RESEARCH and CARE: FROM BENCH, TO BEDSIDE, TO COMMUNITY

Presidenza del Congresso: A. Cingolani, A. Di Biagio, M. Farinella, G. C. Marchetti

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RESEARCH and CARE: FROM BENCH, TO BEDSIDE, TO COMMUNITY

Network analysis of proviral DNA mutations in People with 4-class-resistant HIV-1: Data from the PRESTIGIO Registry

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OSPEDALE SAN RAFFAELE

PRESTIGIO

Potential conflicts of interest

Dr. Sara Diotallevi has no financial relationships with commercial entities to disclose.

Background

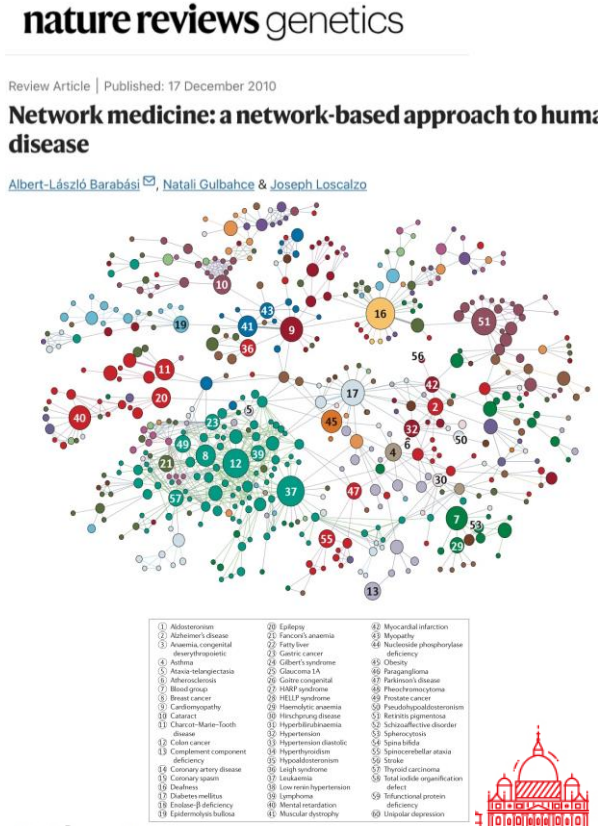
- In highly treatment-experienced people with HIV and multi-drug resistance, mutations interact within a complex system:
  - Long history of ART exposure
  - High HIV-1 DNA and RNA resistance burden[1].
- To date, network-based approach has not been used to study HIV mutations.

1. Armenia D, et al. **Viral resistance burden and APOBEC editing correlate with virological response in heavily treatment-experienced people living with multi-drug resistant HIV.** International Journal of Antimicrobial Agents, 2022.

Aim

To identify clusters of mutations archived in proviral DNA in people with HIV and 4-class drug resistance (PWH-4DR) under virological suppression.

Network Medicine



Methods

- Study population: PWH with 4-class drug resistance and HIV-RNA <50 copies/mL (since >6 months) at sample collection, enrolled in the Prestigio Registry[2]

- NGS genotyping on PBMC samples: mutations frequency (as percentage of reads) detected in HIV-1 DNA by next-generation sequencing (NGS, Illumina MiSeq) with a 5% cut-off was evaluated[3]

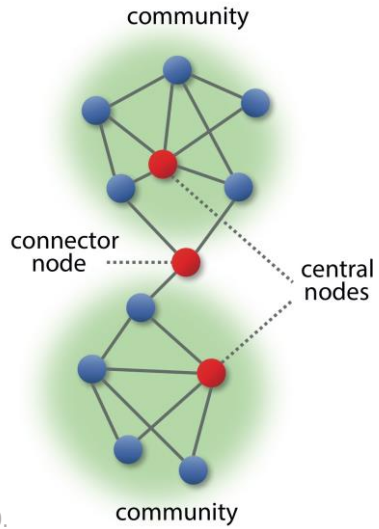
- The following HIV-1 mutations were classified according to the Stanford HIV Drug Resistance Database (version 9.5) rules:
  - Major drug resistance mutations (DRMs)
  - APOBEC context DRMs (including stop codons)
  - Other (including accessory and polymorphic no resistance related) mutations

2. Clemente T, et al. **Cohort profile: PRESTIGIO, an Italian prospective registry-based cohort of people with HIV-1 resistant to reverse transcriptase, protease and integrase inhibitors.** BMJ Open, 2024.
3. Armenia D, et al. **Do minority resistant variants in HIV-DNA predict virological rebound in highly treatment experienced PLWH under virological control? Data from the PRESTIGIO Registry.** ICAR 2023: abstract OC 127; EACS 2023: abstract 343, pag. 355-357; EMHH 2023: abstract OP9. Manuscript under revision.

