Virologic and Immunologic Responses to Raltegravir and Dolutegravir in GALT of HIV+ Men and Women

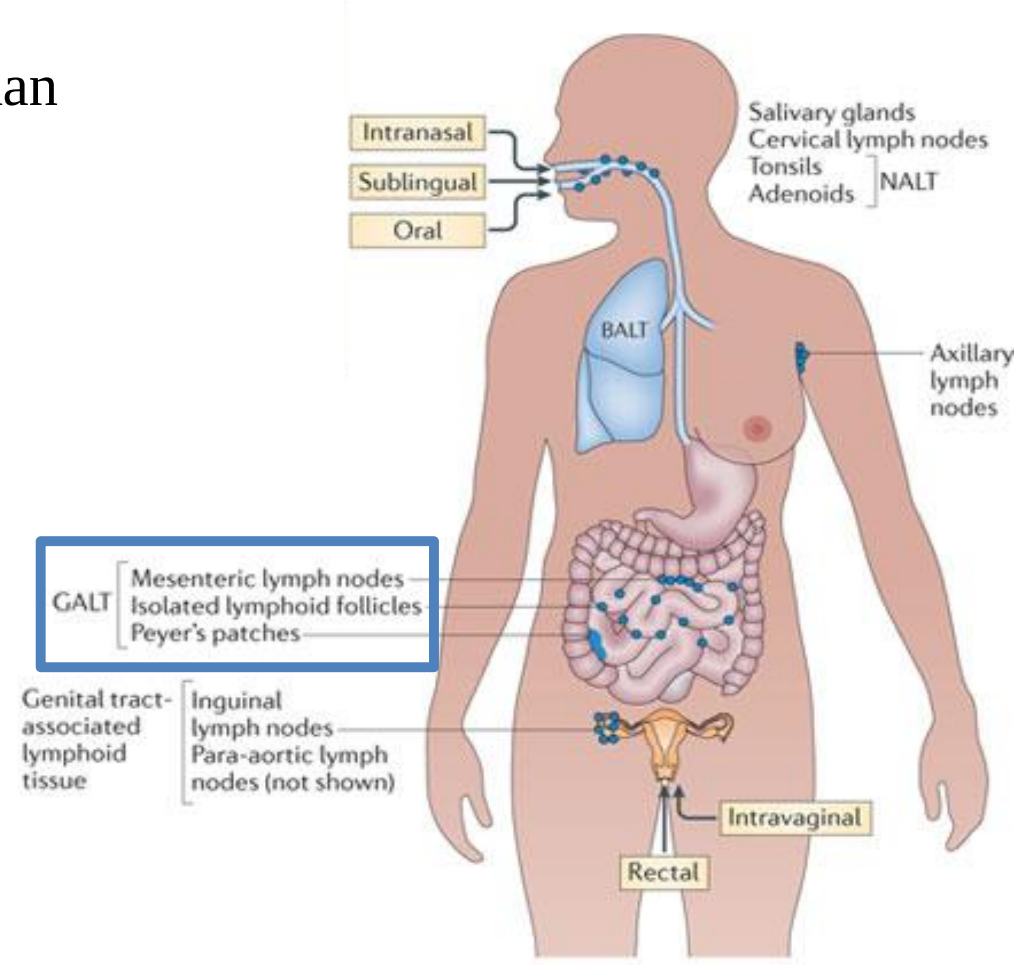
Michael D Weber, Elizabeth Andrews, Heather A Prince, Craig Sykes, Nicholas J Shaheen, Ryan D Madanick, Evan Dellon, Kristina De Paris, Julie AE Nelson, Cynthia Gay, and Angela DM Kashuba

Mike Weber, PharmD
Clinical Research and Drug Development Fellow
University of North Carolina
June 9th, 2016

Gut-Associated Lymphoid Tissue (GALT)

- Largest component of the human lymphoid system
  - High number of CD4+T cells
  - Highest levels of HIV DNA

