Effects of Dolutegravir and Efavirenz-Based Antiretroviral Therapy on the Vaginal Microbiota in Pregnancy

IMPERIAL · Unitaid SAVE LIVES FASTER · DolPHIN2 · 807

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1. BACKGROUND

- Low vaginal bacterial diversity in pregnancy is associated with term birth and considered immune protective
- Transition to high diversity CST IV (bacterial vaginosis), which lacks protective *lactobacilli* species, is less stable and linked with inflammation driven preterm birth (PTB)
- We hypothesised that initiation of antiretroviral therapy (ART) impacts CST stability, local inflammation and differs by regimen

2. METHODS

- Population: South African pregnant women enrolled in the DolPHIN-2 RCT
- Groups: Efavirenz/XTC/TDF (EFV) n =17; Dolutegravir/XTC/TDF (DTG) n = 18
- ART Initiation: ~29 weeks gestation; 4 weeks post ART follow-up sampling ~33 weeks
- Samples: Cervicovaginal fluid (CVF)
- Bacterial composition: (alpha (α) diversity and CSTs by VALENCIA) was determined by PacBio near full-length 16S rRNA gene sequencing
- Cytokines: MSD CVF immunoassay (pre-ART timepoint)
- Correlations and models evaluated group-level and overall effects of ART, days between visits, $\log_{10}$ cytokines, CD4 count, and HIV load on Chao1 diversity change, CST IV transition, and gestational age (GA) at delivery, adjusting for maternal age and GA in R

3. RESULTS

1. Demographics and immune status

Cohort Demographics by Efavirenz (EFV) or Dolutegravir (DTG) Antiretroviral Therapy (ART)

Median (Q1, Q3) or n (%) shown | Group comparisons by Wilcoxon rank-sum or Fisher's exact tests

	DTG N = 18	EFV N = 17	Overall N = 35	P-value
Maternal Characteristics				
Age	26.5 (24.0, 32.0)	28.0 (25.0, 32.0)	28.0 (24.0, 32.0)	0.38
Gestational age (weeks) at visit 1 (Pre ART)[1]	29.0 (27.0, 32.0)	29.0 (28.0, 32.0)	29.0 (27.0, 32.0)	0.62
Gestational age at visit 2 (Post ART)	32.0 (30.0, 35.0)	33.0 (31.0, 35.0)	33.0 (31.0, 35.0)	0.41
Body mass index (BMI) at visit 1	30.7 (27.4, 36.1)	33.7 (26.4, 36.8)	33.1 (26.4, 36.8)	0.44
Tobacco use during pregnancy	4 (22%)	2 (12%)	6 (17%)	0.66
Alcohol use during pregnancy	5 (28%)	3 (18%)	8 (23%)	0.69
Immunological Characteristics				
CD4 cell count (cells/mm³) at visit 1	521.5 (440.0, 735.0)	362.0 (355.0, 554.0)	497.0 (355.0, 647.0)	0.12
HIV viral load (copies/mL) at visit 1	9,549.5 (1,555.0, 31,145.0)	33,622.0 (15,797.0, 94,362.0)	19,000.0 (2,738.0, 55,624.0)	0.08
HIV viral load (copies/mL) at visit 2	20.0 (20.0, 61.0)	268.0 (38.0, 627.0)	48.0 (20.0, 279.0)	<0.001
Detectable HIV (>50 copies/mL) prior to delivery	2 (11%)	12 (71%)	14 (40%)	<0.001
Obstetric History / Outcomes				
Gestational age at delivery	40.2 (39.0, 40.9)	39.7 (38.1, 41.1)	40.0 (38.7, 41.0)	0.83
Term birth (>37 weeks gestational age at delivery)[2]	18 (100%)	16 (94%)	34 (97%)	0.49
Neonatal weight at birth (grams)[3]	3,085.0 (2,720.0, 3,350.0)	3,330.0 (3,106.0, 3,630.0)	3,300.0 (2,920.0, 3,440.0)	0.16
Neonatal vital status				1.00
Alive	17 (94%)	17 (100%)	34 (97%)	
No record	1 (5.6%)	0 (0%)	1 (2.9%)	

