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Immunological and Glycomic Correlates of Time to HIV Rebound in Viremic Controllers

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Introduction

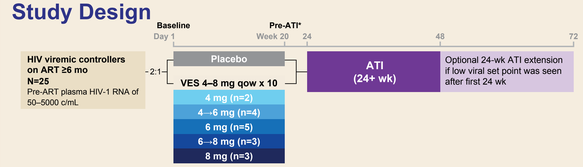
- Analytic treatment interruption (ATI) is the most reliable way to evaluate the efficacy of potential HIV cure strategies, but requires lengthy and intensive monitoring
  - Identifying biomarker correlates of time to HIV rebound or duration of HIV control after antiretroviral therapy (ART) cessation can significantly aid the development of a functional cure for HIV infection
  - Noninvasive biomarkers of viral control during ATI have the potential to improve the safety of ATI by decreasing time off ART and can provide mechanistic insights into the host pathways involved in HIV control during ATI
- Plasma glycoproteins (including antibodies; immunoglobulin G [IgG]) can enter the circulation from tissues through active secretion or leakage; therefore, their levels and chemical characteristics (including glycomic features, and degree and type of carbohydrates added to these proteins) can reflect the overall status of multiple organs, making them excellent candidates for biomarker discovery[1]
  - Glycomic features in total plasma and IgG have been identified as biomarkers for inflammatory bowel disease, cancer, and diabetes[2,3]
  - In addition, glycans on circulating glycoproteins have functional significance, as glycans play essential roles in mediating immunologic functions, including antibody-dependent cell-mediated cytotoxicity, and pro- and anti-inflammatory activities[1]
- Recently, a subset of plasma glycomic biomarkers including N-acetylgalactosamine (GalNAc) glycans, α-GalNAc, and plasma trisialylated N-glycans (A3G3S3) were found to be associated with time to HIV rebound after ART cessation in 2 cohorts of people with HIV[1]
  - In vitro studies also suggested that glycosylation can modulate HIV latency reactivation, myeloid inflammation, and effector cell function
- Vesatolimod (VES) is a toll-like receptor-7 (TLR7) agonist that is being developed as part of an HIV cure strategy; TLR7 agonists can enhance immune responses and potentially lead to improved HIV control in combination with other agents
- Study GS-US-382-3961 (NCT03060447) was a randomized, double-blind, placebo-controlled, Phase 1b clinical trial of VES in ART-suppressed "HIV virologic controllers," a subset of people with HIV with history of maintaining low plasma HIV-1 RNA in the absence of ART; in this study, VES treatment resulted in a modest delay in time to HIV rebound[4]

Objectives

- To identify immunologic and glycomic profiles that are associated with time to HIV rebound or duration of HIV control during ATI in participants from Study GS-US-382-3961

Methods

Study Design



*Pre-ATI was 7 d after Dose 10 for plasma cytokines, 13 d after for interferon (IFN)–stimulated gene and immune cell phenotyping data, and 1 d after for glycomic data.

- 25 ART-treated HIV viremic controllers were randomized 2:1 to receive VES or placebo; 17 participants received 10 doses of VES (orally qow) and 8 received placebo, followed by an ATI for ≤48 wk; a total of 23 participants completed the ATI phase of the study
- ART was reinitiated if HIV-1 RNA was >10,000 c/mL for 4 consecutive wk, HIV-1 RNA did not decrease to <1000 c/mL within 6 wk of virologic rebound, CD4$^+$ cell count was confirmed to decrease >30% from pre-ATI levels, or CD4$^+$ cell count was confirmed at <350 cells/μL
- Changes from baseline in glycomic profile were analyzed and no differences were observed between baseline and pre-ATI time points regardless of treatment regimen; data obtained from immune and glycomic biomarker analyses from both arms were pooled and used to identify biomarkers associated with viral outcomes
- Plasma, serum, and peripheral blood mononuclear cells were collected at baseline and multiple time points following treatment
- Plasma and isolated plasma total IgG glycomic profiles were analyzed using lectin array and capillary electrophoresis
- Several key immune biomarkers (including VES pharmacodynamic biomarkers and immune cell activation markers) were evaluated including:
  - Frequency of cellular subsets: natural killer cells (CD16$^+$ and CD69$^+$), monocytes (CD14$^+$CD16$^+$), CD8$^+$ T cells (Ki67$^+$, CD69$^+$, CD38$^+$, and HLA-DR$^+$CD38$^+$), and CD4$^+$ T cells (Ki67$^+$, CD69$^+$, and HLA-DR$^+$CD38$^+$)
  - Geometric mean fluorescent intensity of CD40 and CD54 on plasmacytoid dendritic cells
  - Plasma cytokines: interleukin-1 receptor antagonist, IFNγ-induced protein 10, IFN-inducible T-cell alpha chemoattractant, and IFNα
- Immune and glycomic biomarkers from baseline and the last collection prior to ATI (pre-ATI) were used to determine associations with time when plasma HIV-1 RNA reached 200 or 1000 c/mL, or the duration of time that plasma HIV-1 RNA remained ≤400 c/mL during ATI using Spearman's correlation method and the Cox proportional-hazards model; changes in intact proviral HIV-1 DNA (IPDA) in relation to biomarkers were also evaluated using Spearman's correlation
  - Nominal and adjusted p-values (false discovery rate [FDR]) are reported