- Cross infection between blood and GALT potential cause of intermittent viremia



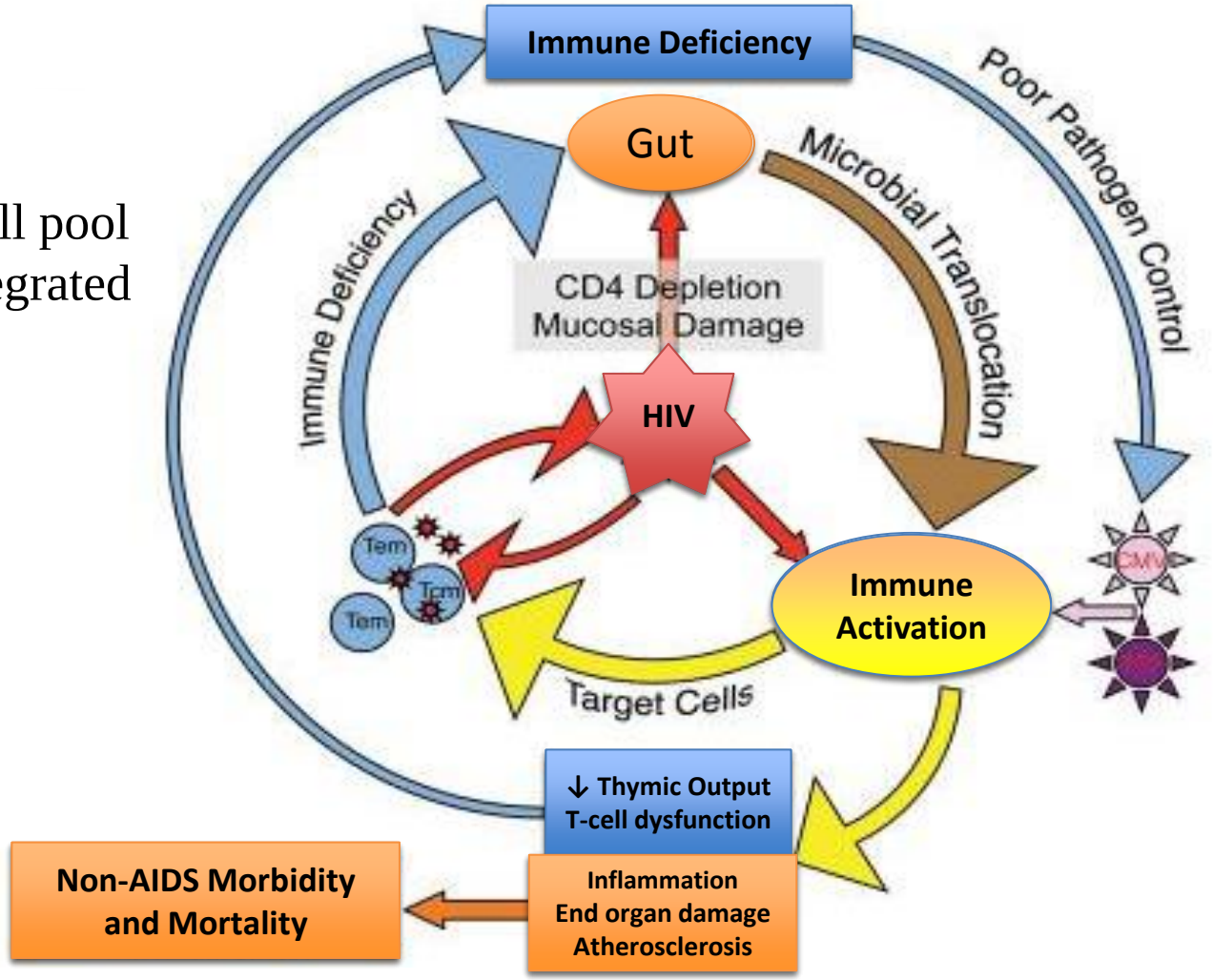
THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Nature Reviews | Immunology

Kulpa D, Chomont N. J Virus Erad. 2015; 1(2):59-66
Yukl et al. *J Infect Dis.* 2010. 202(10): 1553–1561
Yukl et al. *JID* 2008: 197.

HIV Persistence & cART

- Persistence of a small pool of cells carrying integrated HIV DNA can be reactivated
  - "Reservoir"



http://www.cdc.gov/hiv/statistics/basics/ataglance.html
Klatt NR, et al. Immunol Rev 2013;254(1):326-42
Kulpa D, Chomont N. J Virus Erad. 2015; 1(2):59-66

