Rifaximin Has Marginal Impact on Immune Activation in Immune Non-Responders to Antiretroviral Therapy - ACTG A5286

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BACKGROUND

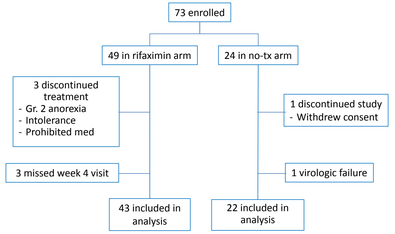
- Immune activation (%CD38⁺HLA-DR⁺ on CD8⁺ T-cells) predicts disease progression in untreated HIV-1 infection & CD4⁺ T-cell rise with ART.
- Immune activation is associated with elevated markers of gut microbial translocation (MT) from HIV-induced gut mucosal CD4⁺ T-cell depletion.
- HIV⁺ persons with CD4⁺ T-cell <350 cells /mm³despite suppressive ART - immune non-responders - have higher levels of MT, immune activation and inflammation.
- Rifaximin, a non-absorbable antibiotic, decreases plasma LPS levels in cirrhotics.
- We hypothesized that rifaximin would decrease gut MT, immune activation & inflammation in immune non-responders.

METHODS

- A5286 was a randomized open-label 2-arm pilot study.
- 73 HIV⁺ adults on ART for ≥ 96 weeks with CD4⁺ T-cell < 350 & viral load below assay limit for ≥ 48 weeks were randomized 2:1 to rifaximin 550 mg BID versus no study treatment for 4 weeks.
- %CD38/HLA-DR⁺ and %Ki67⁺ on CD8⁺/CD4⁺ T-cells, LPS, sCD14, IL-6, D-dimer, CRP, sCD163 and sTNFR-II were measured at baseline & weeks 2, 4, 8 and 12.
  - Cryopreserved blood samples from 20 age-matched HIV-neg men from the Multicenter AIDS Cohort Study were assayed for biomarker levels to serve as controls.
- Wilcoxon rank-sum tests compared changes between arms.
- Spearman correlations assessed correspondence between biomarker levels, & between biomarker responses to treatment.
- P-values are 2-sided at 5% nominal level without adjustments for multiple testing

RESULTS

Subject Disposition



SAFETY

- No deaths reported.
- 36 (49%) subjects had a primary adverse event: 27 in rifaximin arm and 9 in the no-study-treatment arm.
- No significant difference in maximum primary event grade between the 2 arms.
- None of the grade 3 or 4 events were related to study treatment.
- Adverse events that were possibly/definitely related to study treatment: nausea, constipation, flatulence, anorexia, stomach ache, "feeling sick" and elevated lipase.

RESULTS

Table 1: Baseline demographic, immunologic & virologic characteristics of 65 subjects included in the as-treated analyses.

Characteristic		Rifaximin (N=43)	No Treatment (N=22)
Age (years)	Median (Q1, Q3)	48 (43, 56)	51 (45, 55)
	≥ 60	4 (9%)	3 (14%)
Race/Ethnicity	White Non-Hispanic	19 (44%)	12 (55%)
	Black Non-Hispanic	17 (40%)	3 (14%)
	Hispanic	7 (16%)	6 (27%)
Sex	M	39 (91%)	20 (91%)
Baseline CD4 (cells/mm³)	Median (Q1, Q3)	251 (179, 286)	223 (167, 280)
Nadir CD4 (cells/mm³)	Median (Q1, Q3)	50 (22, 71)	40 (10, 88)
Entry HIV-1 RNA	#Undetectable	43 (100%)	22 (100%)
Years since 1st undetectable HIV-1 RNA	Median (Q1, Q3)	3.3 (2.2, 7.3)	3.9 (2.8, 8.8)

Table 2: Baseline immune biomarker levels in subjects compared to levels in HIV-negative age-matched controls.