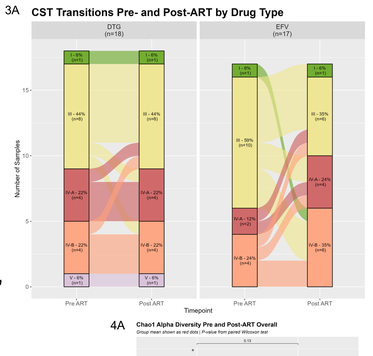
[1] One (n=1) EFV subject started ART 13 days prior to first visit
[2] One preterm birth (n=1) occurred in EFV group (emergency C-section after pathological cardiotocography at 36.4 weeks)
[3] Birth weight data were not recorded for four (n=4) DTG participants

Table 1 Demographics Pre- and Post-ART (visit 1 vs 2) by Drug - Women initiating EFV had significantly higher HIV viral loads after ART and prior to delivery, and trended toward higher viral loads pre-ART

- High prevalence of non-optimal CSTs at baseline (40% CST-IV)
- Alpha diversity decreased in 3rd trimester
- EFV arm showed stronger signal of CST instability driven by an inflammatory interaction (IL-1β × EFV OR≈32)
- DTG arm demonstrated more stable microbial profiles
- No association of microbiota and ART with gestational age at delivery

2. Microbiota composition

- Baseline composition (Fig 1A) and α diversity (Fig 4B) did not differ by ART.
- 40% of women were in CST IV, 51% CST III, 6% CST I and 3% CST V (Fig 1B). There were more transitions in the EFV arm (Fig 3A/B) than DTG arm
- Median alpha diversity decreased in both arms, reaching significance with EFV (p = 0.03), while DTG showed smaller changes (Fig 4B)



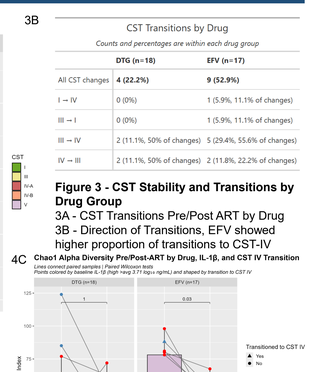


Figure 3 - CST Stability and Transitions by Drug Group
3A - CST Transitions Pre/Post ART by Drug
3B - Direction of Transitions, EFV showed higher proportion of transitions to CST-IV

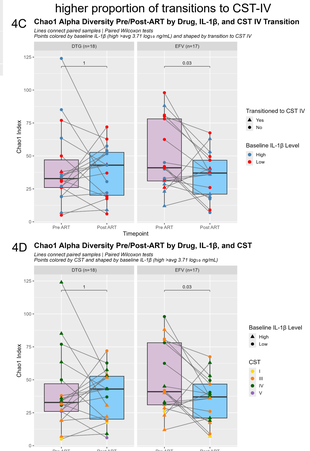


Figure 4 - Alpha Diversity pre and post ART by Drug Group
4A - Overall Chao1 alpha diversity; 4B - Chao1 change pre/post-ART by Drug. Chao1 change with EFV significance (p = 0.03); 4C - Chao1 by Drug highlighted by baseline IL-1β and transition to CST IV; 4D - Chao1 by Drug highlighted by baseline IL-1β and CST at each timepoint

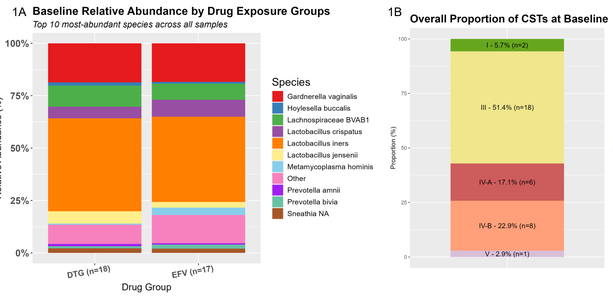


Figure 1 – Baseline Relative Abundance by Drug Group and Baseline CSTs Overall
1A - Top 10 species Pre-ART by Drug; Highlights dominance of *Gardnerella vaginalis*, *L. crispatus*, *L. iners*, BVAB1
1B - CSTs overall at baseline: CST I: 5.7%; CST III: 51.4%; CST IV-A: 17.1%; CST IV-B: 22.9%; CST V: 2.9%