Current Treatment Guidelines

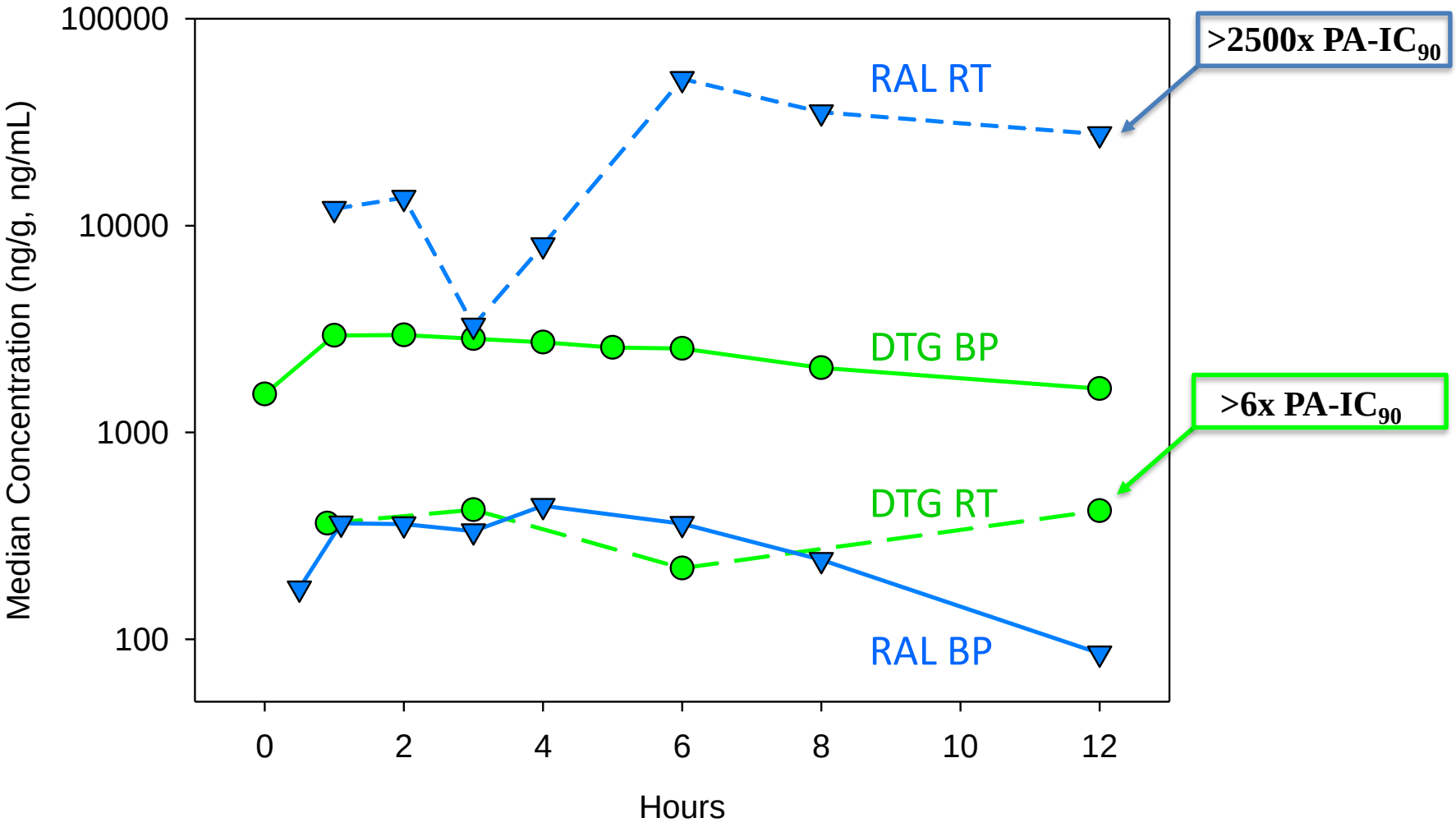
- Integrase Inhibitor Regimens
  - **Dolutegravir** 50mg once daily plus tenofovir/emtricitabine (300/200mg) once daily (Truvada®)
  - **Raltegravir** 400mg BID plus tenofovir/emtricitabine (300/200mg) once daily (Truvada®)
    - Both AI recommendations

- Are there any tissue pharmacokinetic or pharmacodynamic differences?

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents.

