# The clinical research path towards HIV Cure: Focus on the DIORR trial

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## Conflict of interest

- JM's institution receives funding for research from Gilead, Merck, Viiv, Amgen and Shire
- The DIORR study was funded by an unrestricted investigator-initiated study grant from Viiv

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## Overview

- Barriers to HIV cure
- Debate around residual viral replication
- Clinical trials to answer these questions: DIORR trial

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## Barriers to cure

- HIV Latency
- Intact versus defective virus
- Tissue reservoirs
- Clonal expansion of latently infected cells
- Residual viral replication

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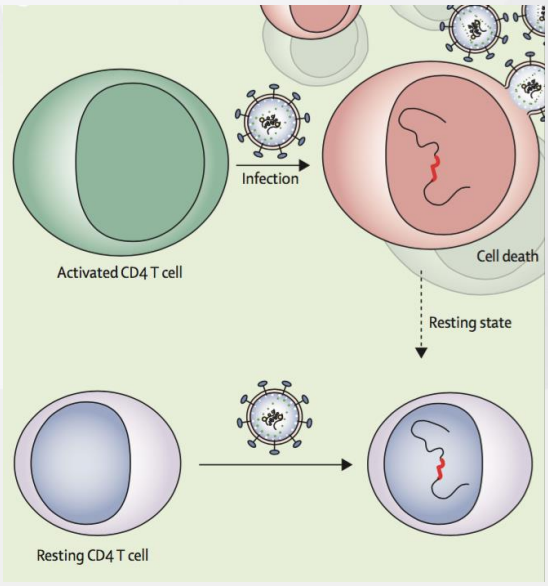
# HIV latency

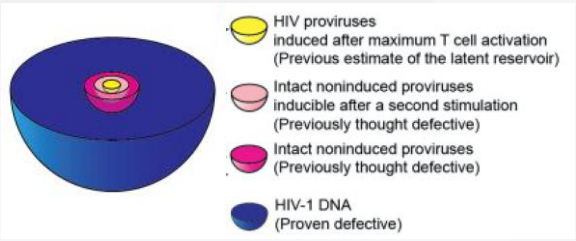
Latent infection = integration of HIV DNA into host genome with virus production

Established by:
- Survival of an activated infected T cell, which reverts to a memory state, or,
- After direct infection of a resting CD4 T cell




Maartens, Lancet, 2014

# Intact versus defective virus




INTACT ~ 60 / million resting CD4+ T-cells

DEFECTIVE > 80%, Contains deletions, mutations

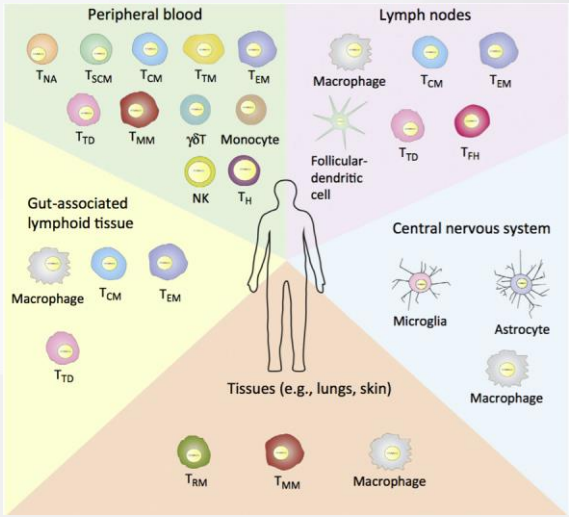
Frequencies of infected resting CD4+ T cells
- Yellow circles = size of the latent reservoir measured by viral outgrowth assay
- Magenta = frequency of cells with intact proviruses. Potential reservoir size if intact noninduced proviruses can be induced in vivo
- Blue = cells with HIV DNA

Ho, Cell, 2013

# Tissue reservoirs

Main sites of latent HIV infection:

- CD4+ memory T cell subsets found in peripheral blood
- Lymphoid tissue
- Gut-associated lymphoid tissue
- Central nervous system




Barton, Trends in Microbiology, 2016

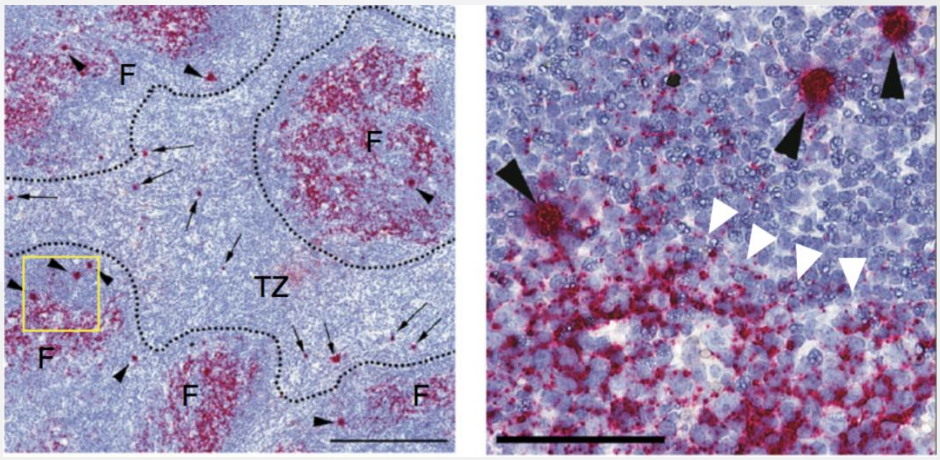
# B cell follicles – a sanctuary for HIV

