DIASTOLIC DYSFUNCTION WITH PRESERVED EJECTION FRACTION IN FEMALE HIV-INFECTED HUMANIZED MICE ON cART (Presentation # 00796)

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JUSTIFICATION FOR WORK: Early-onset diastolic dysfunction has emerged as a major threat to healthy aging in people with HIV-1 infection. Women living with HIV-1 infection (WLWH) are especially vulnerable and develop a unique pattern vascular and myocardial ischemia compared to men. Animal models that recapitulate this pathophysiology remain unreported, and this has left a void in our understanding of the molecular causes of the disease and treatment strategies to alleviate it.

MAIN FINDINGS: Here we show for the first that female humanized mice infected with HIV-1_{ADA} and treated with dolutegravir/tenofovir disoproxil fumarate/emtricitabine combination for fourteen weeks, starting two weeks after productive infection, can recapitulate the diastolic dysfunction, microvascular dysfunction, and ischemia reported in women living with HIV-1 infection (WLWH). This novel model will allow us to conduct pre-clinical studies to identify druggable targets to attenuate early-onset diastolic dysfunction in WLWH.

METHODS and RESULTS: We show using *in vivo* echocardiography that female NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ humanized mice (Hu-mice) infected with HIV-1_{ADA} and treated with the first line regimen, dolutegravir (DTG)/tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC) for fourteen weeks, starting two weeks after productive infection, HIV-infected Hu-mice treated DTG/TDF/FTC develop progressive diastolic dysfunction with preserved ejection fraction (E:A ratio increased by 32.1 ± 5.1% and E:e' increased by 28.2 ± 5.6 % after 16 weeks, compared to uninfected Hu-mice). Left atria, global longitudinal and right ventricular progressively increased (left atria volume increased by 26.6 ± 4.2, global strain increased by 32. 5 ± 4.3% and right ventricular diameter-systole increased by 19.6 ± 1.3%). *In vivo* photoacoustic imaging revealed a 30.4 ± 6.8% reduction in saturated oxygenated hemoglobin in the anterior wall of the heart. *Ex vivo* analyses of hearts DTG/TDF/FTC-treated HIV-1 infected Hu-mice revealed a 37.3 ± 8.2% reduction in intact microvessels, a 30.6 ± 6.2% reduction in the density of perfused microvessels with regions of micro-ischemia There were also 20.2 ± 1.2 and 20.6 ± 2.3 % increases in interstitial and perivascular fibrosis, respectively. Expression of hypoxia-induced transcription factor HIF-1a was increased 2.6 ± 0.5-fold, the cytotoxic glycolysis metabolite, methylglyoxal (MG) was increased 3.2 ± 0.3-fold and the inflammation-induced protein, vascular adhesion protein-1 was increased 123.5 ± 10% in hearts of HIV-infected female Hu-mice compared to uninfected controls.

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MODEL CHARACTERIZATION DATA

	Parameters	At Start (t = 0 weeks)	End (t = 17 weeks)
Hu-NSG mice (n = 6)	Body weight (g)	18.3 ± 0.4	18.3 ± 1.0
	% CD4+ T cell in blood	76.6 ± 0.4	77.2 ± 0.6
	Plasma HSA MG (µg/ml)	26.6 ± 0.4	34.1 ± 0.6
	Plasma SSAO (units/ml/2h)	3.1 ± 0.3	3.1 ± 0.3
Hu-NSG mice infected with HIV-1 (n = 7)	Body weight (g)	18.3 ± 0.4	19.2 ± 0.4
	HIV-1 plasma viral load (RNA copies/ml)	1.0 ± 0.1 X 10^5	1.6 ± 0.2 X 10^5
	Plasma HSA MG (µg/ml)	25.6 ± 0.3	59.6 ± 5.4*
	Plasma SSAO (units/ml/2h)	3.1 ± 0.2	6.2 ± 0.3*
Hu-NSG mice infected with HIV-1 and Treated with DTG/TDF/FTC (n=5)	Body weight (g)	18.3 ± 0.4	19.2 ± 0.4
	HIV-1 plasma viral load (RNA copies/ml)	1.0 ± 0.1 X 10^5	0.5 ± 0.2 X 10^2
	Plasma HSA MG (µg/ml)	27.6 ± 0.3	58.1 ± 5.4*
	Plasma SSAO (units/ml/2h)	3.2 ± 0.1	6.1 ± 0.3*
	Plasma DTG (ng/ml)		7622.5 ± 665.6
	Plasma TDF (ng/ml)		546.7 ± 74.5
	Plasma FTC (ng/ml)		1816.4 ± 278.4

Table 1: General characteristics of animals used in the study.