Previous RAL and DTG Plasma/Tissue (Median) Concentrations



*Tissue Density: 1ng/mL

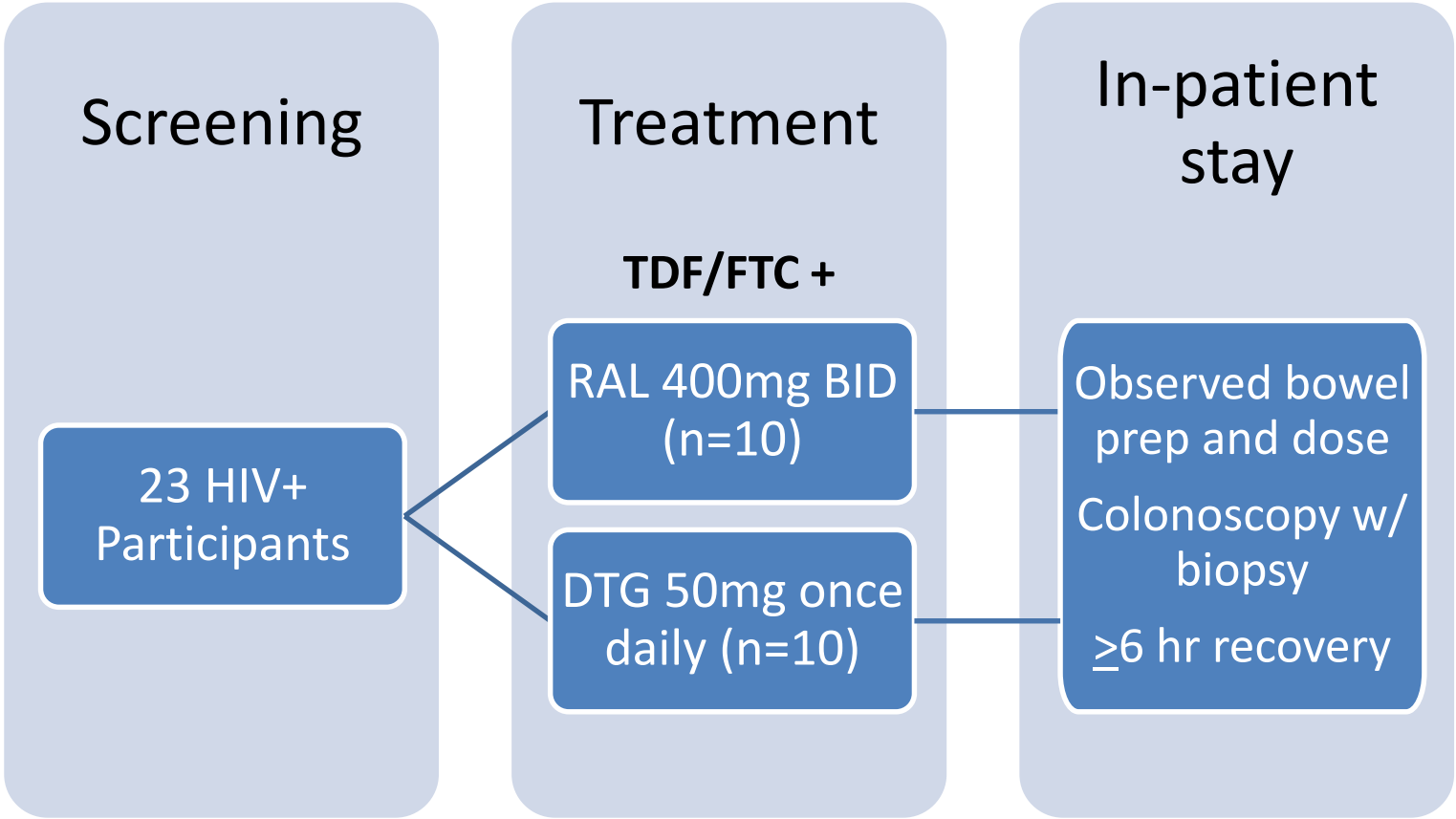
Patterson et al, *AIDS*. 2013; 27(9): 1413-1419
Greener et al., *J Acquir Immune Defic Synd*. 2013; 64(1): 39-44

Study Hypothesis

- Higher RAL exposure in GALT leads to better immunologic and virologic control compared to DTG

Study Design

- Phase IV, open label study



Methods

- Participants underwent standard bowel preparation
  - Low fiber and clear liquid diets
  - Polyethylene glycol
  - Bisacodyl
- Observed medication dose 2-6 hours pre-procedure
- GALT samples (18 biopsies) obtained via colonoscopy using RJ4 forceps

Tissue	Virology	Immunology	Pharmacology
Terminal ileum	✓	✓	
Splenic flexure	✓	✓	
Rectum	✓		✓

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Measures of Interest

Variable	Meaning
CD3+	T-lymphocyte
CD4+	Helper T-cells
CD8+	Cytotoxic T-cells
Gamma delta TCR	Antigen homing and recognition
CCR5+	Marker of activation
CD69+	Marker of activation
CD38+HLA-DR+	Marker of activation
PD1+	Marker of exhaustion
T(N)	Naïve T-cells
T(CM)	Central memory T-cells
T(EM)	Effector memory T-cells
T(Eff)	Effector T-cells
HIV RNA	Marker of replicating virus
HIV DNA	Marker of latent virus

- Markers of Activation
  - Increased activation is correlated with a slower immune reconstitution
- Marker of Exhaustion
  - Increased PD1+ is correlated with limited immune reconstitution

Grabmeier-Pfistershammer K, et al. *JAIDS*. 2011; 56(2):118-24
Hunt PW et al. *J Infect Dis.* 2003; 187(10):1534-43
Yukl S, et al. *J Infect Dis*. 2010; 202(10): 1553–1561.

Sample Analysis

- HIV RNA and DNA
  - Quantified using Droplet Digital PCR
  - LLQ of 1 copy/mg of tissue
  - Cell count determined using CCR5 DNA copies

- Flow Cytometric Analysis
  - Analyzed using BD LSR II with FlowJo Software applying Boolean gating strategies
  - Tissues were combined if $<0.7x10^6$ cells/biopsy

- RAL and DTG concentrations were confirmed in GALT by validated LC-MS/MS in biopsy homogenates
  - LLQ of RAL and DTG was 5ng/mL and 1 ng/mL, respectively

Data Analysis

- Endpoint analysis
  - Each measure was analyzed using an exact Mann-Whitney test with a 2-sided α of 0.05
  - Intrapatient tissue site analysis was done using a paired t-test
  - No adjustments were done for multiple comparisons
  - Any data below the limit of quantitation (BLQ) was imputed at the lower limit of quantitation (LLQ)

