12 (3.9)</td>
<td>2 (0.5)</td>
<td>7.0</td>
<td>1.6-31.4</td>
<td>.01</td>
</tr>
<tr>
<td>Composite*</td>
<td>21 (7.0)</td>
<td>6 (1.6)</td>
<td>4.2</td>
<td>1.7-10.4</td>
<td>.002</td>
</tr>
</tbody>
</table>

*Fatal and nonfatal OD plus serious non-AIDS events.

**START: Immediate vs Deferred Therapy for Asymptomatic, ART-Naive Patients**

- International, randomized trial

**Immediate ART**
- ART initiated immediately following randomization (n = 2326)

**Deferred ART**
- Deferred until CD4+ cell count ≤ 350 cells/mm³, AIDS, or event requiring ART (n = 2359)

- Composite primary endpoint: any serious AIDS-related (AIDS-related death or AIDS-defining event) or non-AIDS-related event (non-AIDS–related death, CVD, end-stage renal disease, decompensated liver disease, non-AIDS–defining cancer)

- Mean follow-up: 3 yrs; median baseline CD4+ cell count: 651 cells/mm³; median baseline HIV-1 RNA: 12,759 copies/mL

- Median CD4+ cell count at initiation of ART for deferred group: 408 cells/mm³

**START:** 57% Reduced Risk of Serious Events or Death With Immediate ART

- 4.1% vs 1.8% in deferred vs immediate arms experienced serious AIDS or non-AIDS–related event or death (HR: 0.43; 95% CI: 0.30-0.62; \( P < .001 \))

---

## START: Primary Endpoint Components
### With immediate vs Deferred ART

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Immediate ART (n = 2326)</th>
<th>Deferred ART (n = 2359)</th>
<th>HR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Rate/100 PY</td>
<td>N</td>
<td>Rate/100 PY</td>
</tr>
<tr>
<td>Serious AIDS-related event</td>
<td>14</td>
<td>0.20</td>
<td>50</td>
<td>0.72</td>
</tr>
<tr>
<td>Serious non-AIDS–related event</td>
<td>29</td>
<td>0.42</td>
<td>47</td>
<td>0.67</td>
</tr>
<tr>
<td>All-cause death</td>
<td>12</td>
<td>0.17</td>
<td>21</td>
<td>0.30</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>6</td>
<td>0.09</td>
<td>20</td>
<td>0.28</td>
</tr>
<tr>
<td>Kaposi’s sarcoma</td>
<td>1</td>
<td>0.01</td>
<td>11</td>
<td>0.16</td>
</tr>
<tr>
<td>Malignant lymphoma</td>
<td>3</td>
<td>0.04</td>
<td>10</td>
<td>0.14</td>
</tr>
<tr>
<td>Non-AIDS–defining cancer</td>
<td>9</td>
<td>0.13</td>
<td>18</td>
<td>0.26</td>
</tr>
<tr>
<td>CVD</td>
<td>12</td>
<td>0.17</td>
<td>14</td>
<td>0.20</td>
</tr>
</tbody>
</table>

N = 19 *Macaca nemestrina* inoculated with *SIVDeltaB670* virus and studied from Jan 2004-April 2009. Animals were euthanized upon development of clinical ‘AIDS’ – CSF and brain removed at death.

SIV encephalitis (SIVE) = presence of SIV in brain tissue + microglial nodules + multinucleated giant cells + profuse perivascular infiltrate of mononuclear cells (definition of SIV encephalitis)

Macaques that developed (viral) encephalitis had evidence of chronic CNS immune activation throughout their post-infection life:

- at acute infection
- during asymptomatic infection
- and at end-stage infection

YKL40 = a protein (glycosyl hydrolase family 18) expressed by synovial cells, neutrophils and macrophages in blood, and astrocytes in CNS tissue and CSF: a protein that assists SIV and HIV in its binding to extracellular matrix cells in the CNS.

Fig. 1. Macaques with SIV encephalitis show SIV-infected microglial nodules and increased SIV viral load and YKL40 expression in frontal cortical tissue.
Fig. 6. The majority of elevated neuroimmune markers became elevated as encephalitis developed and were associated with macrophage recruitment and activation.

Multiplex quantitation of 31 cytokines present in the CSF was performed on samples from baseline (d0), acute infection (d10 and d14), asymptomatic infection (d28 and d42), development of encephalitis (rise of YKL40), and at necropsy (nec). IL-1β (a), MIF (b), IL-8 (c), IFN-γ (d), CXCL9 (e), CXCL11 (f), CCL4 (g), TGF-β (h) were elevated when encephalitis (red) developed or shortly before. SIV-infected non-encephalitic macaques. Bissel SJ., et al. 2016
Fig. 6. The majority of elevated neuroimmune markers became elevated as encephalitis developed and were associated with macrophage recruitment and activation.

