Biomarkers Associated With Risk of Negative Clinical Outcomes in People Living With HIV on Opioid Replacement Therapy

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Conclusions

- Results from SomaScan assay showed that people with HIV (PWH) on treatment with μ-opioid receptor (MOR) agonists methadone (MET) or buprenorphine as medications for opioid use disorder (MOUD) had significantly different plasma levels of 12 unique proteins (HSP70, KERA, NTRI, FLRT2, sCD14, SDF-1, IGLL1, AT1B2, ROR1, MMAC, IGFALS, and ELA2A) compared with control PWH, suggesting that the use of MOR agonists may alter protein profiles and immune responses in PWH
- Differential expression of HSP70, IGLL1, and sCD14 was also confirmed using commercial nondiagnostic ELISA kits. The results showed concordance with the findings from SomaScan protein analysis, suggesting that these proteins could be biomarkers for MOR agonist exposure in PWH
- The SomaSignal data analysis indicated that PWH who received MET showed higher risks for kidney disease and dementia compared with other groups, suggesting that MET treatment in PWH may be associated with increased long-term health risks. PWH on treatment with MOUD also had a higher likelihood of developing progressive chronic renal insufficiency within 4 years, advocating a need for closer monitoring of kidney function
- Overall, these data suggest that PWH chronically exposed to MOR agonists have higher levels of specific circulating protein biomarkers that may be linked to increased risk of negative clinical outcomes in the long term

Plain Language Summary

- HIV infection causes ongoing inflammation even when suppressed by antiretroviral therapy, increasing risks of heart, kidney, and brain problems. Opioid use may worsen inflammation and weaken immune response in people with HIV (PWH). While medications for opioid use disorder (MOUD) like methadone or buprenorphine are commonly prescribed, their long-term effects in PWH are unclear
- Our study compared soluble biomarkers in the plasma and predictive disease prognosis in PWH not using opioids or MOUD with those receiving suboxone/buprenorphine or methadone. Based on advanced protein analysis (SomaScan) and machine learning, we found that treatment with MOUD altered levels of 12 specific proteins found in the blood in PWH. These changes might be linked to increased health risks, especially kidney disease and dementia in methadone recipients
- Our findings highlight the need for careful monitoring of PWH receiving MOUD, particularly their kidney function. This research addresses a crucial gap in understanding how MOUD impacts PWH, paving the way for improved care strategies