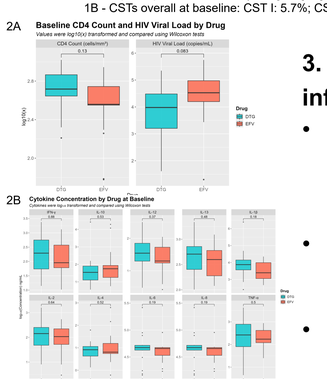


Figure 2 - Immunologic Parameters
2A - CD4/HIV viral load by Drug; 2B – CVF Cytokines by Drug; No significant differences at baseline

3. Modelling the impact of ART, time on ART and vaginal inflammation on bacterial diversity

- Group-level analyses identified that IL-1β was linked to increased α diversity in EFV (model trend p = 0.10; Spearman ρ = 0.53, p = 0.03) (Fig 5A)
- Diversity change in DTG was associated with days between visits (model p = 0.02; ρ = −0.47, p = 0.05) (Fig 5B)
- Models containing both groups identified a significant interaction effect of ART×days between visits on α diversity, as more days was associated with decreased diversity with DTG, but not EFV (p=0.02) (Fig 6B)

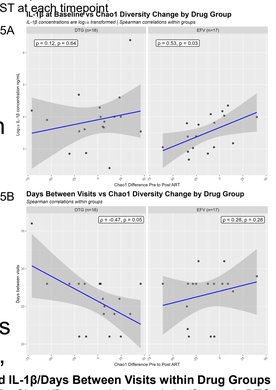


Figure 5 - Correlations between Chao1 and IL-1β/Days Between Visits within Drug Groups
5A - Chao1 Change/IL-1β was significant in EFV, not DTG; 5B - Chao1/Days trended toward significance in DTG

4. Modelling the impact of ART and inflammation on CST change

- Women on EFV trended toward higher odds of transitioning to CST IV (p=0.04), there was a significant ART×IL-1β interaction, as higher IL-1β increased the likelihood of CST IV transition with EFV (OR≈32, 95% CI: 1.2-2476, p = 0.04), but not DTG (Fig 6C)
- No associations were observed between α diversity change, CST IV shifts, IL-1β and GA at delivery

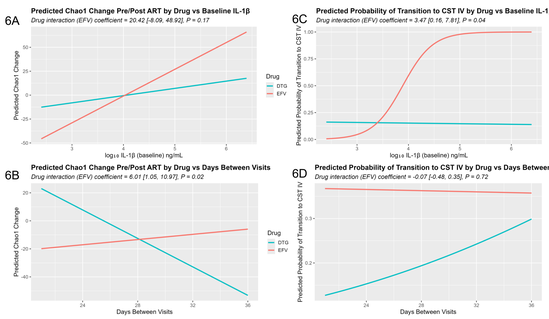


Figure 6 - Prediction Plots of Chao1 Change and CST IV Transition by Drug Group
6A - Chao1/IL-1β; 6B - Chao1/Days between visits, significant interaction (DTG); 6C - CST IV Transition/IL-1β, significant interaction (EFV); 6D - CST IV Transition/Days

4. CONCLUSIONS

- High prevalence of non-optimal CSTs at baseline in late pregnancy
- Alpha diversity declines in the 3rd trimester, especially with EFV
- EFV shows inflammation-linked CST instability
- DTG microbiota changes appear more time-dependent and stable
- No associations between microbiota changes and gestational age at delivery
- Larger studies needed to confirm ART-linked microbiota mechanisms and PTB risk

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PLAIN ENGLISH SUMMARY: We studied how two HIV treatments started late in pregnancy affect vaginal bacteria. The diversity of vaginal bacteria reduced overall in late pregnancy, but in the Efavirenz arm there was greater instability, especially in women with higher inflammation. In this small study, no significant changes in microbiota were seen that can be solely attributed to the treatment. No differences in preterm birth between treatments were seen in this DoLPHIN2 trial sub study