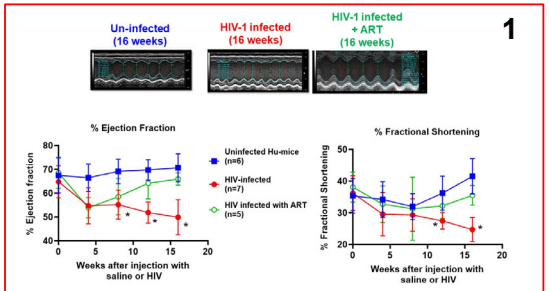


Figure 1: Longitudinal changes in % ejection fraction and % fractional shortening in uninfected, HIV-1 infected and HIV-infected female humanized mice infected treated with combination dolutegravir (DTG 15 mg/kg, an integrase inhibitor), tenofovir disoproxil fumarate (TDF, 131 mg/kg, a nucleotide analogue reverse-transcriptase inhibitor) and emtricitabine (FTC, 87.5 mg/kg, a nucleoside reverse transcriptase inhibitor) via feed based on 4 g/day consumption for fourteen week, starting two weeks after productive infection. All measurements were collected using Fujifilm VisualSonics Vevo 3100 system (Fujifilm VisualSonics, Toronto, ON, CAN) employing a MS550D transducer with a center frequency of 40 Hz and an axial resolution of 40 µM. Echocardiographic data were analyzed using Vevo Lab 5.7.1.
* denotes significantly different from uninfected, p<0.05,
# denotes significantly different from HIV-infected, p.0.05.

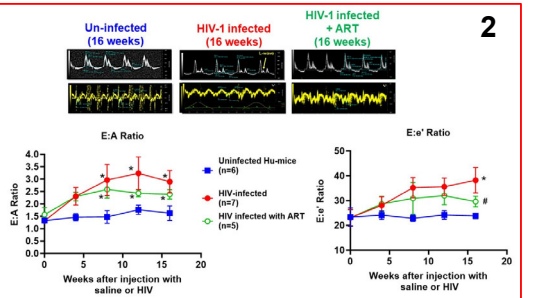


Figure 2: Longitudinal changes in E:A and E:e' ratios in uninfected, HIV-1 infected and HIV-infected female humanized mice infected treated with DTG/TDF/FTC, 87.5 mg/kg, via feed for fourteen week, starting two weeks after productive infection. All measurements were done using Fujifilm VisualSonics Vevo 3100 system (Fujifilm VisualSonics, Toronto, ON, CAN) employing a MS550D transducer with a center frequency of 40 Hz and an axial resolution of 40 µM. Echocardiographic data were analyzed using Vevo Lab 5.7.1.
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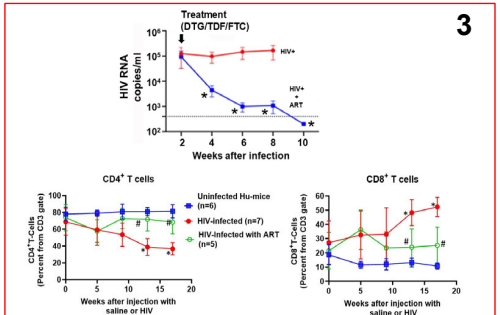