Red = SIV RNA

Black arrows = RNA+ lymphoid cells outside follicles

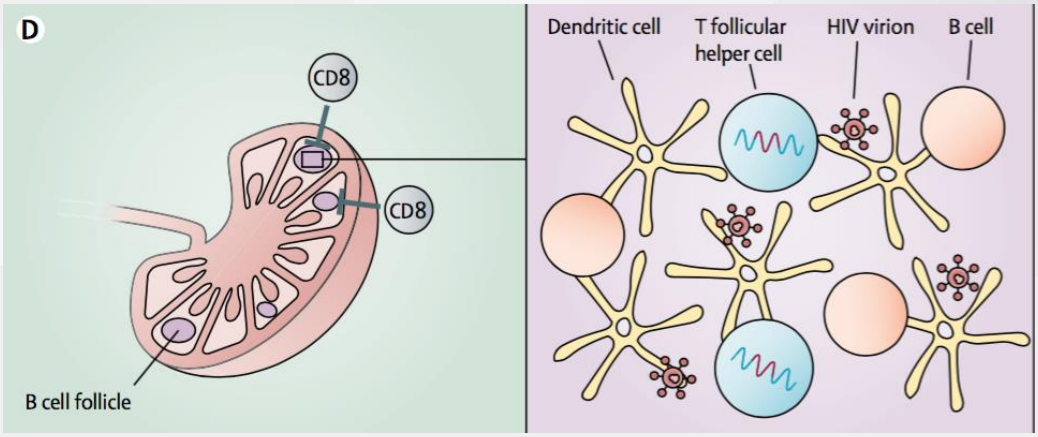
Black arrowheads = RNA+ lymphoid cells in B cell follicles

White arrowheads = extracellular follicular dendritic cell–bound virus within follicles




Fukazawa, Nat Med, 2015

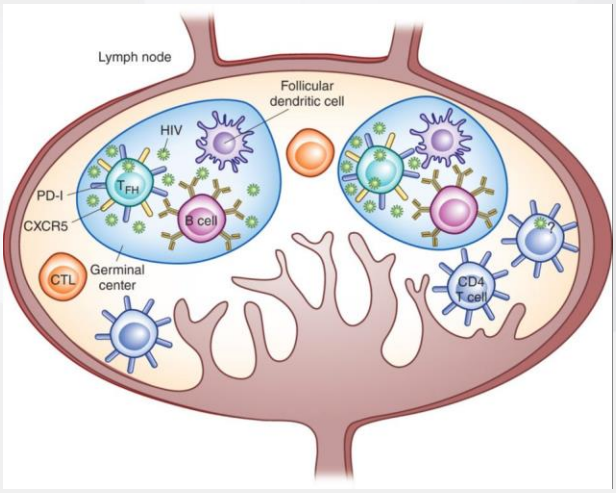
# B cell follicles




Pitman, Lancet HIV, 2018

# T follicular helper cells

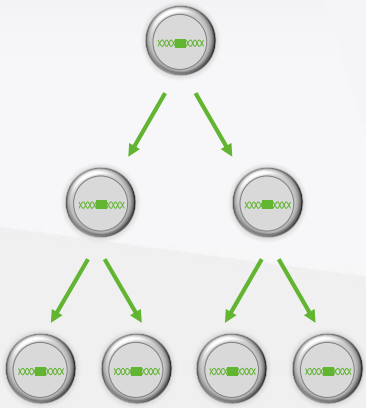
Follicular T helper cells in lymph node germinal centers are enriched for HIV during ART




Paiardini, Nat Med, 2016; Banga, Nat Med, 2016

# Clonal expansion versus residual replication

**T cell proliferation of latently infected cells**



**Residual viral replication (productive infection)**

Finzi Science 1997; Wong Science 1997; Chun PNAS 1997; Palmer PNAS 2008; Chomont Nat Med 2009; Fletcher PNAS 2014, Maldarelli Science 2014

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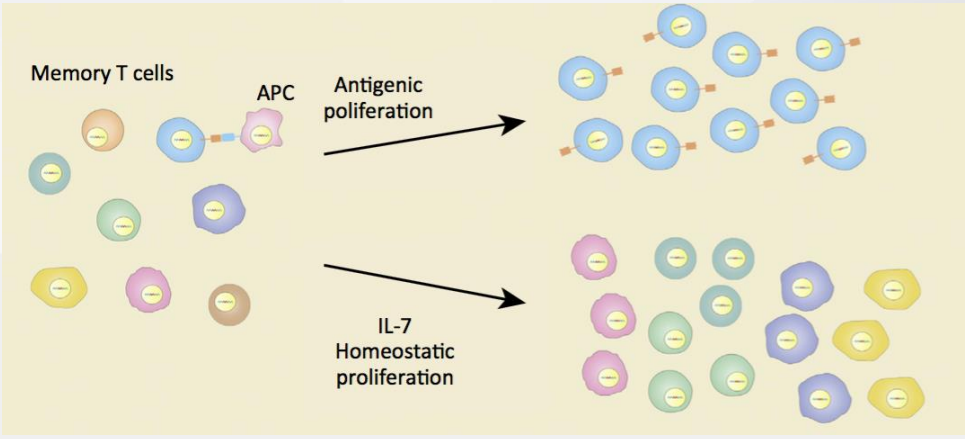
# Drivers of proliferation ➔ antigen / homeostatic / oncogene