- Sample Size
  - n=10 per group needed to achieve 83% power to detect a 1 $\log_{10}$ difference in tissue HIV RNA

- All analysis was done using SAS® Software version 9.4

Demographics Median (Range)

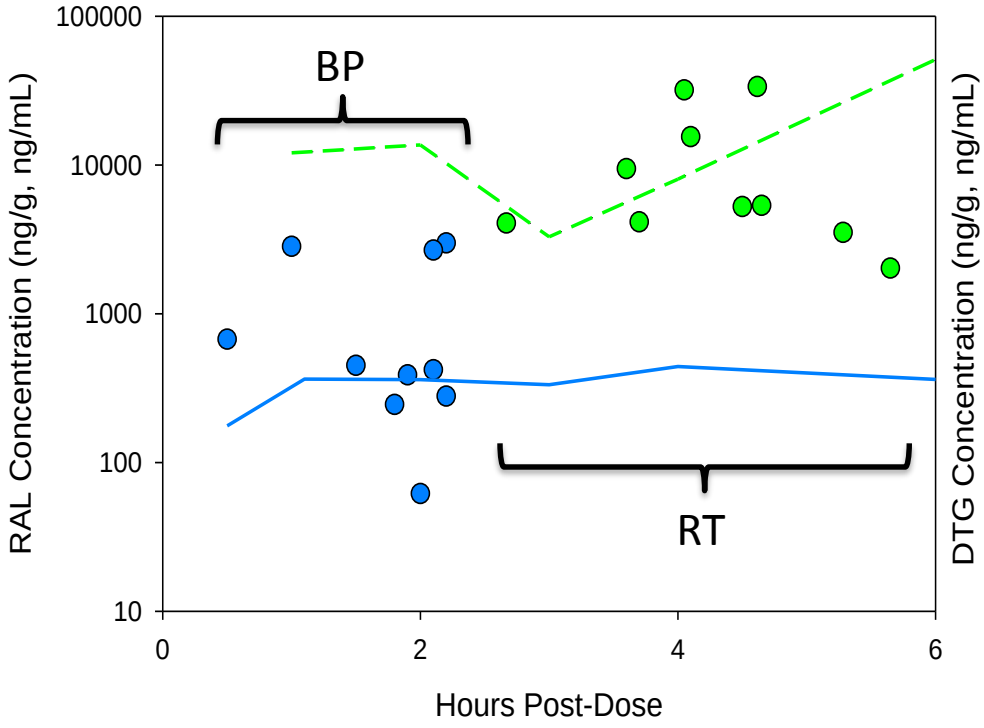
- Enrolled from December 2014 - October 2015
  - All enrolled participants completed the study
- One DTG participant on regimen of 50mg BID

	RAL	DTG
Age*	54.5 (48-64)	49.5 (32-56)
Male	9	6
BMI	30.7 (18.7-41.4)	28.9 (21-35.6)
Diagnosis Length	9.5 years (4-22)	17 years (1-24)
Time on study drug*	5.3 years (2.3-6.7)	1.0 years (0.25-1.5)
Current CD4+	811 (594-956)	620 (223-1300)
CD4+ nadir	356 cells/mm3 (9-476)	74 cells/mm3 (2-458)

