# IMPACT OF IMMUNE-DRIVEN SEQUENCE VARIATION IN POL ON VIRAL REPLICATION CAPACITY AND DISEASE PROGRESSION IN HIV-1 SUBTYPE C INFECTION

*Doty Ojwach[1], Thumbi Ndung'u[1, 2, 3, 4], Jaclyn Mann[1]*

*[1]HIV Pathogenesis Programme, Doris Duke Medical Research Institute, University of KwaZulu-Natal, Durban, South Africa,*
*[2]KwaZulu-Natal Research Institute for Tuberculosis and HIV, K-RITH, University of KwaZulu-Natal, Durban, South Africa,*
*[3]Ragon Institute of MGH, MIT and Harvard, Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States,*
*[4]Max Planck Institute for infection Biology Chariteplatz,D-10117 Berlin, Germany.*

**Background**
CD8+ T cell responses to epitopes, particularly conserved Gag epitopes, in which immune escape mutations result in lower viral replication capacity is a hypothesised mechanism of protection in HIV-1 infected individuals with certain clinically favourable HLA class I alleles. Therefore, a possible vaccine strategy is to direct immune responses to vulnerable HIV regions, aiming to limit immune escape due to fitness constraints or slow virus replication. Substantial replication costs and clinical relevance of several immune escape mutations in the HIV-1 Gag have been demonstrated, however, less is known about consequences of immune-driven mutations in other proteins. We investigated the impact of immune-driven sequence variation in Pol, a highly conserved and immunogenic protein essential for viral replication, on viral replication capacity and disease progression in a large population of individuals chronically infected with HIV-1 subtype C, the most prevalent subtype world-wide.

**Methods**
414 patient-derived RT-integrase NL4-3 recombinant viruses were generated by electroporation of a green fluorescent reporter cell line (GXR cells) with plasma-derived *RT-integrase* PCR products and pNL43Δ*RT-integrase* and sequences were generated for 369 of these thus far. The replication capacities of recombinant viruses were determined by calculating the slope of increase in percentage infected cells, as measured by flow cytometry, from days 3-6 following infection.

**Results**
The mean replication capacity of these viruses, normalised to the growth of wild-type NL4-3, was 0.92 (interquartile range; 0.87 to 0.97). RT-integrase driven replication capacity correlated significantly with log viral load (r= 0.2571 p=<0.0001) and CD4 count (r= -0.2580 p= <0.0001). A preliminary sequence analysis of RT-integrase HLA-associated polymorphisms previously described to reduce replication capacity of subtype B viruses, showed that the HLA-A*33 restricted polymorphism G163E in integrase was associated with significantly reduced replication capacity in subtype C viruses (p=0.02).

**Conclusion**
The data suggest that RT-integrase-driven replication capacity is clinically relevant. Preliminary sequence analyses suggest that immune-driven mutations in Pol may significantly attenuate HIV. Further comprehensive sequence analysis may inform which Pol epitopes are the most vulnerable for an attenuation-based vaccine.