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Barton, Trends in Microbiology, 2016

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## Evolution over time in viral sequence

- No evolution → proliferation
  - Lack of viral evolution over many years in multiple donors[1]
  - Evidence of highly expanded clones across multiple sites in the body that can produce infectious HIV[2]

- Evolution[3] → ongoing replication
  - Viral evolution over first 6 months after starting ART
  - Included modelling that ARV levels low enough in sanctuary sites to prevent the development of drug resistance

1 Kearney, PLoS Pathog, 2014; 2 Malderelli, Science, 2014; Simonetti, PNAS, 2016; 3 Lorenzo-Redondo, Nature, 2016

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## Ongoing HIV Replication During ART Reconsidered

Mary F. Kearney,[1] Ann Wiegand,[1] Wei Shao,[2] William R. McManus,[1] Michael J. Bale,[1] Brian Luke,[2] Frank Maldarelli,[1] John W. Mellors,[3] and John M. Coffin[4]

[1]HIV Dynamics and Replication Program, National Cancer Institute, Frederick, Maryland; [2]Leidos Biomedical Research, Inc. Frederick National Laboratories for Cancer Research, Frederick, Maryland; [3]Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania; [4]Department of Molecular Biology and Microbiology, Tufts University, Boston, Massachusetts

- Re-analysed Lorenzo-redondo data using own methods and analysed their own data using Lorenzo-redondo methods

- "..inappropriate time points to address the question of ongoing replication"
- "Unlike SGS, developed to eliminate errors inherent in bulk PCR amplification, the method of Lorenzo-Redondo restores these errors"
- "extremely limited sampling of the virus populations in the 3 individuals studied is a major flaw … and likely contributed to erroneous conclusions."

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Kearney, Open Forum Inf Dis, 2017

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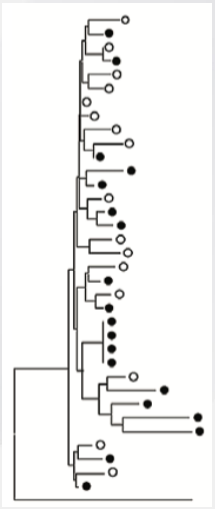
# Major Criticism

- Method to determine change in viral sequence over time flawed
  - Lorenzo-Redondo – rooted on sequence of most prevalent RNA sequence present pre-ART
    - "biases the analysis of viral DNA sequences at later time points in favor of showing the appearance of evolution"
  - Kearney – rooted on consensus pre-ART DNA sequence → assumes there is a common ancestor for all sequences from the time of infection



Example of PBMC DNA samples from one person at baseline (open circles) and > 7 years later (filled circles)

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Kearney, Open Forum Inf Dis, 2017

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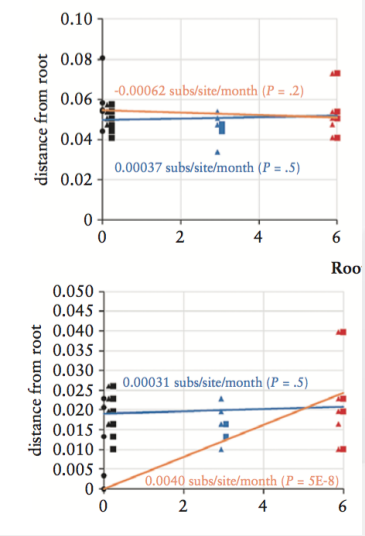
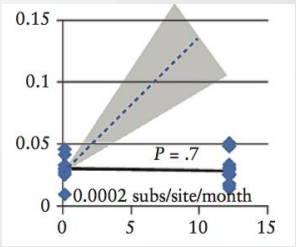
Focus on orange lines →

Bottom panel shows evolution of sequence diversity when comparing to the most common RNA sequence pre-ART

Top panel shows no evolution when comparing to consensus pre-ART sequence




Sequence evolution in someone from NIH on > 10 years ART

Dashed line = evolution using Lorenzo-Redondo method

Solid line = evolution using consensus sequence

"the conclusions in the publication by Lorenzo-Redondo et al. are not valid and that the concept of ongoing HIV replication during ART remains unproven"

