Poster 0716

Inflammation and Mitochondrial Dysfunction Not NRTIs Drive Events in ACTG A5241

Carl J. Fichtenbaum,[1] T Stone[2], GS Gojanovich[3], R Jandarov[2], JJ Eron[4], RT Gandhi[5], K Tashima[6], NS Utay[7], M Gerschenson[3]

[1] Division of Infectious Diseases, University of Cincinnati College of Medicine; [2]University of Cincinnati, Department of Environmental Health, Division of Biostatistics, [3]University of Hawaii at Manoa, Department of Cell and Molecular Biology, [4]University of North Carolina, Department of Internal Medicine, Division of Infectious Diseases, [5]Massachusetts General Hospital, [6]Brown University, Department of Internal Medicine, Division of Infectious Diseases, [7] University of Texas Health Science Center in Houston, Department of Internal Medicine, Division of General Medicine

Background

- ACTG A5241 (OPTIONS) was a randomized trial of adding or omitting NRTIs to optimized therapy in persons with triple class experience or resistance that were failing a protease inhibitor based regimen.
- AIDS-related opportunistic illnesses (AIDS-OIs), serious non-AIDS clinical events (SNAEs) and death were more common in those randomized to add NRTIs.
- Analysis of CD4, HIV-1 RNA, and other clinical variables did not explain the observed association between clinical events and the use of NRTIs in the main study.
- We hypothesized that inflammation or mitochondrial dysfunction might explain differences in these clinical outcomes.

Objective

The objectives of this analysis was to evaluate the association of circulating inflammatory biomarkers and measures of mitochondrial function with clinical events.

Methods

Study design and population:

- Analysis of randomly selected cohort enhanced for those with clinical events defined as death, AIDS-OI or SNAEs.
- Participants were randomly selected from 413 individuals enrolled in OPTIONS.
- All individuals with clinical events (CE, N=58) were compared to 116 participants that were randomly selected if they had samples available for analysis at baseline, weeks 24 and 48.
- Main entry criteria for OPTIONS included:
  - A history of triple class experience and/or resistance (NRTIs, NNRTIs and PIs)
  - HIV RNA ≥ 1,000 copies/mL
  - Current use of PI-based regimen

Inflammatory Biomarkers and Mitochondrial Measures:

- Circulatory biomarkers were measured in batched frozen plasma samples obtained at baseline, weeks 24 and 48; NADH dehydrogenase assay was measured on frozen cell pellets at baseline. Samples were diluted per manufacturer's suggestions.

Biomarker assays:

- Multiple Luminex assays (Life Science Technologies) were used to measure insulin, sCD14, CRP, sTNFr-I and sTNFr-II. IL-6 was measured with a high sensitivity Luminex assay (Millipore Corp).
- Multiple ELISAs were used to measure NADH dehydrogenase (Complex I), FGF-21 and GDF-15 (Abcam, Inc).

Statistical analyses:

- Participants were censored from analysis after the occurrence of an event.
- We created logistic regression models including variables with P<0.05 on univariate analysis.
- Sensitivity models included variables with P<0.1 with removal of variables likely to have interactions (e.g., Absolute CD4 and CD4 percentage)

Results

Table 1 – Characteristics at baseline, weeks 24 and 48

	Baseline CE Group (N=58)	Baseline No CE Group (N=116)	P value	Week 24 CE Group (N=52)	Week 24 No CE Group (N=116)	P value	Week 48 CE Group (N=49)	Week 48 No CE Group (N=116)	P value
Median Age (years) (range)	47.5 (18-69)	47 (17-68)	NS	48 (18-61)	47 (17-68)	NS	48 (18-61)	47 (17-68)	NS
Sex at birth									
Male	62%	62%	NS	60%	61%	NS	59%	61%	NS
Female	38%	38%		40%	39%		41%	39%	
Race/Ethnicity									
White	36%	33%	NS	38%	33%	NS	38%	33%	NS
Black or African-American	47%	41%		46%	41%		46%	41%	
Hispanic	14%	22%		11%	22%		12%	22%	
Other	3%	4%		4%	4%		4%	4%	
Treatment Arm									
Add NRTIs	38	74	NS	32	74	NS	30	74	NS
Omit NRTIs	20	42		20	42		19	42	
Median CD4 count (cells/mm^3) (range)	**148 (3-894)**	**212 (2-865)**	**0.03**	**219 (6-1424)**	**333 (5-1171)**	**0.009**	**228 (0-1158)**	**371 (4-1135)**	**0.001**
Median CD4:CD8 ratio (range)	**0.18 (0.01-0.89)**	**0.24 (0.01-1.03)**	**0.02**	**0.25 (0.02-1.09)**	**0.30 (0.02-1.09)**	**0.02**	**0.24 (0.0-1.23)**	**0.35 (0.02-1.34)**	**<0.001**
Median Log HIV RNA (range)	4.33 1.81-6.26	4.22 1.7-5.62	0.24	**1.42 (0.09-5.92)**	**1.30 (0.78-4.85)**	**0.01**	**1.42 (0.78 - 5.89)**	**1.19 (0.79 - 5.87)**	**0.005**
Median VACS (range)	**46 (12-104)**	**32 (11-70)**	**<0.001**	-	-	-	-	-	