Baseline Results

Association of Baseline Glycomic Biomarkers With Time to HIV Rebound and Duration of HIV Control During ATI*

		Spearman's Correlation								
		Time to 1st Rebound to Plasma HIV-1 RNA of 200 c/mL			Time to 1st Rebound to Plasma HIV-1 RNA of 1000 c/mL			Duration of Plasma HIV-1 RNA ≤400 c/mL During ATI		
Glycomic Biomarkers	n	p-Value	R	FDR	p-Value	R	FDR	p-Value	R	FDR
IgG N-glycans										
G2FB	23	0.10	-0.36	0.981	0.12	-0.34	0.926	0.011	-0.53	0.775
A1FB	23	0.029	-0.47	0.981	0.17	-0.31	0.926	0.13	-0.34	0.775
A2FB	23	0.028	-0.47	0.981	0.14	-0.33	0.926	0.043	-0.44	0.775
A2B	23	0.25	-0.26	0.981	0.18	-0.3	0.926	0.048	-0.43	0.775
Plasma total glycans										
GNA-binding glycans	23	0.06	-0.40	0.981	0.65	-0.10	0.937	0.040	-0.43	0.775
HPA-binding glycans	23	0.005	0.57	0.981	0.001	0.64	0.247	0.016	0.50	0.775
MPA-binding glycans	23	0.07	0.39	0.981	0.041	0.43	0.926	0.019	0.49	0.775
PHA (E)-binding glycans	23	0.035	-0.44	0.981	0.21	-0.27	0.926	0.97	-0.01	0.998
G total group	23	0.23	-0.27	0.981	0.008	-0.55	0.556	0.11	-0.35	0.775
G0 group	23	0.23	0.27	0.981	0.008	0.55	0.556	0.11	0.35	0.775
FA2G0	23	0.23	0.27	0.981	0.009	0.54	0.556	0.14	0.33	0.775
FA2BG0	23	0.50	0.15	0.981	0.037	0.45	0.926	0.36	0.21	0.998
S total group	23	0.31	-0.23	0.981	0.045	-0.43	0.926	0.42	-0.18	0.998
S0 group	23	0.31	0.23	0.981	0.045	0.43	0.926	0.42	0.18	0.998

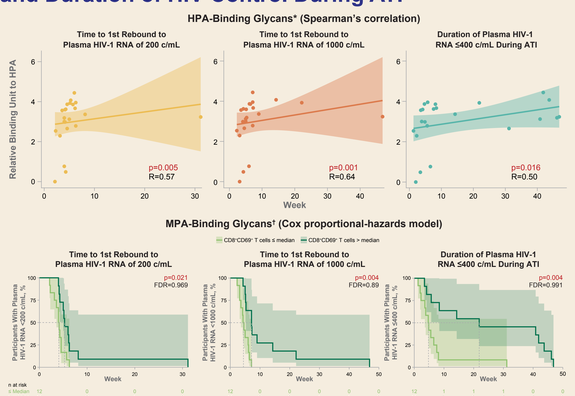
*Data only include participants with nominal ps0.05 for ≥1 plasma HIV-1 RNA c/mL level. GNA, *Galanthus nivalis* agglutinin; HPA, *Helix pomatia* agglutinin; MPA, *Maclura pomifera* agglutinin; NPA, *Narcissus pseudonarcissus* agglutinin; PHA, *Phaseolus vulgaris* phytohaemagglutinin.