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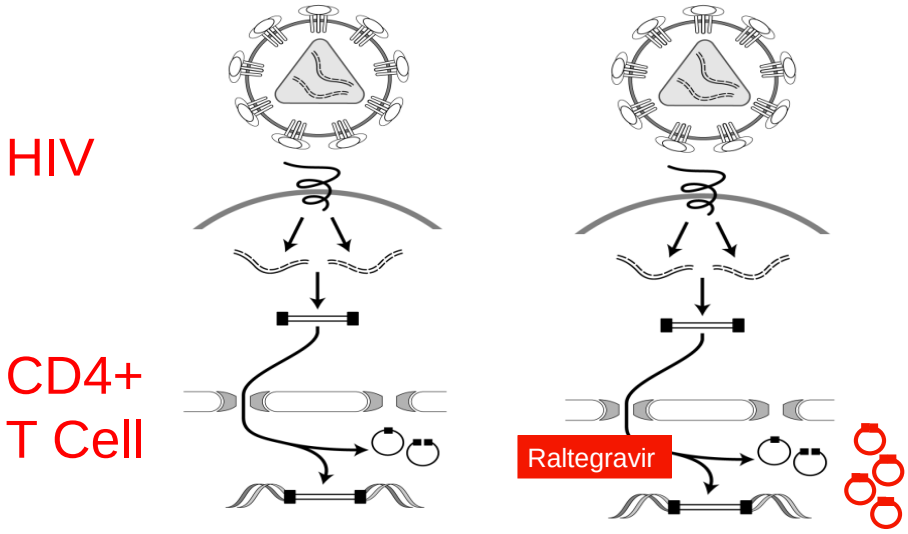
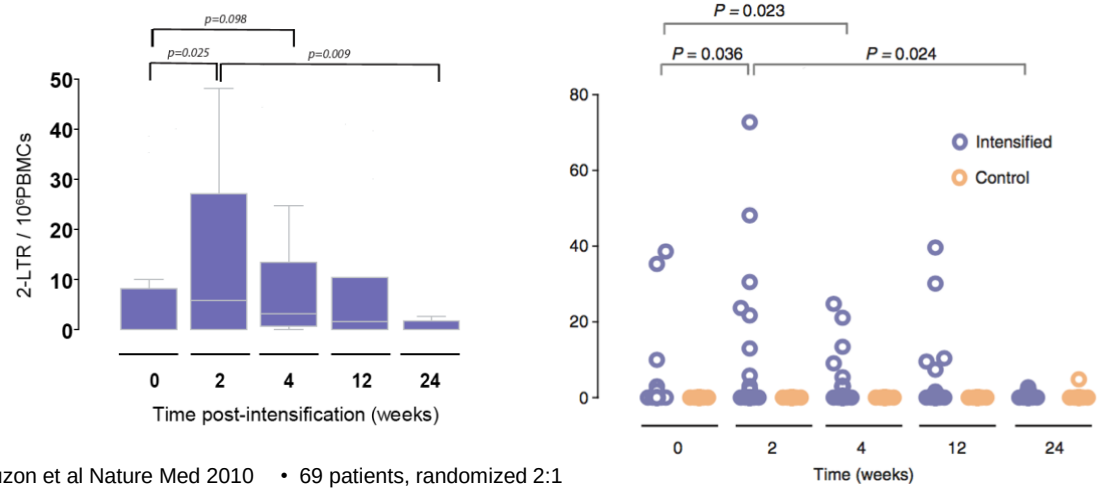
# Raltegravir intensification and argument for ongoing replication




Buzon et al Nature Med 2010, Hatano et al JID 2013

# Raltegravir intensification




Buzon et al Nature Med 2010

- 69 patients, randomized 2:1
- 13/45 (29%) in the RAL arm with detectable 2-LTR

## Raltegravir intensification

- 2-LTR circles measured at 0, 2, 4, 12 and 24 weeks post initiation of raltegravir intensification
- Intensification maintained throughout this period
- Detected 2-LTR circles at one or more time points in 29% (13 of 45) of the subjects given raltegravir and 5% (1 of 22) of controls

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Buzon et al, Nature Med 2010

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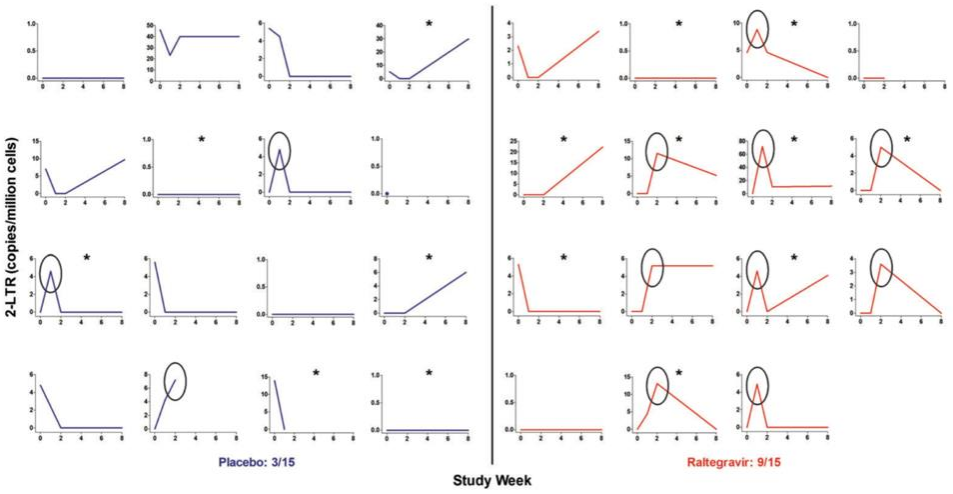
## Raltegravir intensification