Commonly Used Abbreviations

CE- Clinical Events

NRTIs – Nucleoside/tide reverse transcriptase inhibitors

VACS – Veterans Aging Cohort Study

Complex I – NADH Dehydrogenase

IL-6 – Interleukin 6

sTNFR-I – Soluble Tumor Necrosis Factor Receptor I

sTNFR-II – Soluble Tumor Necrosis Factor Receptor II

CRP – C-reactive protein

sCD14 – Soluble Cluster of differentiation 14

FGF-21 – Fibroblast Growth Factor-21

GDF-15 – Growth differentiation factor-15

Table 2 – Median biomarkers at baseline, weeks 24 and 48

Biomarker	Baseline CE Group (N=58)	Baseline No CE Group (N=116)	P value	Week 24 CE Group (N=52)	Week 24 No CE Group (N=116)	P value	Week 48 CE Group (N=49)	Week 48 No CE Group (N=116)	P value
Complex I (mcg/mL) (range)	24.63 (3.67 – 200)	26.48 (2.25 – 200)	NS	-	-	-	-	-	-
IL-6 (pg/mL) (range)	7.47 (0.70 – 429)	8.24 (1.59 – 233)	NS	8.24 (1.01 – 253)	9.37 (0.64 – 229)	NS	8.25 (1.23 – 750)	8.32 (1.22 – 218)	NS
Insulin (pg/mL) (range)	2, 030 (138 – 6,604)	2,290 (138- 11,026)	0.13	2,022 (138–16,035)	1,960 (138 – 7,819)	NS	2,388 (138 – 12,556)	1,912 (138 – 7,296)	NS
sTNFR-I (pg/mL) (range)	1,491 (134-11,550)	1,379 (134–12,022)	NS	1,617 (134–13,458)	1,302 (134–12,045)	0.13	1,672 (134 – 11,856)	1,364 (134 – 6,929)	0.058
sTNFR-II (pg/mL) (range)	60 (18 – 272)	69 (15 – 343)	NS	66 (7.6–266)	61 (12.7–353)	NS	63 (17.5 – 258)	58 (14.6 – 309)	NS
CRP (mg/L) (range)	0.78 (0.02 – 6.95)	0.87 (0.02–8.68)	NS	1.01 (0.02 –7.85)	0.90 (0.02 – 5.57)	NS	1.27 (0.01 – 7.70)	0.78 (0.02 – 9.00)	NS
sCD14 (pg/mL) (range)	**555,263 (192,453-1,702,348)**	**448,584 (111,764-1,467,008)**	**0.03**	**545,043 (115,661 – 1,558,520)**	**387,479 (98826 – 1039589)**	**<0.001**	**572,375** (127,200 – 2,351,872)	**363,165** (109,275 – 1,627,899)	<0.001
FGF-21 (pg/mL) (range)	168.7 22.1-1006.4	124.2 21.3-16,753.1	0.08	**146.5 (22.1 - 2776.5)**	**102.2 (22.1 - 4148.5)**	**0.02**	**169.7 (22.1 – 1,329.6)**	**123.9 (19.7 – 2,000)**	**0.0486**
GDF-15 (pg/mL) (range)	2514 398-64,788	2241 433-145,301	NS	2508 (429.3 - 40779.1)	2037 (320.4 - 37246.9)	0.13	2,142.6 (402.5 – 29,414.9)	1,994.4 (416.5 – 49,597.8)	NS