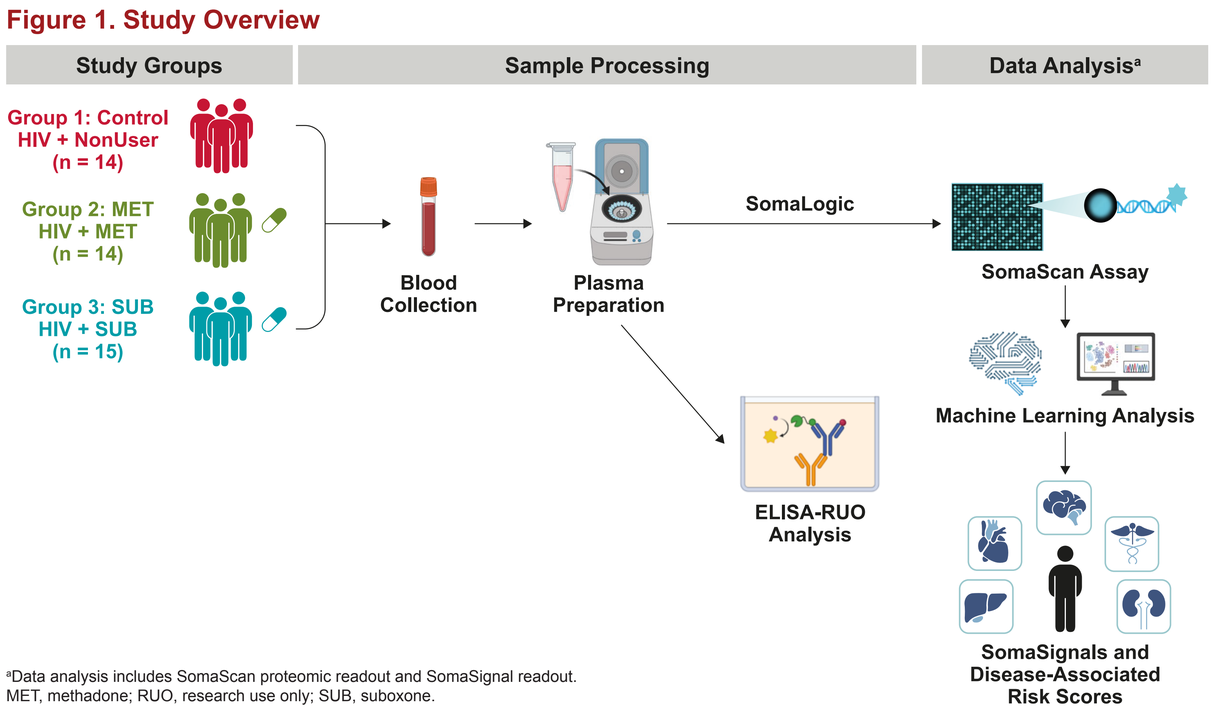
Background

- HIV infection, even when suppressed by antiretroviral therapy (ART), causes chronic inflammation, increasing the risk of cardiovascular, kidney, and neurocognitive disorders[1]
- Opioid use impairs immune response, potentially exacerbating chronic inflammation in PWH and possibly limiting ART-mediated immune reconstitution, but the exact mechanism remains unknown[2]
- Full (MET) and partial (buprenorphine) MOR agonists are standard MOUD, but their long-term impact on PWH is not fully understood[3]
- Here, we present results from an HIV pilot study to identify immune biomarkers in PWH receiving MOUD, and to assess their potential association with clinical outcomes, addressing a significant knowledge gap in HIV and substance use research

Methods

- The overview of the study is illustrated in **Figure 1**. Three groups of virally suppressed PWH on ART were enrolled at the Jonathan Lax Treatment Center/Philadelphia FIGHT, Philadelphia, PA, USA:
  - Group 1, Control: HIV + NonUser; PWH receiving ART with no opioid or MOUD use
  - Group 2, MET: HIV + MET; PWH receiving ART with daily oral MET
  - Group 3, SUB: HIV + SUB; PWH receiving ART with suboxone (SUB; buprenorphine + naloxone) or buprenorphine extended release
- Plasma samples were prepared by centrifugation from citrate dextrose-anticoagulated blood collected from study participants. Frozen samples (−80°C) were sent to SomaLogic (Boulder, CO, USA) for SomaScan assay measurements
- Circulating proteome was analyzed with the SomaScan platform
  - Upon completion of the assay, the SomaScan readouts were processed and normalized via their standard data processing pipeline to account for the multiple readout steps per 96-well plate
  - SomaScan data underwent multistep normalization: hybridization control adjustment, median signal normalization of calibrators, and ratio-based scaling to correct for both overall and SOMAmer-specific variations between runs
  - Analyte selection and determinations were gated with a signal-to-noise ratio of > 3 prior to statistical analysis
- Clinical outcomes, predicted by SomaSignal, were chosen for analysis based on machine learning (ML) and data prediction algorithms
  - The SomaScan assay quantitatively transforms the proteins present in a small amount of biological sample (55 μL of plasma/serum) into a specific SOMAmer-based DNA signal (~ 7k)
  - Based on protein signature modeling, SomaSignal tests were evaluated for clinically relevant information about patient health and risk status
  - ML was used to derive disease-associated risk scores from the SomaScan assay (named SomaSignal)
- Biomarkers of interest identified by SomaScan were independently analyzed with an ELISA-RUO (research use only) assay at the Wistar Institute
- Associations between clinical outcomes and biomarkers were determined by 2 separate statistical approaches: the Kruskal–Wallis test followed by Dunn's test for all 21 SomaSignals and beta regression for probability and likelihood SomaSignals
- Differences between groups were analyzed by Wilcoxon rank sum test. *P* values were adjusted for false discovery rate

Figure 1. Study Overview



[a]Data analysis includes SomaScan proteomic readout and SomaSignal readout.
MET, methadone; RUO, research use only; SUB, suboxone.

Results

- The mean age of participants (48.6 years) and mean length of ART (10.4 years) were similar across all cohorts (**Table 1**)
- The percentage of females was lower in the control group (non-MOUD recipient) (14.3%) compared with the SUB (40.0%) and MET groups (42.9%)