Week 1 to week 0 ratio was 8.8-fold higher in the RAL group, compared to placebo

Asterisks for those on PI regimens

Hatano et al, J Infect Dis 2013

## Raltegravir intensification studies

- Increase in 2LTR circles more common if on a PI regimen[1,2]
- Associated with a decrease in immune activation – reduced T-cell activation[1] and D-dimer[2]
- Unclear if effect on residual viral replication is unique to raltegravir
  - High uptake in GI tract[3]
  - 2 other Raltegravir intensification studies did not detect increase in 2-LTR but did not sample early[4]

[1] Buzon, Nat Med 2010; [2] Hatano, J Infect Dis 2013; [3]Patterson, AIDS 2013; [4]Gandhi, JAIDS 2012; Vallejo, AIDS, 2012

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## Many other negative intensification studies

- Maraviroc
- Efavirenz
- Enfurvitide
- Other antiretroviral combinations
- No change in HIV DNA, cell associated or plasma HIV RNA

Dinoso, PNAS, 2009; McMahon Clin infect Dis, 2010; Gutierrez, PLoS One, 2011; Hatano, J Infect Dis, 2011; Llibre, Antivir Ther, 2012; Gandhi, J Infect Dis, 2010; Anonwaronich, J Virus Erad, 2015; Tiraboschi, AIDS Res Hum Retro, 2017

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## Other arguments – HIV drug resistance

- No development of drug resistance despite suppressive ART argues against ongoing replication[1]
- BUT…
- As there are low ARV levels in lymph nodes[2] ongoing replication could occur in these sanctuary sites without the development of drug resistance[3]

1 Hermankova M JAMA 2001; Persaud D J Virol 2004; 2 Fletcher PNAS 2014; 3 Lorenzo-Redondo, Nature, 2016

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**The effect of antiretroviral intensification with dolutegravir on residual virus replication in HIV-infected individuals: a randomised, placebo-controlled, double-blind trial**

*Thomas A Rasmussen*, James H McMahon*, J Judy Chang, Jennifer Audsley, Ajantha Rhodes, Surekha Tennakoon, Ashanti Dantanarayana, Tim Spelman, Tina Schmidt, Stephen J Kent, Vincent Morcilla, Sarah Palmer, Julian H Elliott, Sharon R Lewin*

Lancet HIV. 2018 May;5(5):e221-e230

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# Hypothesis

**Dolutegravir intensification will inhibit and reveal residual virus replication in individuals on suppressive ART**

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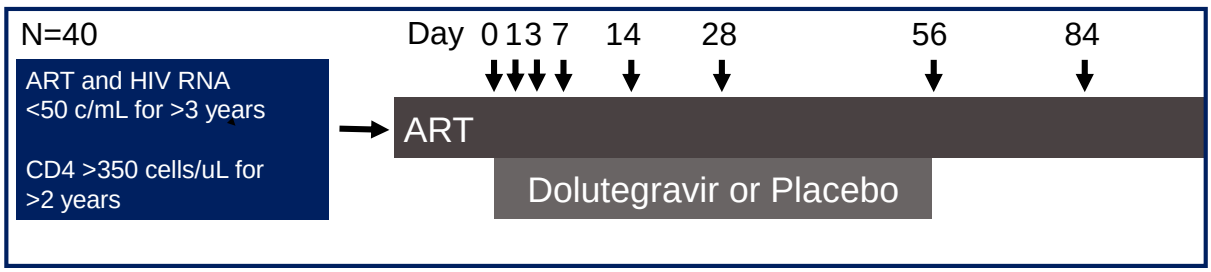
## DIORR: Dolutegravir Intensification effect On Residual virus Replication on ART

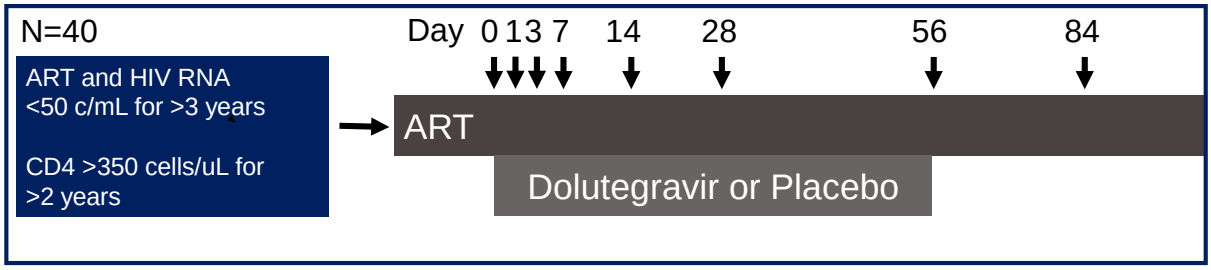



**Design:** Investigator-initiated, randomized, placebo-controlled, double-blind clinical trial

**Study sites**

- Alfred Hospital, Melbourne, Australia
- Melbourne Sexual Health Centre, Melbourne, Australia