Figure 3: Panel A shows longitudinal changes in plasma viremia in HIV-infected female humanized mice treated with and without DTG/TDF/FTC in feed for 10 weeks. Data shown are for n= 6 animals per group. *denotes significantly different from untreated. **Panels B and C** shows longitudinal changes in $CD4^+$ and $CD8^+$T cells in uninfected, HIV-1 infected and HIV-infected female humanized mice infected treated with combination DTG/TDF/FTC via feed for fourteen week, starting two weeks after productive infection as a percent of CD3 gate. All measurements were collected using LSR-II FACS analyzer (BD Biosciences, Mountain View, CA, USA).
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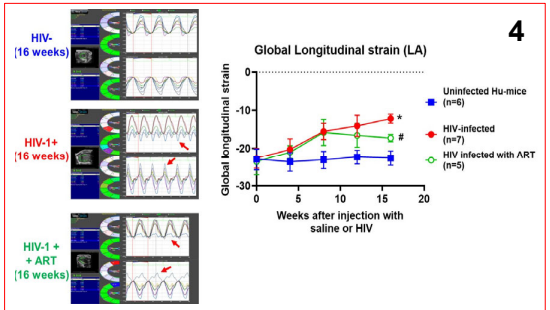


Figure 4: Longitudinal changes in left ventricular strain in uninfected, HIV-1 infected and HIV-infected female humanized mice infected treated with DTG/TDF/FTC, via feed for fourteen week, starting two weeks after productive infection. Red arrows shows dyskinesia in opposite segments in hearts from HIV-infected and HIV-infected female humanized mice infected treated with DTG/TDF/FTC. All measurements were collected using Fujifilm VisualSonics Vevo 3100 system (Fujifilm VisualSonics, Toronto, ON, CAN) employing a MS550D transducer with a center frequency of 40 Hz and an axial resolution of 40 µM. Echocardiographic data were analyzed using Vevo Lab 5.7.1.
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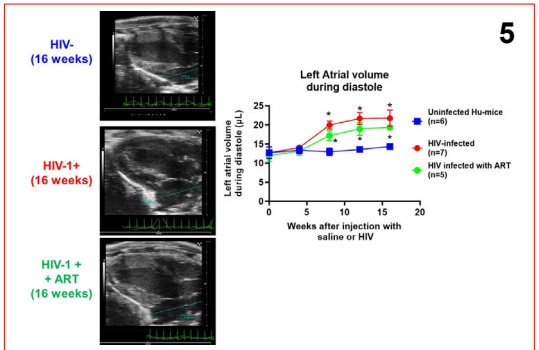


Figure 5: Longitudinal changes in left atrial volume in uninfected, HIV-1 infected and HIV-infected female humanized mice infected treated with DTG/TDF/FTC, via feed for fourteen week, starting two weeks after productive infection. All measurements were done using Fujifilm VisualSonics Vevo 3100 system (Fujifilm VisualSonics, Toronto, ON, CAN) employing a MS550D transducer with a center frequency of 40 Hz and an axial resolution of 40 µM. Echocardiographic data were analyzed using Vevo Lab 5.7.1. * denotes significantly different from uninfected, p<0.05.

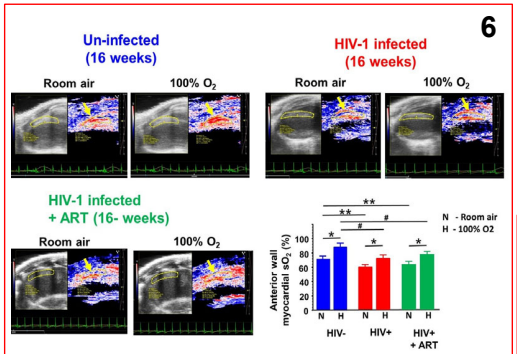


Figure 6: Photoacoustic images of saturated oxygenated hemoglobin in the anterior wall of the left ventricle (septum) of hearts from uninfected HIV-1 infected and HIV-infected female humanized mice infected treated with DTG/TDF/FTC for fourteen weeks in normal air and 100% oxygen (compare yellow arrows). Graph at lower right is mean ± SEM from N = 5 female mice per group, * denotes significantly different from control in room air, p < 0.05, # denotes significantly different from control with 100% oxygen, p<0.05. All images were collected using Fujifilm VisualSonics Vevo 3100 system with a LAZR-X (Fujifilm VisualSonics, Toronto, ON, CAN), employing a MX550D transducer with a center frequency of 40 Hz and an axial resolution of 40 µM. Photoacoustic image data were analyzed using Vevo Lab 5.7.1.