Multiplex quantitation of 31 cytokines present in the CSF was performed on samples from baseline (d0), acute infection (d10 and d14), asymptomatic infection (d28 and d42), development of encephalitis (rise of YKL40), and at necropsy (nec). IL-1β (a), MIF (b), IL-8 (c), IFN-γ (d), CXCL9 (e), CXCL11 (f), CCL4 (g), TGF-β (h) were elevated when encephalitis (red) developed or shortly before. SIV-infected non-encephalitic macaques. Bissel SJ., et al. 2016

Admitted: No evidence of trauma. Apart from disorientation, exam was normal. CT brain without contrast = prominent areas of low attenuation in both hemispheres, no bleeds, no contusion, no infarct.

PMH: Tested HIV+ve 4 years earlier following a PAP smear and high grade cervical dysplasia.

Started on ART: TDF + FTC +LPV/r
Baseline CD4 = 211 c/mm³ VL = 476,000 cp/ml

Daily H/A started 2yr before admission. LP = CSF pleocytosis. Tx IV acyclovir.
H/A improved over next 2 months. But recurred after 6 months. Accompanied by general malaise, photophobia, N & V, gait instability.

MRI Brain with gadolinium = diffuse lepomeningeal enhancement, extensive asymmetric hyperintensity on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images of the subcortical, basal ganglia, dorsal pons.
NB. CSF from the patient reveals a persistent abnormality i.e. raised white cells and protein (meningitis) for which the usual viral pathogens test ‘negative’.

A meningitis that lasts for 2 years without killing the patient!!

…or a chronic meningoencephalitis caused by….?

Table 2. Results of Hematologic and Serum Chemical Studies.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference Range, Adults†</th>
<th>12 Mo before Admission, in Neurology Clinic</th>
<th>2 Mo before Admission</th>
<th>On Admission</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 T-lymphocyte count (per mm³)</td>
<td>348–1456</td>
<td>581</td>
<td>665</td>
<td>397</td>
</tr>
<tr>
<td>CD8 T-lymphocyte count (per mm³)</td>
<td>148–1173</td>
<td>533</td>
<td>582</td>
<td>546</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/liter)</td>
<td>9–32</td>
<td>23</td>
<td>24</td>
<td>35</td>
</tr>
<tr>
<td>Alanine aminotransferase (U/liter)</td>
<td>7–30</td>
<td>27</td>
<td>33</td>
<td>38</td>
</tr>
<tr>
<td>Antinuclear antibody</td>
<td>Negative at 1:40 and 1:160 dilutions</td>
<td>Positive at 1:640 dilution, speckled pattern</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG antibodies to EBV viral capsid antigen</td>
<td>&lt;1:10, negative</td>
<td>&gt;1:10,240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBV DNA (copies/ml)</td>
<td>&lt;200, negative</td>
<td>2100</td>
<td>&lt;200</td>
<td></td>
</tr>
<tr>
<td>Human immunodeficiency virus RNA (PCR) (copies/ml)</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>228</td>
</tr>
</tbody>
</table>

* EBV denotes Epstein–Barr virus, and PCR polymerase chain reaction.
† Reference values are affected by many variables, including the patient population and the laboratory methods used. The ranges used at Massachusetts General Hospital are for adults who are not pregnant and do not have medical conditions that could affect the results. They may therefore not be appropriate for all patients.

Comment:
CD4:CD8 ratio = <1 on admission.
Previously CD4:CD8 ratio > 1
Figure. Fluid-attenuated inversion recovery (FLAIR) images obtained from the initial MRI of the brain reveal hyperintense signal abnormalities involving the subcortical and deep white matter at the level of the lateral ventricles (Panel A). FLAIR images from the MRI performed two years later, at the time of the current admission, show a significant progression of the white-matter signal abnormalities (Panel B). FLAIR images obtained 1 year after the initiation of glucocorticoid treatment reveal multifocal regions of hyperintense signal abnormality (Panel C). However, the abnormalities are far less extensive than those seen in the earlier images.
A. Biopsy of the right frontal lobe was performed. The specimen from the cerebral cortex shows normal architecture and multifocal perivascular infiltrates but no notable leptomeningeal cellularity (Panel A).

The perivascular infiltrate is composed of small, benign inflammatory cells, without evidence of vascular-wall injury (Panel B).

Immuno-histochemical staining for CD3 shows that the infiltrate consists of T cells, with extension of the lymphocytic infiltrate into the parenchyma (Panel C). And that the majority of T cells express CD8 (Panel D).
Assessment: “Diffuse abnormalities on imaging studies, similar to those seen in this patient have been described in patients with neuro-IRIS and no evidence of coinfection. Similarly lymphocytic pleocytosis has been reported. In order to make a definitive diagnosis of neuro-IRIS and definitively rule out other causes, an open brain biopsy of the right frontal lobe and meninges was performed on the sixth hospital day.”

“Since her symptoms had waxed and waned in the past, since the biopsy suggested neuro-IRIS, and since opportunistic infection had been ruled out, prednisone 60mg per day was initiated... The ART was not changed. The steroid was tapered during the next year to 5 mg per day and administration of steroid was discontinued after 2.5 years. She has been evaluated clinically at regular intervals and with repeated MRI scans. Most of her white-matter abnormalities have resolved and the patient has returned to full time work.