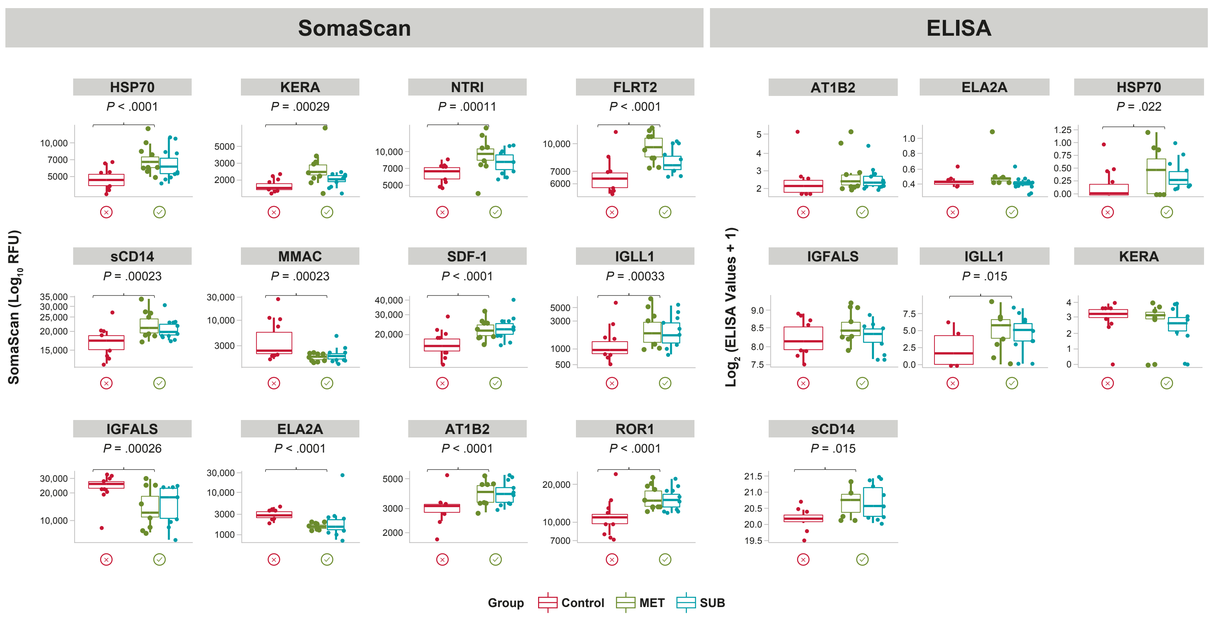
Table 1. Key Characteristics of Participants

	Control (n = 14)	MET (n = 14)	SUB (n = 15)	Overall (N = 43)
Gender, n (%)				
Female	2 (14.3%)	6 (42.9%)	6 (40.0%)	14 (32.6%)
Male	12 (85.7%)	8 (57.1%)	9 (60.0%)	29 (67.4%)
Age, years				
Mean (SD)	46.1 (9.43)	49.2 (11.70)	50.4 (6.33)	48.6 (9.30)
Median (min, max)	47.5 (26.0, 60.0)	51.0 (25.0, 62.0)	52.0 (41.0, 61.0)	50.0 (25.0, 62.0)
Years on ART				
Mean (SD)	10.2 (5.60)	12.0 (9.21)	9.0 (3.02)	10.4 (6.37)
Median (min, max)	10.0 (2.00, 19.0)	10.0 (0.750, 29.0)	10.0 (2.00, 14.0)	10.0 (0.750, 29.0)

ART, antiretroviral therapy; MET, methadone; SUB, suboxone.

- The levels of 12 unique proteins identified by 13 SOMAmers were differentially expressed between the control and MOR agonist groups. Among the 12 proteins of interest identified by SomaScan, HSP70, KERA, NTRI, FLRT2, sCD14, SDF-1, IGLL1, AT1B2, and ROR1 levels were higher in PWH exposed to MOR agonists, but MMAC, IGFALS, and ELA2A were higher in the control group (*P* < .001) (**Figure 2**)
- The levels of HSP70, IGLL1, and sCD14 measured by ELISA were significantly different between PWH exposed to MOR agonists and controls (adj *P* values .022, .015, and .015, respectively) and were concordant with the SomaScan results (**Figure 2**)

Figure 2. Differential Biomarkers Between MOUD Recipients vs Controls in PWH



MET, methadone; MOUD, medications for opioid use disorder; PWH, people with HIV; RFU, relative fluorescence units; SUB, suboxone.