Correlation Network

A network is a visual representation of data focused on associations (links) between elements (nodes):

- Nodes: mutations
- Links: pairwise Spearman correlations, selected with both following thresholds[3]:
  - ✓ Correlation coefficient values were <5th percentile OR >95th percentile
  - ✓ Adjusted Benjamini-Hochberg pvalue were <0.001



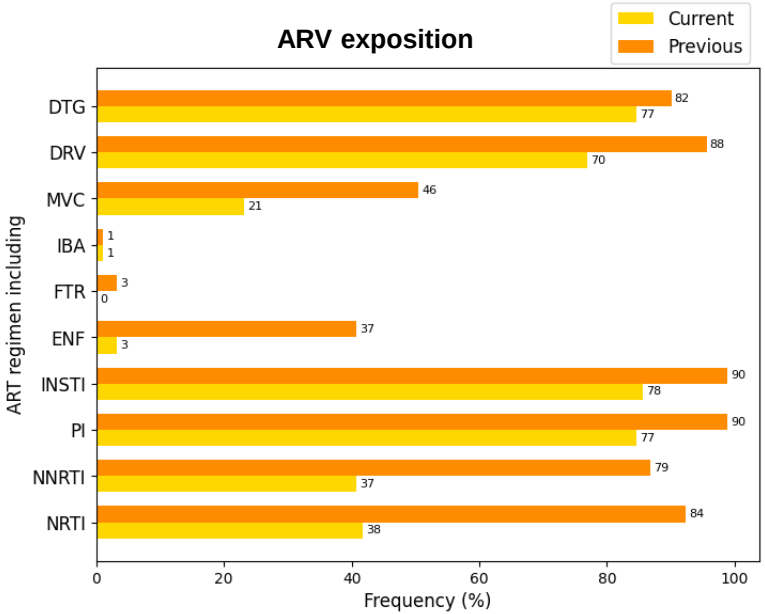
Elements of network theory[4]:

- ❑ *Connected components* = sub-networks that are disconnected from each other but whose nodes are connected internally
- ❑ *Module* or *community* = highly interlinked local regions in the network
  → shared biological function?
- ❑ *Connector node* = node that connects distinct modules by acting as a "bridge"

4. Silverman E, **Molecular Networks in Network Medicine: Development and Applications.** Wiley Interdiscip Rev Syst Biol Med, 2020.
5. Farina L, **Network as a language for precision medicine**. Ann Ist Super Sanità 2021, Vol. 57, No. 4: 330-342

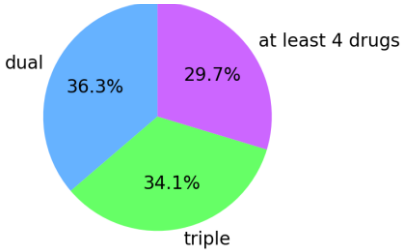
Individuals' characteristics at sample collection

Characteristics at sample	Overall (N=91)
Age (Years), median (IQR)	54.3 [50.0;59.0]
Sex at birth: male, n (%)	70 (76.9%)
Years of ART therapy, median (IQR)	22.8 [21.0;25.0]
Years since HIV diagnosis, median (IQR)	27.0 [23.0;30.9]
Years since multiresistance, median (IQR)	5.31 [3.57;7.28]
HIV-RNA (copies/mL), median (IQR)	20.0 [0.90;36.0]
Years under virological suppression, median (IQR)	3.18 [1.66;4.99]
$CD4^+$ T-cell nadir >200 cells/mm^3, n (%)	25 (29.8%)
$CD4^+$ T-cell count (cells/mm^3), median (IQR)	655 [484;890]
$CD4^+/CD8^+$ ratio, median (IQR)	0.71 [0.49;0.97]
Total HIV-1 DNA (copies/million CD4+ T-cell), median (IQR)	2377 (1274-4949)
Number of PI drug mutations, median (IQR)	21.0 [13.5;30.5]
Number of INSTI drug mutations, median (IQR)	16.0 [12.0;20.5]
Number of NRTI or NNRTI drug mutations, median (IQR)	33.0 [25.0;38.0]
Number of previous ART regimen, median (IQR)	15.0 [9.50;19.0]