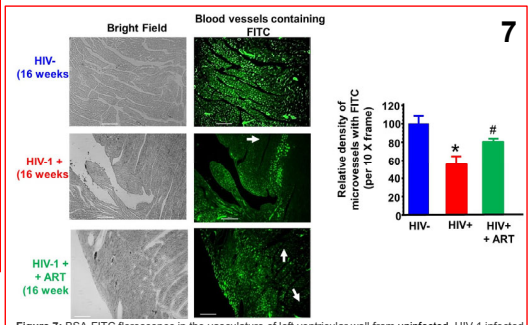


Figure 7: BSA-FITC florescence in the vasculature of left ventricular wall from uninfected, HIV-1 infected and HIV-infected female humanized mice infected treated with DTG/TDF/FTC for fourteen weeks. For this, BSA-FITC was injected into a tail vein 5 minutes prior to euthanasia to visually density of perfused microvessels in heart tissues. White arrows show regions of ischemia in left ventricular walls of HIV-1 infected and HIV-infected female humanized mice infected treated with DTG/TDF/FTC. Graph on right shows relative density of microvessels perfused with BSA-FITC per 10-x frame. White scale bar at the bottom indicates 200 µm.
* denote significantly different from uninfected, p < 0.05,
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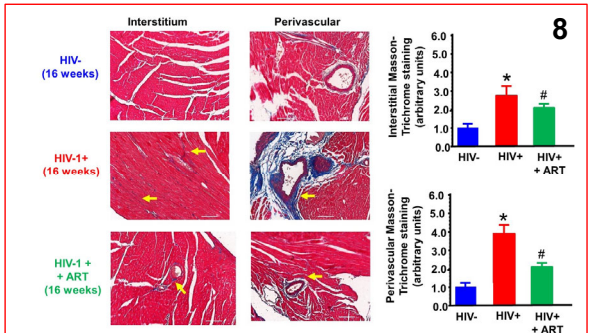


Figure 8: Masson-Trichrome staining in let ventricular wall from uninfected, HIV-1 infected and HIV-infected female humanized mice infected treated with DTG/TDF/FTC for fourteen weeks. Yellow arrows show regions of increased interstitial and perivascular staining in HIV-1 infected and HIV-infected female humanized mice infected treated with DTG/TDF/FTC. Graphs on right shows relative density of Masson-Trichrome staining per 10-x frame. White scale bar at the bottom indicates 200 µm
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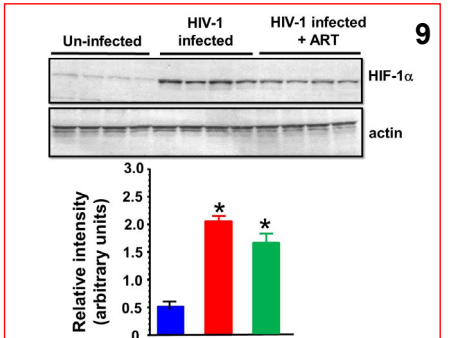


Figure 9: Western blot analysis for total hypoxia-induced factor alpha (HIF-1α) in hearts from uninfected, HIV-1 infected and HIV-infected female humanized mice infected treated with DTG/TDF/FTC. Graphs on below shows relative density of HIF-1α.
* denote significantly different from uninfected, p < 0.05

REFERENCES

1. Dash PK, Alomar FA, Hackford B, Su H, Conaway A, Poluektova LY, Gendelman HE, Gorantla S, and Bidasee KR (2020). HIV-1-Associated Cardiac Deficits in Humanized Mice, Scientific Reports, 6;10(1):9746-9760. PMCID: 7297773. PMID: 32546795
2. Alomar FA, Tian, C, Dash, P, McMillan, JM, Gendelman HE, Gorantla, S and Bidasee, KR (2021). Skeletal Muscle Intracellular Ca^{2+} Homeostasis and Antiretroviral Drugs. 187:104975. PMID: 33450312.
3. Dash PK, Alomar FA, Cox JL, McMillan J, Hackfort BT, Makarov E, Morsey B, Fox HS, Gendelman HE, Gorantla S, Bidasee KR. (2021). AI link between heart failure and methylglyoxal during HIV-1 infection. Front Cardiovasc Med. 2021 Dec 14;8:792180