Association of Baseline Glycomic Biomarkers With Time to HIV Rebound and Duration of HIV Control During ATI*

		Cox Proportional-Hazards Model								
		Time to 1st Rebound to Plasma HIV-1 RNA of 200 c/mL			Time to 1st Rebound to Plasma HIV-1 RNA of 1000 c/mL			Duration of Plasma HIV-1 RNA ≤400 c/mL During ATI		
Glycomic Biomarkers	n	HR (95% CI)	p-Value	FDR	HR (95% CI)	p-Value	FDR	HR (95% CI)	p-Value	FDR
IgG N-glycans										
A2B	22	1.3 (0.5, 3.2)	0.54	0.969	1.9 (0.7, 4.8)	0.18	0.984	4.3 (1.4, 13.4)	0.013	0.991
G1FB	22	2.6 (1.0, 7.0)	0.05	0.969	1.9 (0.8, 4.6)	0.17	0.984	2.4 (1.0, 6.1)	0.06	0.991
Plasma total glycans										
A3G3S3	22	3.3 (1.1, 10.4)	0.037	0.969	3.6 (1.1, 11.1)	0.028	0.984	1.7 (0.6, 4.8)	0.32	0.991
G2 group	22	1.6 (0.7, 3.7)	0.31	0.969	2.7 (1.1, 6.8)	0.036	0.984	3.0 (1.1, 8.0)	0.029	0.991
GSL II	23	1.9 (0.8, 4.5)	0.16	0.969	3.0 (1.1, 7.9)	0.029	0.984	2.2 (0.9, 5.3)	0.09	0.991
MPA-binding glycans	23	0.4 (0.1, 0.9)	0.021	0.969	0.2 (0.1, 0.6)	0.004	0.890	0.2 (0.1, 0.6)	0.004	0.991
NPA-binding glycans	23	2.2 (0.9, 5.2)	0.09	0.969	1.2 (0.5, 2.8)	0.64	0.984	2.4 (1.0, 5.9)	0.05	0.991
WGA-binding glycans	23	3.5 (1.3, 9.4)	0.011	0.969	2.4 (1.0, 5.7)	0.05	0.984	1.6 (0.7, 3.7)	0.30	0.991

*Data only include participants with nominal ps0.05 for ≥1 plasma HIV-1 RNA c/mL level. CI, confidence interval; HR, hazard ratio; WGA, wheat germ agglutinin.

Higher Baseline HPA- and MPA-Binding Glycans Were Associated With Longer Time to HIV Rebound and Duration of HIV Control During ATI



*Nominal p-value and correlation coefficient (R) are reported; †HPA selectively binds to α-GalNAc residues and type A erythrocytes; HPA recognizes aberrant O-linked α-GalNAc in cancer and metastasis-associated glycosylation changes; HPA-binding glycoproteins by cancer cells were deemed as a poor prognosis marker[5,6]; ‡MPA selectively binds to tumor-associated T-antigen (Galb1-3GalNAc and α-GalNAc) and O-linked glycol peptides.[7]

Pre-ATI Results

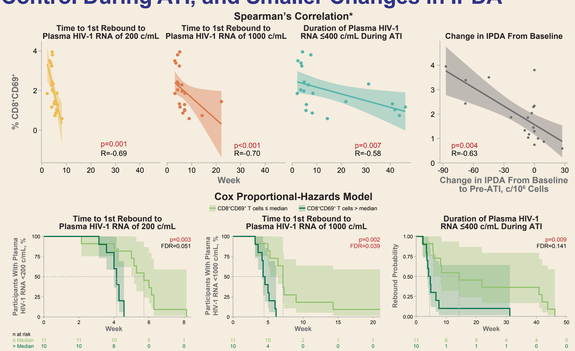
Association of Pre-ATI Glycomic Biomarkers With Time to HIV Rebound and Duration of HIV Control During ATI*

		Spearman's Correlation								
		Time to 1st Rebound to Plasma HIV-1 RNA of 200 c/mL			Time to 1st Rebound to Plasma HIV-1 RNA of 1000 c/mL			Duration of Plasma HIV-1 RNA ≤400 c/mL During ATI		
Glycomic Biomarkers	n	p-Value	R	FDR	p-Value	R	FDR	p-Value	R	FDR
IgG N-glycans										
A2B	23	0.24	-0.25	0.981	0.18	-0.29	0.926	0.036	-0.44	0.775
Plasma total glycans										
DSA-binding glycans	23	0.22	0.27	0.981	0.14	0.32	0.926	0.006	0.56	0.775
LCA-binding glycans	23	0.08	0.38	0.981	0.042	0.43	0.926	0.011	0.52	0.775
GNA-binding glycans	23	0.13	-0.33	0.981	0.46	-0.16	0.932	0.025	-0.47	0.775