Biomarkers	Normal Control (N=20)	Pooled Study Arms at Baseline (N=65)	P-Value
	Median (Q1, Q3)	Median (Q1, Q3)	
Advanced Flow Markers			
% HLA-DR⁺ CD38⁺ of CD8⁺	4.05 (2.68 , 6.04)	8.14 (5.18 , 13.70)	<.001
%HLA-DR⁺ CD38⁺ of CD4⁺	1.76 (1.46 , 2.42)	4.95 (3.64 , 6.06)	<.001
%Ki67⁺ of CD8⁺	0.50 (0.31 , 0.74)	0.71 (0.52 , 0.99)	0.014
%Ki67⁺ of CD4⁺	0.58 (0.46 , 1.11)	1.65 (1.39 , 2.50)	<.001
Gut Microbial Translocation Markers			
LPS (pg/mL)	84.0 (70.7 , 91.3)	103.7 (73.7 , 121.2)	0.036
sCD14 (ng/mL)	1,270.9 (1,100.2 , 1,464.0)	1,808.9 (1,604.8 , 2,161.6)	<.001
Soluble Markers			
IL-6 (pg/mL)	0.9 (0.6 , 1.4)	1.2 (0.9 , 2.2)	0.016
CRP (ng/mL)	1,126.9 (346.7 , 3,175.6)	1,446.9 (730.9 , 2,989.2)	0.35
D-dimer (ng/mL)	192.2 (157.1 , 239.9)	129.6 (96.0 , 213.8)	0.018
sTNFR-II (pg/mL)	2,995.8 (2,319.0 , 3,776.8)	4,214.0 (3,186.0 , 6,354.4)	<.001
sCD163 (ng/mL)	658.4 (610.3 , 771.5)	611.4 (438.1 , 743.9)	0.278

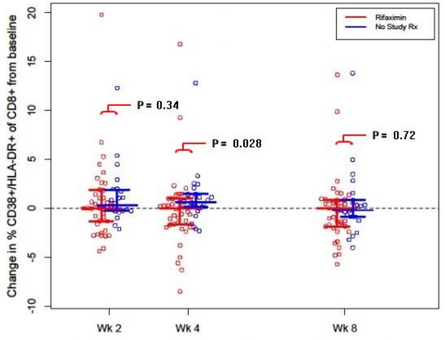


Figure 1: Changes in %CD38+HLA-DR+ of CD8+ T-cells from baseline

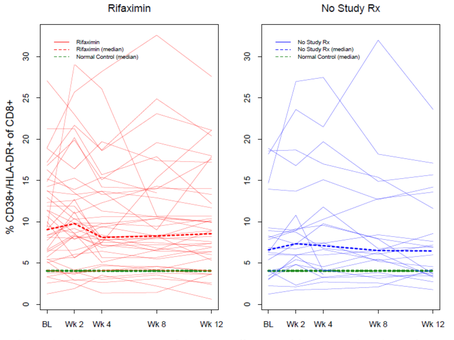


Figure 2: %CD38+HLA-DR+ of CD8+ T-cells per subject over time in each arm

Table 3: Median log_{10} change in soluble biomarkers.

Biomarker	Time Period	Treatment Arm	N	Median (Q1, Q3)	95% CI for Median	p-value
Gut Microbial translocation biomarkers						
LPS (log_{10} pg/mL)	1. BL – Week 2	Rifaximin	43	-0.01 (-0.09, 0.02)	(-0.05, 0.01)	**0.013**
		No Study Treatment	22	0.03 (-0.03, 0.11)	(-0.01, 0.15)	
	2. BL – Week 4	Rifaximin	43	0.00 (-0.08, 0.05)	(-0.04, 0.02)	0.44
		No Study Treatment	22	-0.01 (-0.04, 0.05)	(-0.02, 0.05)	
	3. BL – Week 8	Rifaximin	42	-0.01 (-0.12, 0.06)	(-0.06, 0.03)	0.22
		No Study Treatment	21	0.02 (-0.02, 0.08)	(-0.03, 0.08)	
sCD14 (log_{10} ng/mL)	1. BL – Week 2	Rifaximin	43	-0.00 (-0.06, 0.04)	(-0.04, 0.02)	**0.033**
		No Study Treatment	22	0.05 (-0.02, 0.13)	(-0.00, 0.09)	
	2. BL – Week 4	Rifaximin	43	-0.03 (-0.07, 0.06)	(-0.05, 0.01)	0.97
		No Study Treatment	22	-0.03 (-0.05, 0.01)	(-0.07, 0.02)	
	3. BL – Week 8	Rifaximin	42	-0.04 (-0.09, 0.03)	(-0.08, -0.01)	**0.049**
		No Study Treatment	21	0.01 (-0.05, 0.08)	(-0.03, 0.07)	
Soluble Inflammation Biomarkers						
IL-6 (log_{10} pg/mL)	1. BL – Week 2	Rifaximin	43	0.02 (-0.11, 0.15)	(-0.07, 0.07)	0.94
		No Study Treatment	22	-0.05 (-0.11, 0.20)	(-0.10, 0.20)	
	2. BL – Week 4	Rifaximin	43	-0.03 (-0.14, 0.08)	(-0.08, 0.03)	0.33
		No Study Treatment	22	0.05 (-0.13, 0.12)	(-0.06, 0.13)	
	3. BL – Week 8	Rifaximin	42	-0.05 (-0.18, 0.07)	(-0.12, 0.01)	**0.025**
		No Study Treatment	21	0.05 (-0.08, 0.18)	(-0.02, 0.19)	
CRP (log_{10} ng/mL)	1. BL – Week 2	Rifaximin	43	0.00 (-0.26, 0.21)	(-0.14, 0.08)	0.14
		No Study Treatment	22	0.04 (-0.09, 0.32)	(-0.04, 0.27)	
	2. BL – Week 4	Rifaximin	43	-0.08 (-0.29, 0.15)	(-0.20, 0.03)	0.72
		No Study Treatment	22	-0.09 (-0.21, 0.16)	(-0.22, 0.12)	
	3. BL – Week 8	Rifaximin	42	-0.04 (-0.26, 0.13)	(-0.18, 0.03)	**0.046**
		No Study Treatment	21	0.09 (-0.02, 0.28)	(-0.06, 0.31)	
sTNFR-II (log_{10} pg/mL)	1. BL – Week 2	Rifaximin	42	-0.03 (-0.11, 0.03)	(-0.08, 0.01)	**0.043**
		No Study Treatment	22	0.04 (-0.06, 0.17)	(-0.03, 0.13)	
	2. BL – Week 4	Rifaximin	42	-0.04 (-0.17, 0.09)	(-0.09, 0.02)	0.08
		No Study Treatment	22	0.07 (-0.05, 0.13)	(-0.06, 0.11)	
	3. BL – Week 8	Rifaximin	42	0.04 (-0.07, 0.12)	(-0.02, 0.08)	0.35
		No Study Treatment	21	-0.01 (-0.15, 0.10)	(-0.11, 0.06)	