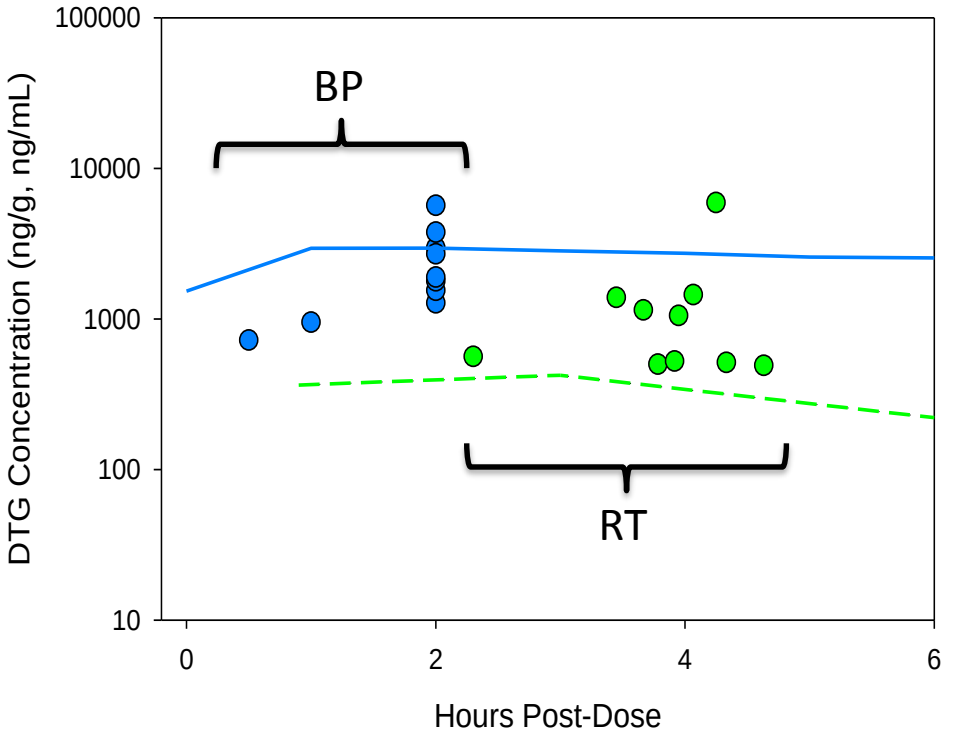
*P-value <0.05

Pharmacology Results

Raltegravir



Dolutegravir



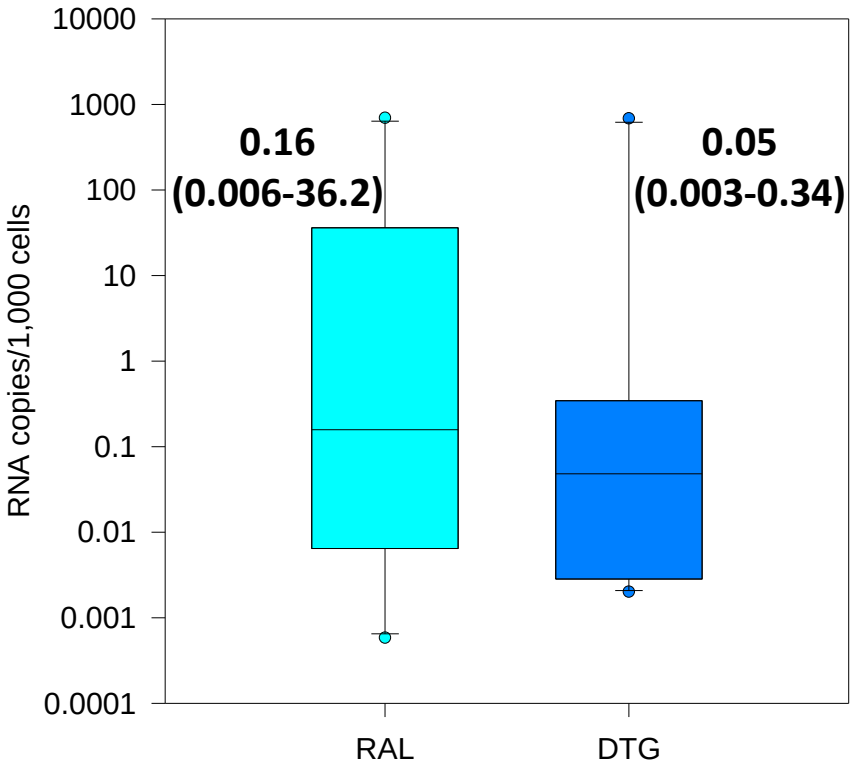
	RAL	DTG
Tissue Concentration	5,308ng/g (3,938-19,600)	810ng/g (510-1,408)
Tissue:plasma	11.3 (7.7-25.5)	0.44 (0.29-0.65)

Median (IQR)

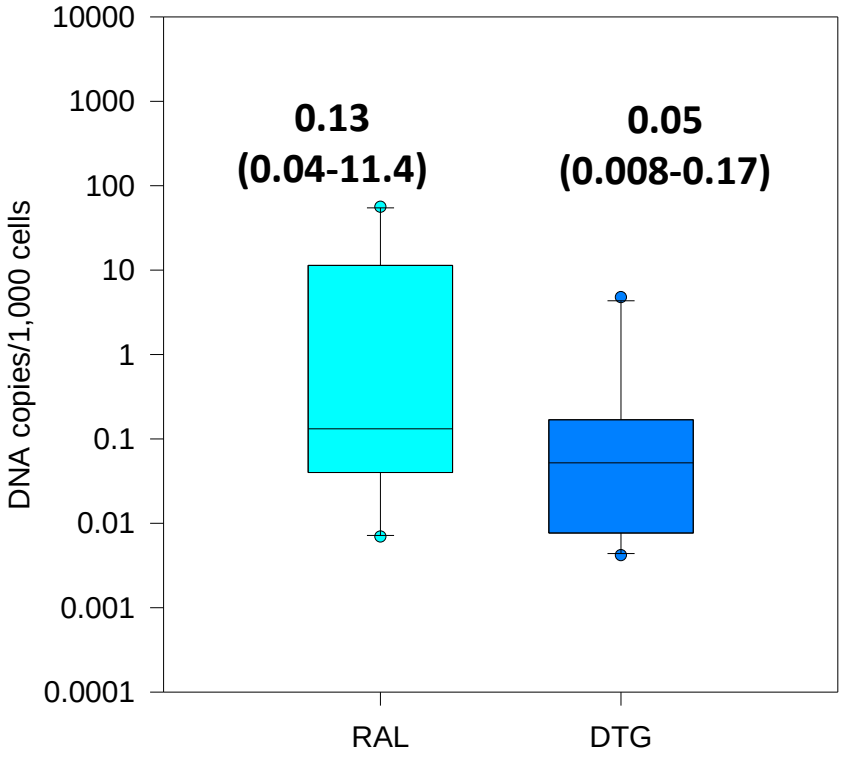
Virology Results

- All tissue sites combined for analysis

RNA per 1,000 cells (p=0.47)