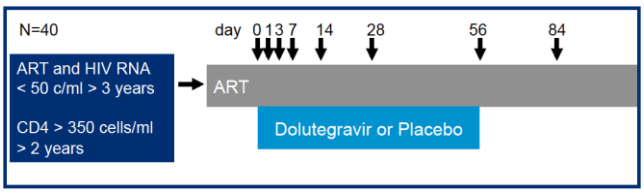
# DIORR: Dolutegravir Intensification effect On Residual virus Replication on ART




## Study strategy

- Dolutegravir 50 mg or placebo once daily for 56 days, added to background ART
- Dolutegravir/placebo BD if concomitant nevirapine or efavirenz
- Randomised 1:1 in blocks of 2 or 4
- Stratified by PI use

# DIORR Study




## Primary endpoint

- The level of 2-LTR circles in circulating CD4+ T cells after 7 days of intensification

## Secondary endpoints

- 2-LTR circles at other time points
- Plasma HIV RNA (single copy assay – LLOD 0.3 c/mL)
- Total and integrated HIV DNA
- Cell-associated unspliced HIV RNA
- Inflammation and T-cell activation – hsCRP, IL-6, d-dimer, sCD14, HLA-DR, CD38, PD1
- Safety
- Concentration of dolutegravir in plasma

## Statistical analysis

- **Sample size:** powered to detect a 3-fold difference in 2-LTR
- **A priori analysis for primary and numerical secondary endpoints:** Repeat measures ANOVA
  - ANOVA compares change from baseline between groups. Means of triplicates used at each timepoint
  - Baseline and screening values also averaged
  - Repeated measures testing can incorporate data between baseline and day 7



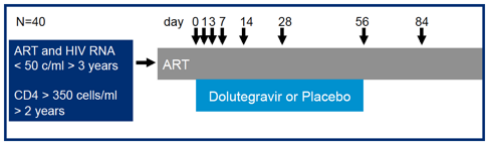
## Exploratory analyses

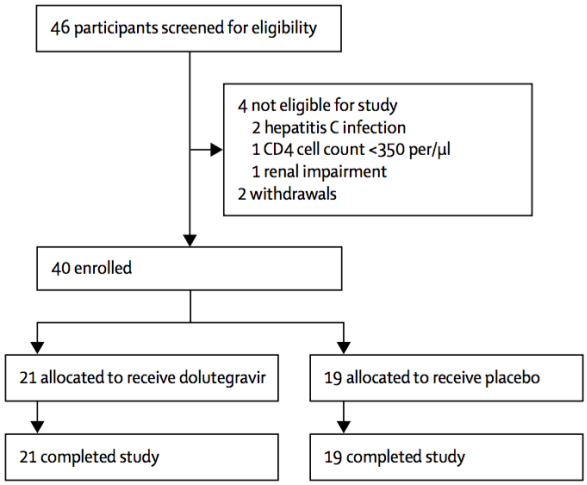
- Analysis of covariance (ANCOVA): Tests for difference between groups at a certain time point *adjusting* for baseline
- Negative binomial regression[1]
  - Outcome variable = Number of copies
  - Exposure variables include PCR input quantity (total RNA, total DNA or plasma volume)
  - Accounts for variation in amounts of input RNA, DNA or plasma volume. Specimens with higher input provide more weight than specimens with lower input quantity
  - Handles overdispersed data (data with increased variability [variance] than expected)
  - Advantages – can model positively skewed data, accounts for variation in amount of input RNA or plasma, can include values of zero without need for ad-hoc adjustments



1 Elliott Lancet HIV 2015

## Study flow chart




## Baseline characteristics

| Characteristics | Dolutegravir (n=21) | Placebo (n=19) |
|---|---|---|
| Age (years), mean (+/- SD) | 49.4 +/- 10.8 | 48.5 +/- 8.0 |
| Male gender, n (%) | 18 (86%) | 19 (100%) |
| CD4+ T-cell count (cells/µL), median (IQR) | 721 (648 – 953) | 664 (545 – 891) |
| **ART Regimen** | | |
| NNRTI, n (%) | 18 (86%) | 17 (89%) |
| Protease inhibitor, n (%) | 3 (14%) | 2 (11%) |

