Transcriptomics and Proteomics Reveal Differential Pathways in DTG/3TC versus Three-drug Regimens in PLHIV

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2000 HIV

Key Concepts

Oxidative Stress: An imbalance in the body between reactive oxygen species (ROS) and antioxidants. Too many ROS can damage our cells, DNA, and proteins, leading to various diseases and accelerated aging.
Mitochondria and NRTIs: Nucleoside reverse transcriptase inhibitors (NRTIs), a type of antiviral drug, can affect mitochondria by inhibiting the function of an enzyme called polymerase gamma, which is responsible for replicating mitochondrial DNA. This can lead to an increase in ROS, contributing to oxidative stress.
Benefit of Reducing Oxidative Stress: Prevents cell damage, potentially slowing aging and disease development.

BACKGROUND

Drug toxicity in people living with HIV (PLHIV) using cART is a concern. Nucleoside reverse transcriptase inhibitors (NRTI) are associated with mitochondrial damage. Two-drug regimens (2DR) may reduce drug toxicity, but it is unknown whether they suppress inflammation as a three-drug regimen (3DR). Systemic effects and immune activation of dolutegravir (DTG) and lamivudine (3TC) and 3DR were studied using multi-omics.

METHODS

Study design.
2000HIV study (clinicaltrials NTC03994835) included 1895 PLHIV in the Netherlands, separated into a discovery and validation cohort to test for reproducibility (see **Figure 1**). Plasma proteins (Olink Explore) and gene expression (PBMC Bulk RNA-seq) were explored, comparing DTG/3TC to 3DR, containing integrase strand inhibitors (3DR-INSTI) or not (3DR-non-INSTI). DTG/3TC were also compared to cART without NRTIs (cART-no-NRTI). Whole blood samples immunophenotyped by flow cytometry were also studied to assess immune activation. Adjustments were made for confounders and pre-cART conditions.

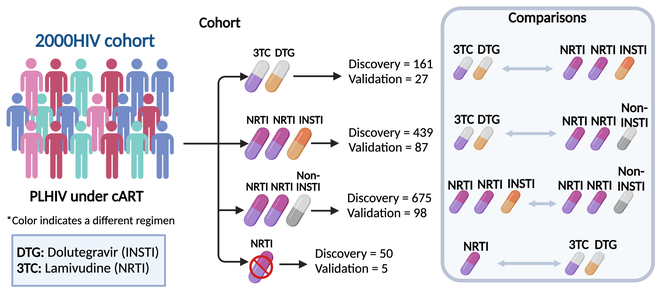


Figure 1. Graphic representation of study setup and comparison groups. Created with BioRender.com

Definition Differentially Expressed Genes (DEG) and Proteins (DEP):
- False discovery rate (FDR) < 0.05.
- Absolute fold change (FC) > 1.25 (captures significant biological changes while minimizing false positives).
- Concordant sign of FC in discovery and validation cohorts.

Enriched Pathways. Genes and proteins were ranked based on the combined score: –log10(p-value) * sign(log2(FC)). Pathway regulation was associated with normalized enrichment scores (NES). The top 10 significant upregulated (NES>0) and downregulated (NES<0) pathways were reported (FDR-adjusted).

Immune Activation Assessment. HLA-DR, CD38, PD1, PDL-1, CD40, CD307d, and CD81 were evaluated. Distinctions in activation, exhaustion, maturation status, and intercellular communication within B cells, T cells, and innate immune cells were discerned.

RESULTS

- **DTG/3TC vs. 3DR-INSTI and 3DR-non-INSTI:** Limited DEG (2 down) and DEP (6 down) against 3DR-INSTI (see **Figure 2**). 3DR-non-INSTI revealed no DEG and 24 DEP (1 up, 23 down). Both comparisons show down-regulation of Biological oxidations pathway with GSTA1 DEP as leading marker, with stronger effects against 3DR-non-INSTI group.
- **3DR-INSTI vs. 3DR-non-INSTI:** No DEG, limited DEP (3 up, 10 down). Downregulation of Metabolism of lipids and Fat digestion and absorption pathways with FABP1 and FABP2 DEP as leading protein markers.

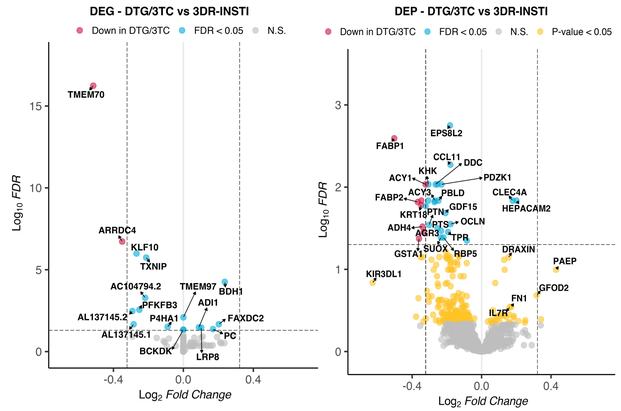


Figure 2. Volcano plots illustrating DEG and DEP of DTG/3TC versus 3DR-INSTI

- **cART-no-NRTI vs. DTG/3TC:** DEG (2 up), DEP (9 up). Suppression of oxidoreductase activity pathways in genes but activation in proteins for cART-no-NRTI, see **Figures 3 and 4**.
- **Immune activation:** No significant differences in innate or adaptive immune cell populations between DTG/3TC vs. 3DR-INSTI.