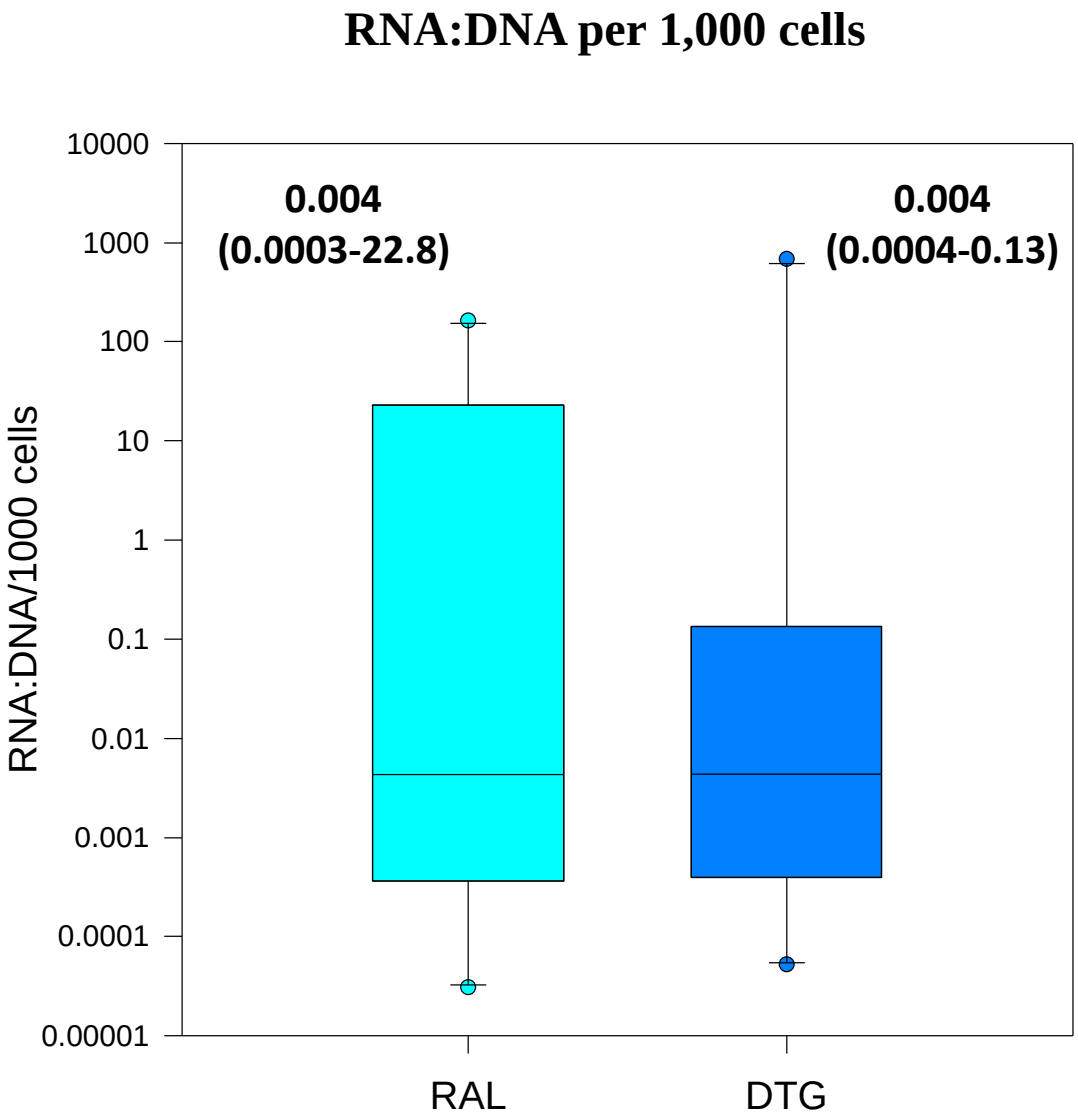
DNA per 1,000 cells (p=0.14)