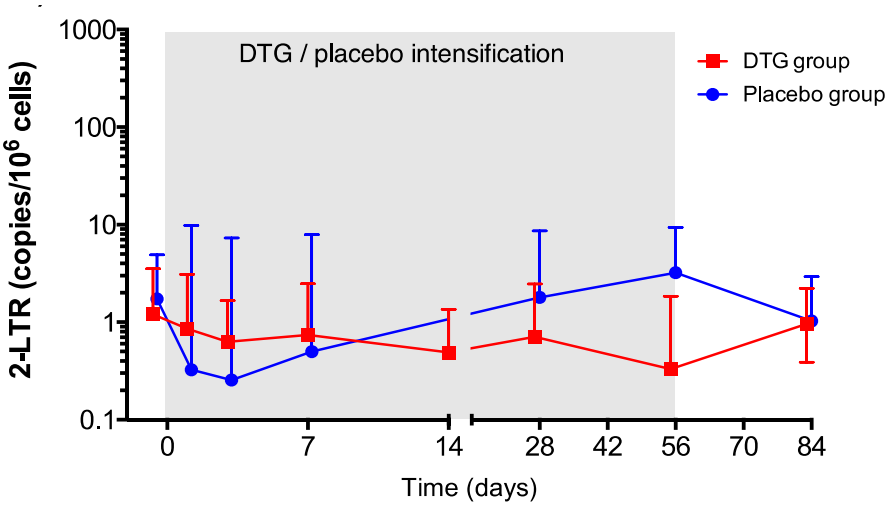
## Safety data

| Adverse events | Dolutegravir (n=21) | Placebo (n=19) |
|---|---|---|
| **Treatment-related** | | |
| Clinical | 4[#] | 11^ |
| Laboratory | 4 | 1 |
| **Treatment-unrelated** | | |
| Clinical | 21* | 14 |
| Laboratory | 3 | 7 |

[#] Fatigue, anxiety, dry mouth, rash
^ Vivid dreams, diarrhoea, dissociation, nausea, fatigue, dizziness, oesophageal pain, bloating
* Included one SAE (hospital admission for IV antibiotics due to cellulitis)

- All treatment-related AEs grade 1
- No treatment-related SAEs
- No AE led to study withdrawal

## No increase in 2-LTR circles on dolutegravir



## Primary Endpoint 2-LTR

- No significant difference between arms over 7 days RM-ANOVA (p=0.17)
- Median (IQR) baseline to day 7 fold-change was -0.17 (-0.90 to 0.90) DTG and -0.26 (-1.00 to 1.17) placebo

- Exploratory analyses
  - ANCOVA - no significant difference comparing baseline to day 7 (trend to increase in placebo compared to DTG (p=0.06))
  - Binomial regression - significant reduction from baseline to day 7 in DTG compared to placebo (p<0.001), if intervening time points (Days 1 and 3) included, then not significant

- 3 methods did not identify a significant increase in DTG compared to placebo at any time point from baseline to day 84

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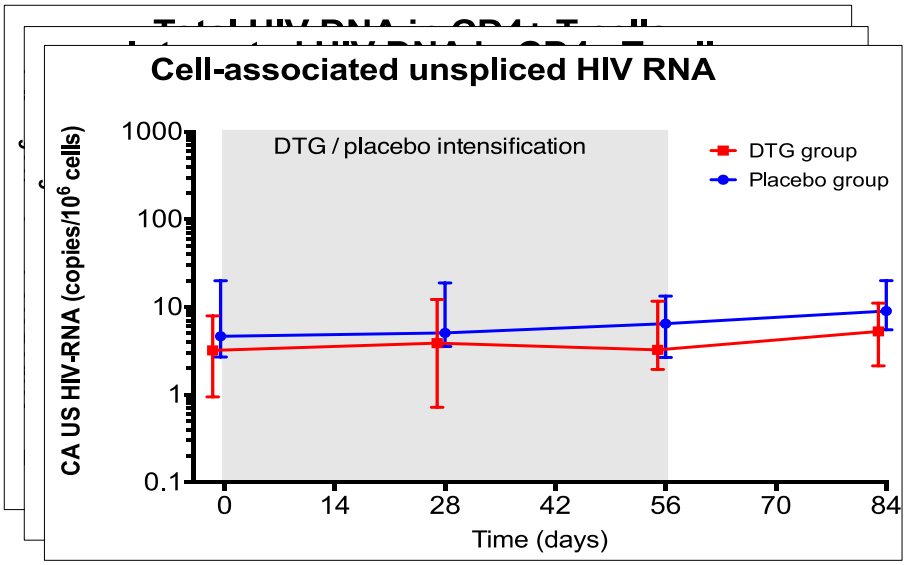
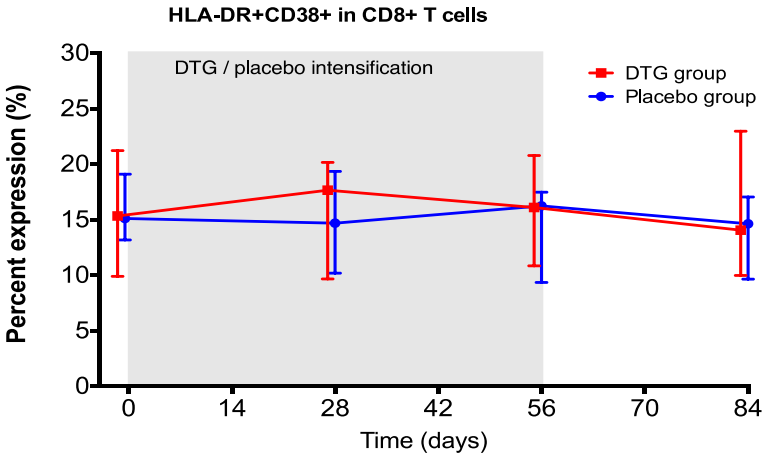
## No change in HIV DNA, cell-associated or plasma HIV RNA