- SomaSignal analysis indicated that PWH receiving MET had a higher risk for kidney disease, heart failure, and dementia, and a trend for lower visceral fat and alcohol impact, compared with controls (**Table 2**)
- The SomaSignal kidney prognosis test for patients with chronic kidney disease showed significance (*P* = .04), indicating that MOUD recipients were more likely to develop progressive chronic renal insufficiency in 4 years
  - The median kidney prognosis probabilities were 0.08 (control), 0.14 (MET), and 0.12 (SUB), which suggests lower risk in the control group (**Table 2**)

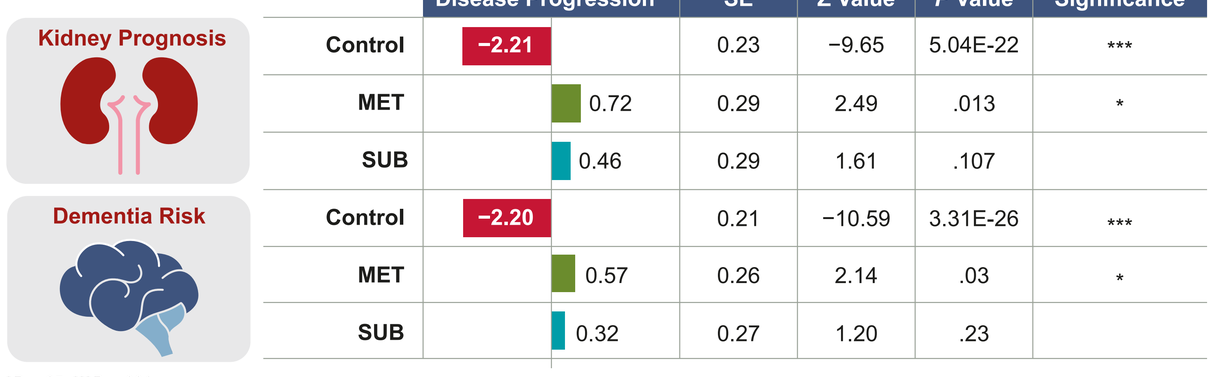
Table 2. Summary of SomaSignal Results From Kruskal–Wallis Test

Category	SomaSignals Test	Unit	Control	MET	SUB	*P* Value
Continuous	Body fat percentage	%	28.95 (5.2)	30.35 (8)	31.1 (11)	.97
	Cardiorespiratory fitness - VO_2 max	mL/kg/min	29.25 (6.2)	27.6 (4.3)	27.4 (7.9)	.723
	Lean body mass	kg	55.85 (7.6)	53.7 (10)	54.7 (16.2)	.763
	Resting energy rate	Calories/day	2160.5 (222.5)	2334 (482.2)	2185 (474)	.189
	Visceral fat	g	1001 (564)	673.5 (610.2) ↓	607 (484.5)	.151
Likelihood	Heart failure prognosis - HFpEF - 12 months	%	2 (1.8)	2.75 (2.8)	2 (2.2)	.291
	Heart failure prognosis - HFpEF - 6 months		1.15 (1)	1.55 (1.7)	1.2 (1.3)	.299
	Heart failure prognosis - HFrEF - 12 months		2.5 (2)	4.85 (3.2)	3.5 (2)	.096
	Heart failure prognosis - HFrEF - 6 months		1.45 (1.2)	2.8 (1.8) ↑	2 (1.1)	.09
	Primary cardiovascular risk - 4 years		2.9 (2.2)	3.4 (5.2)	2.6 (1.8)	.628
	Secondary cardiovascular risk - 4 years		15.6 (18.8)	30 (27.6)	22.4 (15.3)	.39
Probability	Alcohol impact	Probability	0.47 (0.3)	0.3 (0.1) ↓	0.38 (0.2)	.265
	Dementia risk		0.08 (0.1)	0.15 (0.1) ↑	0.09 (0.1)	.103
	Glucose tolerance		0.64 (0.5)	0.74 (0.6)	0.46 (0.5)	.185
	Kidney prognosis		0.08 (0.1)	0.14 (0.1) ↑	0.12 (0.1)	.04
	Liver fat		0.72 (0.5)	0.65 (0.5)	0.53 (0.3)	.505

Results show median (IQR) for each group.
HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; IQR, interquartile range; MET, methadone; SUB, suboxone; VO_2, volume of oxygen.

- Kidney prognosis and predicted dementia risk were significantly elevated in the MET group relative to the control group (**Figure 3**)

Figure 3. Summary of Beta Regression Model Results



*P < .05. ***P < .001.
MET, methadone; SE, standard error; SUB, suboxone.

Limitations

- The study analysis was limited due to small sample size
- The study lacked data from people without HIV actively using opioids
- The magnitude and quality of data obtained from SomaScan (relative fluorescence units) and ELISA (pg/mL) are not comparable. SomaScan used aptamers to detect and identify proteins based on their unique peptidomic readouts, while ELISA used an antibody-binding approach to identify proteins based on their specific structural features. The ELISA assay used in this study is intended for research use only and is not fully validated for clinical use
- The SomaSignal algorithm prediction is based on data with heterogeneous populations but not limited to PWH, and those data were collected from blood with a distinct anti-coagulant (EDTA) instead of citrate dextrose

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