OTHER RESULTS

- Significant difference was seen in the change in %Ki67⁺CD8⁺ T-cell from baseline to week 4 (p=0.013): median (Q1, Q3) change for the rifaximin arm: -0.12% (-0.27%, 0.12%) vs no-study-treatment arm: 0.12% (-0.07%, 0.27%).
  - No significant differences in the change from baseline to weeks 2/8.
- No significant differences were seen between the arms in change in CD4 T-cell count, CD4 T-cell activation and turn-over, D-dimer, and sCD163.

Table 4: Spearman correlations between baseline biomarker levels & between changes from baseline to week 4 in biomarkers.

Biomarker	LPS			sCD14		
	N	r	P-value	N	r	P-value
Correlation between biomarkers at baseline						
%HLA-DR⁺CD38⁺ of CD8⁺	65	-0.03	0.796	65	-0.10	0.41
IL-6	65	0.21	0.097	65	0.32	**0.009**
LPS	.	.		65	0.19	0.13
CRP	65	0.16	0.193	65	0.26	**0.036**
sCD14	65	0.19	0.129	.	.	
D-dimer	65	0.34	**0.006**	65	0.32	**0.009**
sTNFrII	65	0.02	0.864	65	0.10	0.44
Correlation between changes in biomarkers from baseline to week 4						
%HLA-DR⁺CD38⁺ of CD8⁺	43	0.12	0.460	43	-0.01	0.95
IL-6	43	-0.20	0.202	43	0.32	**0.038**
LPS	.	.		43	-0.01	0.96
CRP	43	-0.29	0.057	43	0.30	**0.054**
sCD14	43	-0.01	0.956	.	.	
D-dimer	43	-0.05	0.758	43	0.23	0.14
sTNFrII	42	0.02	0.897	42	-0.11	0.47

CONCLUSIONS

- While rifaximin for 4 weeks was well-tolerated, it only led to a transient decline in gut MT & had a marginal impact on immune activation in immune non-responders to virally suppressive ART.
- Rifaximin was associated with a significant but minimal post-treatment decrease in systemic inflammation (IL-6 & CRP). Whether longer use of rifaximin will impact systemic inflammation is unknown, and may be worth exploring.
- The absence of correlations between baseline T-cell activation, inflammation & MT levels, & between rifaximin-mediated changes in T-cell activation & MT suggest that while MT & T-cell activation are high in immune non-responders, these processes may only be weakly or indirectly linked mechanistically.
- Changes in sCD14 were more closely linked to changes in systemic inflammatory markers (IL-6) that are associated w/ morbidity in HIV⁺ persons on ART.

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