		Cox Proportional-Hazards Model								
		Time to 1st Rebound to Plasma HIV-1 RNA of 200 c/mL			Time to 1st Rebound to Plasma HIV-1 RNA of 1000 c/mL			Duration of Plasma HIV-1 RNA ≤400 c/mL During ATI		
Glycomic Biomarkers	n	HR (95% CI)	p-Value	FDR	HR (95% CI)	p-Value	FDR	HR (95% CI)	p-Value	FDR
IgG N-glycans										
A2B	23	1.3 (0.5, 3.0)	0.60	0.969	1.9 (0.7, 4.7)	0.18	0.984	2.5 (1.0, 6.4)	0.05	0.991
Plasma total glycans										
DSA-binding glycans	23	0.8 (0.3, 1.9)	0.63	0.969	0.6 (0.2, 1.4)	0.20	0.984	0.2 (0.1, 0.7)	0.011	0.991
SNA-binding glycans	23	1.0 (0.4, 2.2)	0.93	0.995	0.9 (0.4, 2.0)	0.77	0.984	0.4 (0.1, 0.9)	0.028	0.991
GNA-binding glycans	23	1.5 (0.6, 3.5)	0.36	0.969	1.6 (0.7, 3.7)	0.31	0.984	2.5 (1.1, 6.2)	0.038	0.991
LTL-binding glycans	23	1.3 (0.6, 3.1)	0.55	0.969	1.9 (0.8, 4.6)	0.17	0.984	2.4 (1.0, 5.7)	0.049	0.991

*Data only include participants with nominal ps0.05 for ≥1 plasma HIV-1 RNA c/mL level. DSA, *Datura stramonium* agglutinin; LCA, *Lens culinaris* agglutinin; LTL, *Lotus tetragonolobus* lectin; SNA, *Sambucus nigra* agglutinin.

Higher Level of Pre-ATI CD8$^+$CD69$^+$ T Cells Correlated With Faster HIV Rebound, Shorter Duration of HIV Control During ATI, and Smaller Changes in IPDA



Nominal p-value and R are reported.

Conclusions

- This exploratory analysis highlights specific host pro-inflammatory immunologic and glycomic factors as potential correlates of the duration of viral control post-ART cessation in HIV viremic controllers
  - Baseline levels of several plasma glycomic markers were found to be positively associated with time to HIV rebound or duration of HIV control during ATI: O-linked GalNAc glycans (binding to HPA and MPA), N-acetylglucosamine (binding to WGA), α-GalNAc, and plasma trisialylated N-glycans (A3G3S3)
  - Baseline bisecting A2FB glycan trait in IgG glycome inversely correlated with time to HIV rebound and duration of HIV control during ATI
  - Higher pre-ATI frequency of activated CD8$^+$CD69$^+$ T cells, perhaps associated with inflammation and immune cell dysfunction, correlated with faster HIV rebound, shorter duration of HIV control during ATI, and smaller changes in IPDA from baseline (nominal p<0.01 in both methods regardless of viral milestone)
- Consistent with previous reports,[1,4] these data suggest that host inflammatory pathways may be linked to greater HIV replication and foster viral rebound during ATI; interventions aimed at sustained HIV control during ATI may ultimately require a balanced enhancement of innate and adaptive immune responses, likely only achievable with combination treatments
- This analysis was limited by the small number of participants and further studies with larger independent cohorts, including those without history of natural pre-ART viral control, are needed to validate these biomarkers, as well as to investigate their prognostic and functional significance

References: 1. Giron LB, et al. Nat Commun 2021;12:3922; 2. Keser T, et al. Diabetologia 2017;60:2352-60; 3. Vuckovic F, et al. Clin Cancer Res 2016;22:3078-86; 4. SenGupta D, et al. Science Transl Med 2021;13:eabg3071; 5. Khosrowabadi E, et al. Oncotarget 2022;13:73-89; 6. Rambaruth NDS, et al. Glycobiology 2012;22:839-48; 7. Zhang L, et al. mAbs 2016;8:205-15.

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