

Next Generation Sequencing (NGS): redefining management of chronic viral infections

OC 48 Comparison between Next-Generation Sequencing and Historical Genotypic Resistance Testing at Virological Failure in ART-Experienced Individuals: Data from the ARCA Cohort and the Italian NGS Network

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ABSTRACT

Background: Next-generation sequencing (NGS) increases sensitivity for detecting HIV-1 drug resistance mutations, including minority variants, but its real added value may depend on clinical scenarios.

Materials and Methods: We conducted a cross-sectional analysis within the ARCA cohort and Italian NGS Network, including adult people with HIV (PWH) who failed an antiretroviral regimen between 2022 and 2025 and for whom genotypic resistance testing by NGS (NGS-GRT) on plasma samples at the time of virological failure and historical Sanger-GRT (hGRT) were available. hGRTs were compared with NGS-GRT at detection thresholds of 5–20% (minority variants) and $\geq 20\%$ (high-frequency variants). Major resistance mutations (MRMs) were assessed according to the Stanford HIVdb algorithm v.10.1.

Results: We included 54 PWH; all had prior PR/RT hGRT, while integrase sequences were available for 31 PWH. Participants had a median treatment history of 14 (IQR: 9–18) years and a median of 4 (IQR: 2–8) prior regimens. At the time of NGS-GRT, median HIV-RNA was 3.9 (IQR: 3.0–4.8) log₁₀ copies/mL. PWH were receiving bicitgravir/dolutegravir-based (37.0%), raltegravir/elvitegravir-based (22.2%), protease inhibitor-based (29.6%), and NNRTI-based (11.1%) regimens, for a median duration of 5 (IQR: 3–7) years.

Overall, mutation patterns were heterogeneous, combining newly emerging and historically re-detected MRMs. At a 5% threshold, 63.0% of PWH harbored ≥ 1 MRM, decreasing to 48.1% at $\geq 20\%$. MRMs exclusively detected in hGRT were observed in 11.1% of individuals.

Historical MRMs were re-detected in 16 individuals (29.6%), with complete re-detection in only eight (14.8%). New

MRMs detected exclusively by NGS were observed in 40.7% and 25.9% of individuals at 5% and $\geq 20\%$ thresholds, respectively.

MRMs in hGRT and/or NGS-GRT were observed in 10 (18.5%), 25 (46.3%) and 27 (50.0%) individuals for PIs, NRTIs and NNRTIs, respectively. Historical MRMs not detected by NGS were considerable ($>25\%$) for NRTIs and NNRTIs, while modest ($<12\%$) for PIs and INSTIs (Figure 1). NGS re-detected historical RTIs resistance in a modest proportion of individuals ($<20\%$) while re-detection was poor for PIs or INSTIs resistance with a minimal contribution of minority variants (1.8% for PI MRMs). Emergent resistance was observed across all drug classes, mainly as high-frequency variants (PIs: 3.7%; NRTIs: 13.1%; NNRTIs: 9.3%; INSTIs: 12.9%).

Emerging mutations were consistent with regimen-specific selective pressure (Figure 2). MRMs were also identified in 4 individuals without prior integrase hGRT.

Conclusion: NGS performed at virological failure effectively identifies emerging resistance, with a moderate contribution of low-frequency variants. However, its ability to fully recover previously documented resistance is limited. Therefore, integration of virological failure history and historical resistance data remain crucial for appropriate treatment tailoring in PWH experiencing virological failure.

Figure 1. Prevalence of resistance detected through hGRT and NGS in cART-experienced individuals at virological failure.

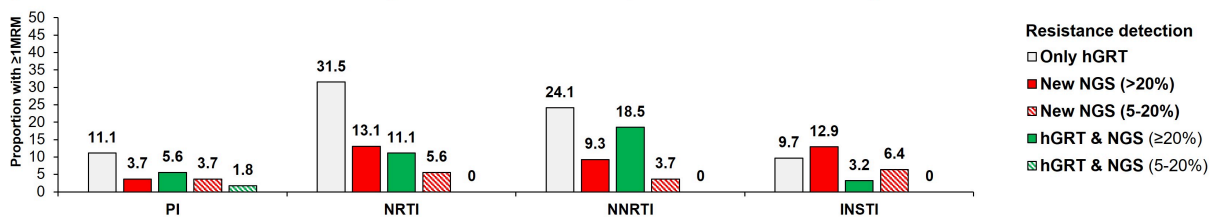
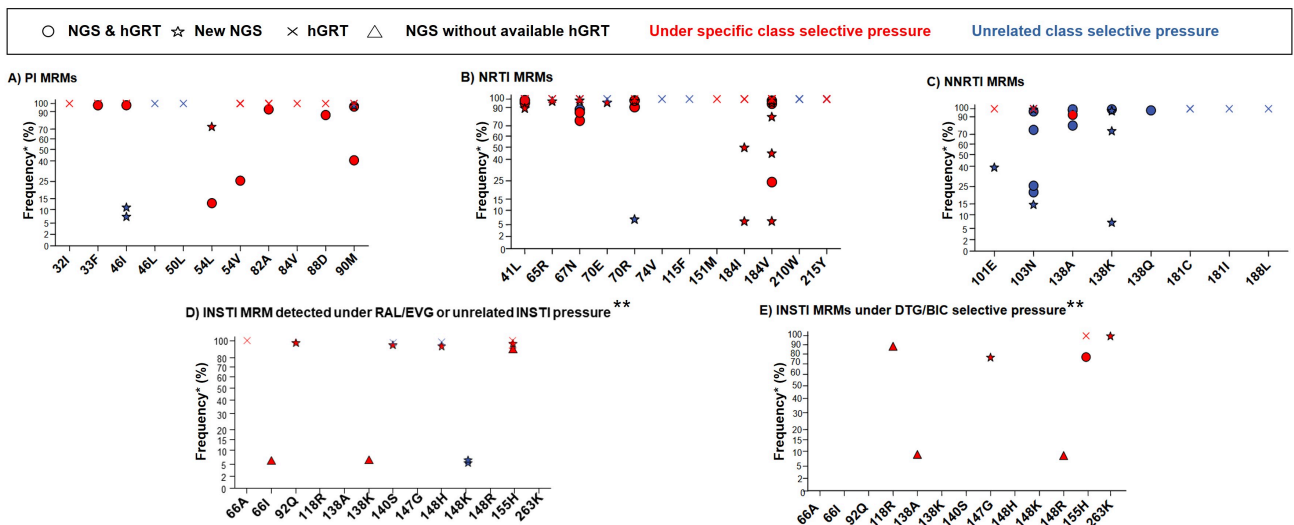


Figure 2. Overview of PI, NRTI, NNRTI and INSTI MRMs detected through hGRT and NGS in cART-experienced individuals at virological failure. Symbols indicate detection category: historical MRM re-detected by NGS (dots), newly identified MRM by NGS at virological failure (stars), MRM detected only by hGRT (X) and MRM identified by NGS without available hGRT (triangles). Red denotes class-specific selective pressure; blue denotes unrelated class selective pressure. Mutations detected only in hGRT were conventionally considered with a frequency of 100%.



*Y-axis power-transformed for improved visualization of low-frequency variants **Integrase hGRT available for 31 individuals