Odds Ratios Associated with Clinical Events

Table 3 – Baseline Multivariate Analysis

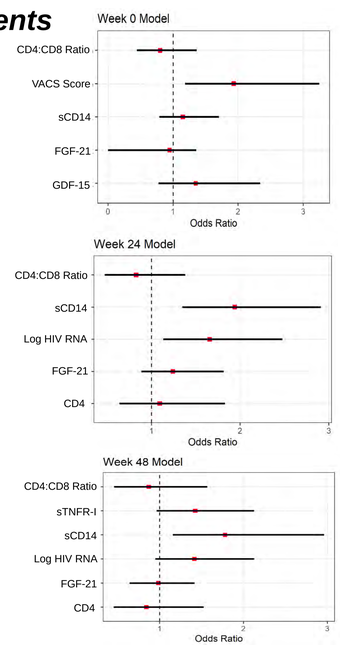
Parameter	Odds Ratio	Lower Bound	Upper Bound
sCD14	1.15	0.79	1.70
FGF21	0.94	0	1.36
VACS SCORE	**1.93**	**1.19**	**3.24**
CD4 COUNT	1.35	0.78	2.34
CD4:CD8 RATIO	0.80	0.44	1.36

Table 4 – Week 24 Multivariate Analysis

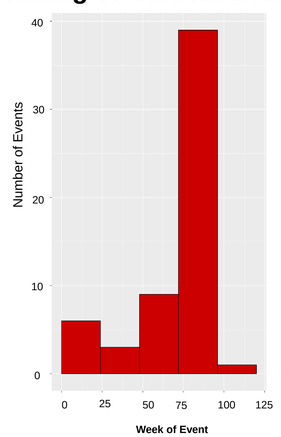
Parameter	Odds Ratio	Lower Bound	Upper Bound
sCD14	**1.94**	**1.35**	**2.91**
LOG HIV RNA	**1.65**	**1.13**	**2.47**
FGF21	1.24	0.89	1.81
CD4 COUNT	1.09	0.64	1.83
CD4:CD8 RATIO	0.83	0.47	1.38

Table 5 – Week 48 Multivariate Analysis

Parameter	Odds Ratio	Lower Bound	Upper Bound
sCD14	**1.78**	**1.15**	**2.96**
FGF21	0.98	0.64	1.42
sTNFR-I	1.42	0.96	2.13
LOG HIV RNA	1.41	0.95	2.13
CD4 COUNT	0.84	0.45	1.52
CD4:CD8 RATIO	0.87	0.45	1.57



Timing of Clinical Events



Clinical Events

- **Deaths – 11**
- **AIDS-defining OIs – 20**
- **SNAEs – 27**

Discussion

- At study entry higher VACS scores, lower CD4 counts and lower CD4:CD8 ratios were associated with a greater chance of developing a clinical event in the OPTIONS study.
- In time-updated analyses, higher sCD14 and FGF-21 levels, lower CD4 counts and higher HIV-1 RNA levels were associated with the occurrence of subsequent clinical events.
- Randomization to omit or add NRTIs was not associated with a later occurrence of a clinical event.
- Of the biomarkers, only higher sCD14 levels at baseline, weeks 24 and 48 were consistently associated with the development clinical events.

Limitations

- The study was limited in that viable PBMCs were not stored at multiple endpoints restricting analyses.
- Although individuals generally improved on the OPTIONS study with nearly two-thirds having successful control of HIV replication, clinical events still occurred throughout the study.
- Many events occurred after week 48 of the study and it is possible that measurements of assays closer to the time of the event might have a greater ability to predict the occurrence of events.
- The design of this analysis did not include all participants from the OPTIONS study introducing the possibility of ascertainment bias.

Conclusions

- In persons failing antiretroviral treatment who were randomized to omit or add NRTIs plus optimized therapy in the OPTIONS study, clinical events were associated with several biomarkers of inflammation and mitochondrial dysfunction.
- Less control of HIV replication and lower CD4 counts were also associated with a greater chance of clinical events.
- Use of NRTIs was not associated with the subsequent occurrence of clinical events.
- The driving force behind the higher levels of inflammation and mitochondrial dysfunction remain unclear.

Acknowledgements

The authors thank all the participants of the OPTIONS study. This work was supported in part by NIAID Grant UM1-AI069501, 1P20GM113134, and the University of Hawaii at Manoa.

Corresponding author:
Carl J. Fichtenbaum
231 Albert Sabin Way
Cincinnati, OH 42567-0560
E-mail: carl.fichtenbaum@uc.edu