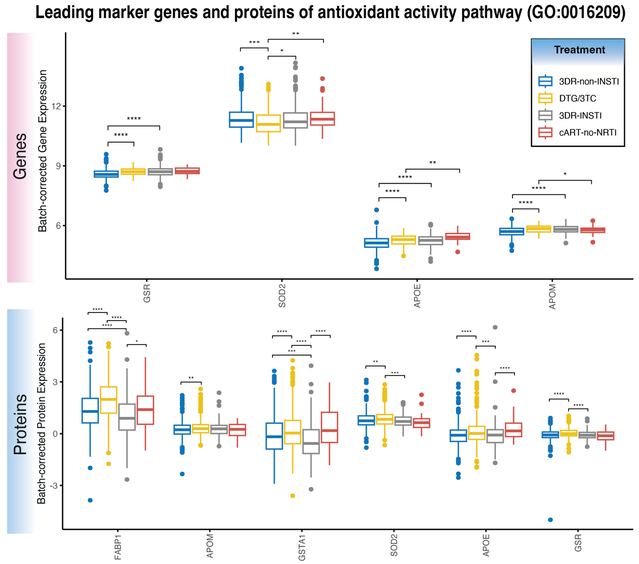


Figure 3. Gene and protein expression of antioxidant activity pathway (GO:0016209)

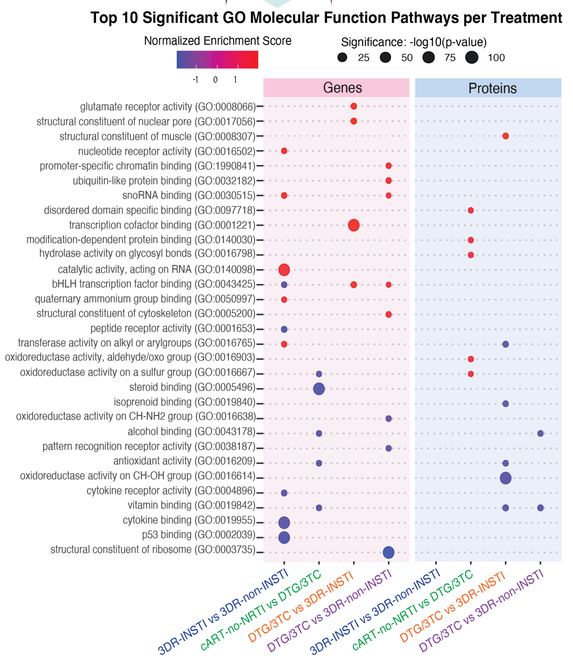
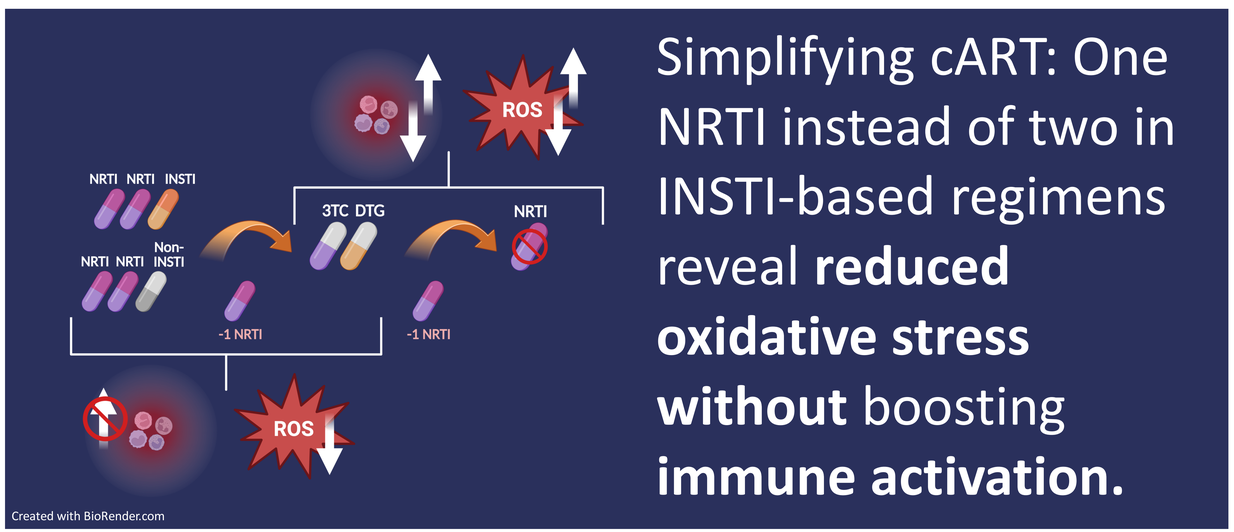


Figure 4. Pathways were ranked based on the FDR and the top 10 most significant categories were selected from each of positive and negative pathways based on normalized enrichment score [1].



CONCLUSIONS

- INSTI regimens containing 1 NRTI (DTG/3TC) show reduced oxidative stress without increased immune activation compared to INSTI regimens containing 2 NRTIs.
- INSTIs appear to downregulate lipid metabolism compared to non-INSTI-based 3DR.
- Excluding NRTIs does not consistently show reduced oxidative stress or drug toxicity compared to using 1 NRTI in INSTI-based 2DR for plasma proteomics and PBMCs bulk transcriptomics.

ADDITIONAL KEY INFORMATION

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References:
1. The Gene Ontology Consortium. The Gene Ontology knowledgebase in 2023. Genetics. 2023 May 4;224(1):iyad031. DOI: 10.1093/genetics/iyad031

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www.2000HIV.com