

Impact of *Gag* and *Nef* Gene Mutations on Integrase Drug Resistance Among Patients Failing Second-Line ART

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BACKGROUND AND OBJECTIVES: The introduction of integrase strand transfer inhibitors (INSTIs), particularly dolutegravir (DTG), into South Africa's antiretroviral therapy (ART) program has improved viral suppression due to their high efficacy and genetic barrier to resistance. However, evidence suggests that mutations outside the *pol* gene, including those in *gag* and *nef*, may influence the emergence of INSTI resistance. In this study, we characterize resistance-associated mutations (RAMs) in the *gag*, *IN*, and *nef* genes isolated from nucleic acid extracts from patients failing to respond to Human Immunodeficiency Virus-1 (HIV-1) second-line Protease Inhibitor (PI)-based ART.

METHODS: This retrospective study analyzed 273 HIV-1 nucleic acid extracts collected between 2018 and 2020 at Dr. George Mukhari Academic Hospital, South Africa. Nested RT-PCR was performed to amplify *gag* (1502 bp), *IN* (1238 bp), and *nef* (770 bp) regions, followed by Sanger sequencing. Sequences were analyzed using Chromas Pro, the Stanford HIV Drug Resistance Database, and REGA subtyping tools.

RESULTS: Sequencing was successful for 184 *gag*, 98 *IN*, and 147 *nef* amplicons. *Gag* analysis identified cleavage site mutations L449F (18.5%) and P453L (1.1%), detected even in the absence of major PI RAMs, suggesting a compensatory role in resistance pathways. Integrase analysis revealed no major RAMs; however, a minor variant (Q95K) was observed at 1.0% of sequences. *Nef* displayed high sequence variability; however, the 3'-polypurine tract (3'-PPT) region remained highly conserved despite frameshifts, indicating possible functional constraints. These findings suggest that *gag* and *nef* variation may indirectly contribute to the development of integrase resistance by supporting viral persistence under drug pressure.

CONCLUSION: Although major INSTI RAMs were rare, *gag* cleavage site mutations and *nef* variability may provide a permissive genetic background for integrase resistance. Incorporating non-*pol* genes into resistance monitoring could strengthen surveillance strategies.