	RAL	DTG
RNA undetectable	20%	40%
DNA undetectable	10%	40%

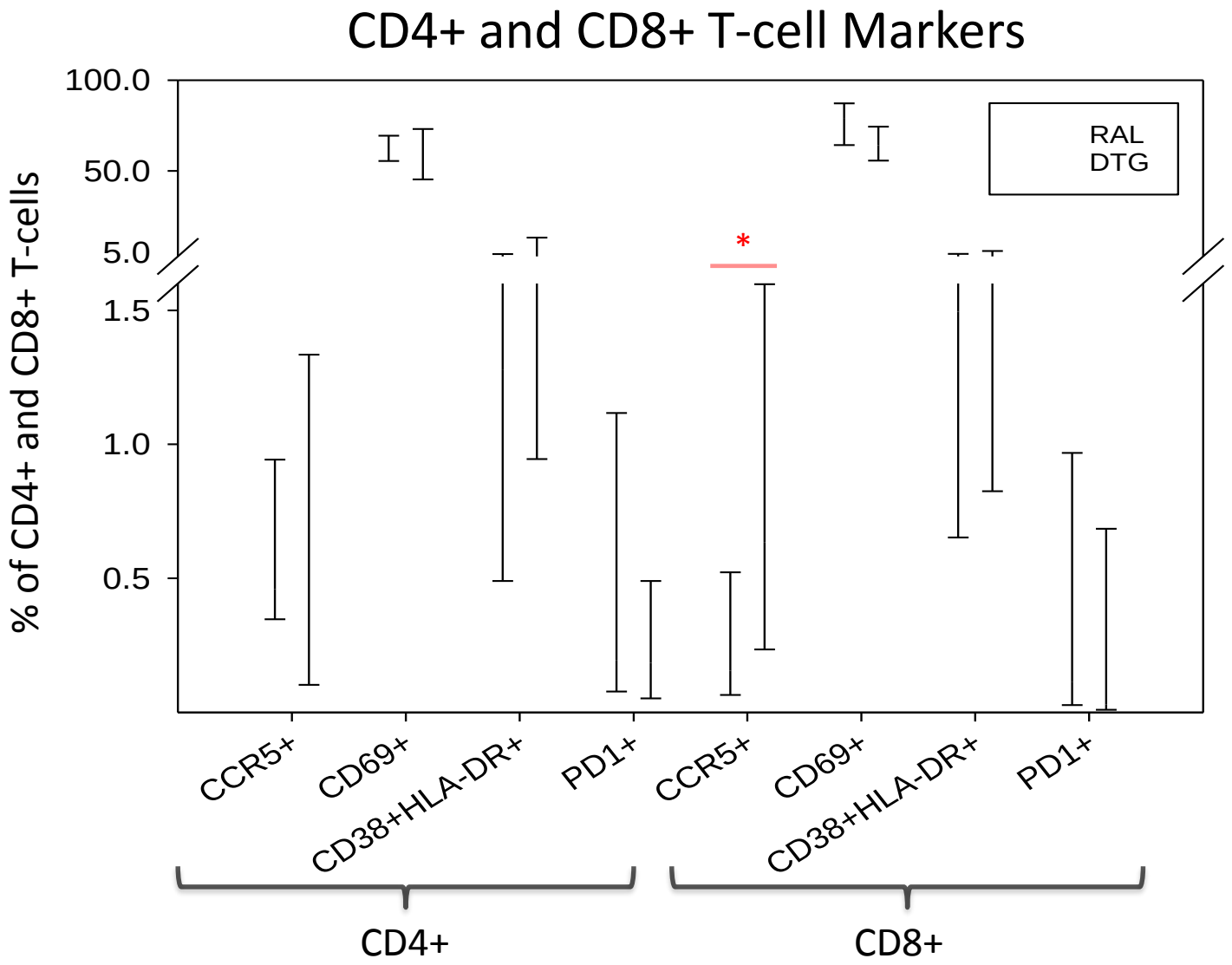
Median (IQR)

Virology Results



Median (IQR)

Immunology Results



*p-value<0.05
Median (IQR)

Limitations

- Pooling of tissues for DNA and RNA analysis limited potential site specific findings
- Nine participants had site specific immunology analysis
  - 11 had combined analysis
  - No consistent statistical difference found between sites
- Statistical analysis did not adjust for multiple comparisons
- Tissue homogenates do not show tissue localization
  - IR-MALDESI imaging currently ongoing

Conclusions

- RAL produced higher tissue exposures than DTG consistent with previous studies
- No significant differences in tissue HIV RNA, DNA, or most immunological markers were observed
- Observed higher tissue exposure of RAL did not correlate with superior immunologic or virologic control compared to DTG

Acknowledgements

- Kashuba Lab:
  - Dr. Angela DM Kashuba
  - Heather Prince
  - Craig Sykes
  - Dr. Eli Rosen
- UNC Center for AIDS Research
  - Virology, Immunology & Microbiology Core
    - Dr. Kristina De Paris
    - Dr. Julie Nelson
- UNC Department of Medicine
  - Dr. Nicholas Shaheen
  - Dr. Ryan Madanick
  - Dr. Evan Dellon
  - Dr. Cynthia Gay
- Clinical Trials Research Center Staff
- Funding by Merck & Co., Inc.
- United Therapeutics Corporation
  - Dr. Kevin Laliberte
- Study participants

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL