Multiomics Profiling Links Galectin-9 and Interferon Signaling to Inflammation-Driven Comorbidities

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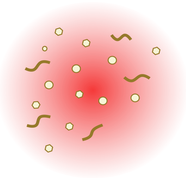
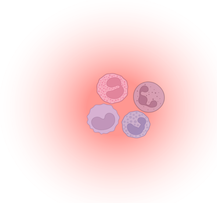


Inflammatory and immune activation markers remain elevated in people with HIV (PWH) despite early antiretroviral therapy (ART) and sustained viral suppression.

Although biomarkers such as IL-6, D-dimer, and CD4/CD8 ratio predict severe non-AIDS events (SNAEs), their clinical interpretation and underlying biological pathways remain **incompletely defined**.



We hypothesized that **distinct, outcome-specific inflammatory patterns during suppressive ART predict differential risks** of cardiovascular events and malignancies.

Galectin-9 identified PWH at increased risk of SNAEs within a type I interferon–enriched inflammatory network, supporting its prioritization as a biomarker and potential therapeutic target to mitigate persistent immune activation under ART

Cohort of the Spanish AIDS Resarch Network (CORIS)
N = 18,573 PWH

Nested case-control study
on ART, virologically suppressed, follow-up > 2 years

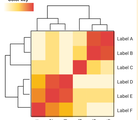
Cases/controls - 1:1 matching by propensity score
age, sex, risk factor for HIV transmission, AIDS history, level of education, country of origin, smoking, CD4/CD8 ratio at year 2, ART regimen, total cholesterol, HDL cholesterol

89 cases and 89 controls
Primary outcome: 1) nonaccidental death (n = 22) or
2) severe cardiovascular event (n = 28) or
3) non-AIDS cancers (n = 61) from year 2 through year 10

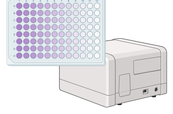
Proximity Extension Assay (Olink Proteomics) on plasma
RNA-seq from PBMC
Outcomes through **year 10** since ART initiation
Samples obtained from patients **on ART for 24 ± 6 months**

Proteomic assay of 368 inflammatory proteins

Transcriptomic analysis



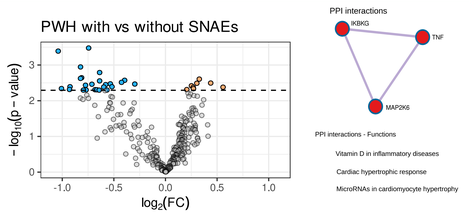
Data integration



ELISA validation of 4 targets

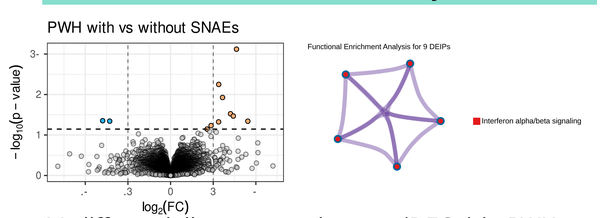
Participants were predominantly middle-aged (mean 46.5 years) with similar baseline characteristics at baseline and a median follow-up of 63 months (IQR 42–82) in cases and 94 months (IQR 62–133) in controls.

1) Proteomic screening identifies inflammatory signatures associated with SNAEs and mortality



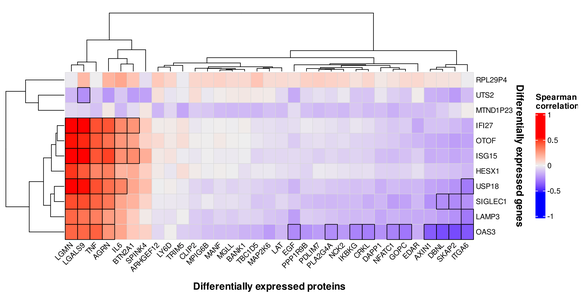
- 33 differentially expressed proteins (DEPs) in PWH with vs without SNAES
- 52 DEPs in PWH with vs without neoplasia
- 7 DEPs in PWH with vs without mortality

2) Transcriptomic profiling revealed a shared type I interferon signature associated with SNAEs and mortality



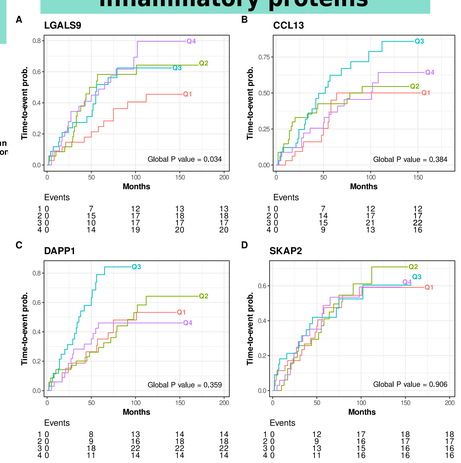
- 11 differentially expressed genes (DEGs) in PWH with vs without SNAES
- 15 DEGs in PWH with vs without neoplasia
- 32 DEGs in PWH with vs without CV events
- 10 DEGs in PWH with vs without mortality

3) Cross-omic correlations between differentially expressed genes and proteins



Higher levels of inflammatory proteins including LGMN, LGALS9, TNF, AGRN, IL6, and BTN2A1 showed positive correlations with interferon-stimulated genes such as IFI27, OTOF, ISG15, HESX1, USP18, SIGLEC1, LAMP3, and OAS3.

4) Predictive ability of selected inflammatory proteins



Higher LGALS9 levels were associated with increased SNAEs risk over the full follow-up (HR per quantile increase = 1.25, 95% CI 1.02 - 1.54; p = 0.034).

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Unlike conventional biomarker studies immediately preceding clinical events, we identified an **early inflammatory signature during suppressive ART associated with subsequent SNAEs**.

This signature supports a coherent **interferon–galectin-9 axis linked to adverse outcomes**, while revealing substantial biological heterogeneity across non-AIDS complications and arguing against a one-size-fits-all model of residual risk.

Further research is needed to:

i) Determine whether interferon signalling or the galectin-9/Tim-3 pathway represents a modifiable driver of risk or a stable correlate of immune dysfunction.

ii) Refine predictive strategies to identify individuals who may benefit from targeted monitoring and preventive interventions.

PLAIN LANGUAGE SUMMARY

In people whose HIV is successfully treated, hidden immune system activity may still predict future illness or death. A molecule called galectin-9 stood out as a reliable warning signal in the blood.