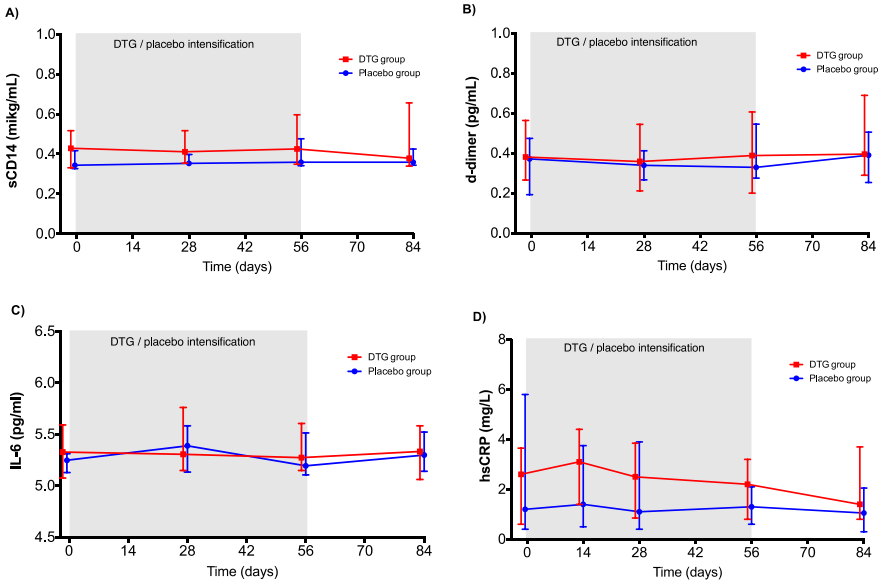


# No change in T cell activation




**No change in PD1 or HLA-DR/CD38 expression in CD4+ or CD8+ T cells**

# No change in plasma markers of inflammation



## Trough levels

- Dolutegravir trough levels (n=19)
  - range 0.773 to 5.670 microg/mL
  - mean of 3.08 microg/mL

- Therapeutic levels in a phase 3 RCT (n= 399) receiving dolutegravir 50 mg daily mean trough of 1.18 microg/mL[1]

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1 Raffi, Lancet Infect Dis 2013

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## Discussion

- Negative findings consistent with some studies of treatment intensification with raltegravir and other ARVs (efavirenz, maraviroc, boosted PIs)[1]
- Lack of change in immune activation consistent with an intervention that did not interfere with residual viral replication
  - ↓ in immune activation possibly expected if residual replication disrupted
  - However → possible that virus production from infected cells that persists on ART could stimulate immune activation in the absence of ongoing infection of new cells.

1 Dinoso, PNAS 2009; McMahon, Clin Infect Dis, 2010; Gandi, J Infect Dis. 2010; Gutierrez C, PLoS One, 2011; Vallejo, AIDS, 2012; Gandhi R, PLoSMed 2010; Ananworanich J, J Virus Erad, 2015; Tiraboschi J AIDS Res Hum Retro, 2017

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## Discussion

- Discrepancy between this study and the 2 raltegravir intensification studies that showed increase in 2-LTR[1]
- Different population studied
  - Prior studies 30-68% on PIs where 2-LTR increase more commonly seen
  - This study 12.5% on PIs
- Hatano study used droplet digital PCR → has greater range in detection of a target compared to real-time PCR

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1 Buzon, Nature Med 2010; Hatano, J Infect Dis 2013

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## PK differences between RAL and DTG

- DTG concentration in rectal tissue estimated at 17% of plasma[1]
- Concentrations of RAL in GIT can be 600 x higher than plasma[2]
- If residual replication occurring in GIT where most virus persists,[3] higher GIT RAL concentration may allow for a greater effect
- However, no difference in the frequency of cell-associated HIV RNA and DNA in GALT from PLHIV receiving RAL or DTG[4]
  - Suggests similar frequency of infected cells in GALT on both drugs, however a cross sectional study would not address residual replication

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1 Greener, JAIDS, 2013; 2 Patterson K, AIDS 2013; 3 Estes J, Nat Med 2017;
4 Weber M, 2016 17th Int. Workshop on Clinical Pharmacology of HIV and Hepatitis Therapy.

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## Limitations

- Powered to detect a 3-fold change in 2-LTR so small differences may have been missed
- Only 5 people on PI regimens included in this study
- No LN or GALT samples therefore cannot exclude the possibility that dolutegravir intensification impacted residual replication in tissue

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## Conclusion

- **In a randomised, placebo-controlled study of dolutegravir intensification there was**
  - No change in 2-LTR (no interference with residual replication)
  - No change in cell-associated markers of HIV persistence
  - No change in T cell activation or in plasma markers of immune activation

**Dolutegravir intensification did not reveal or impact residual virus replication in blood in HIV infected individuals on ART**

## Where does this leave residual replication in 2018?

- The great majority of evidence does not support the presence of residual viral replication on virologically suppressive ART as a key driver to maintain the HIV reservoir
- Targeting residual replication is not a focus of current HIV cure focused clinical trials when compared to work to understanding driver of, or potential ways to interfere with, proliferation of latently infeected T-cells


## Acknowledgements

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