Type of the current ART regimen

22 out 27 (81%) participants under a dual regimen received boosted **DRV** plus **DTG**



Results

1226 mutations detected among 91 PWH-4DR with plasma HIV-RNA<50 cps/mL

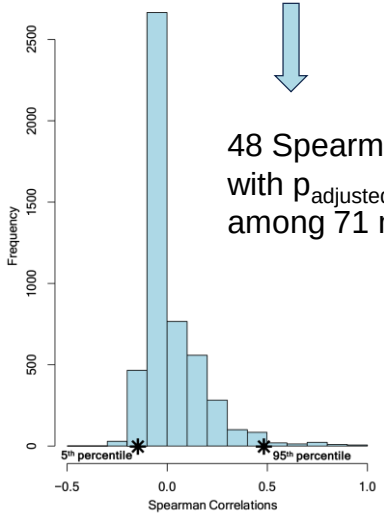
71 major mutations ≥5% NGS cut-off:

- 15 INSTI RMs
- 18 NNRTI RMs
- 13 NRTI RMs
- 25 PI RMs

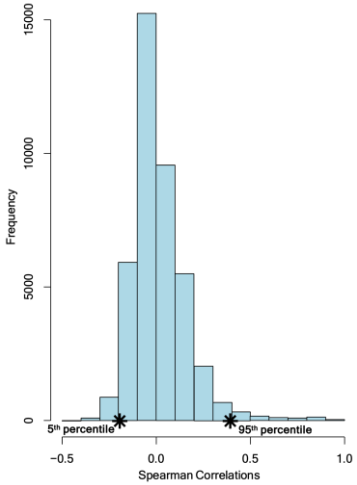
131 additional mutations ≥5% NGS cut-off and ≥10% of study population:

- 125 other (accessory or polymorphic) mutations
- 6 stop codons or APOBEC context DRMs

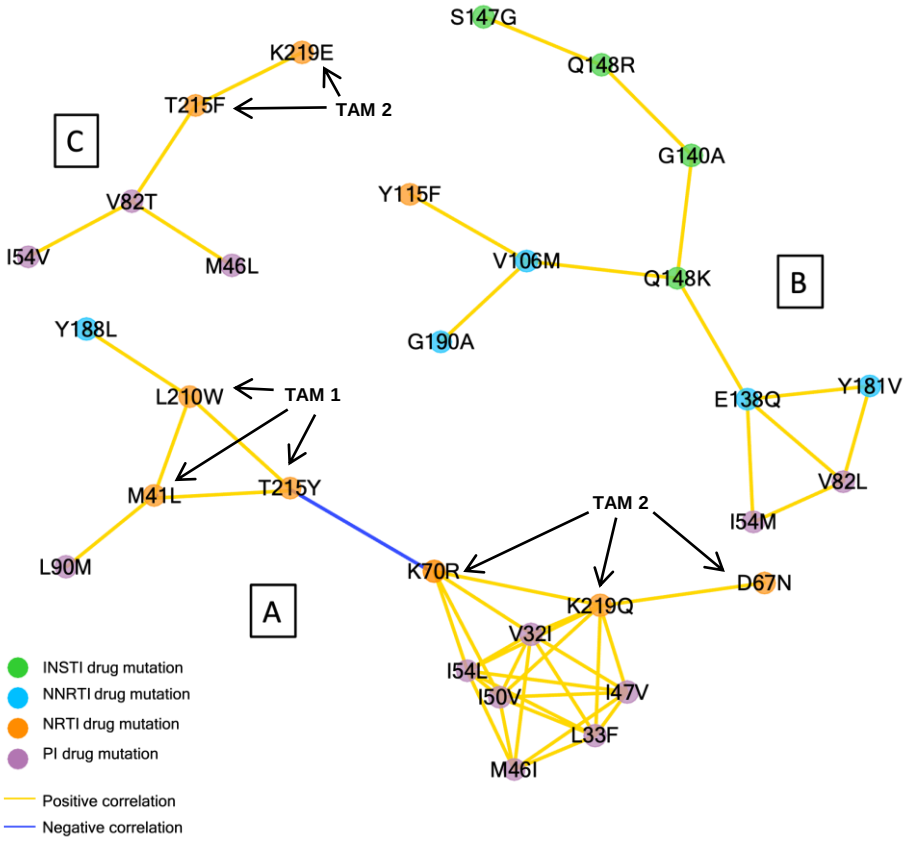
48 Spearman correlations with $p_{adjusted}$ <0.001 among 71 mutations

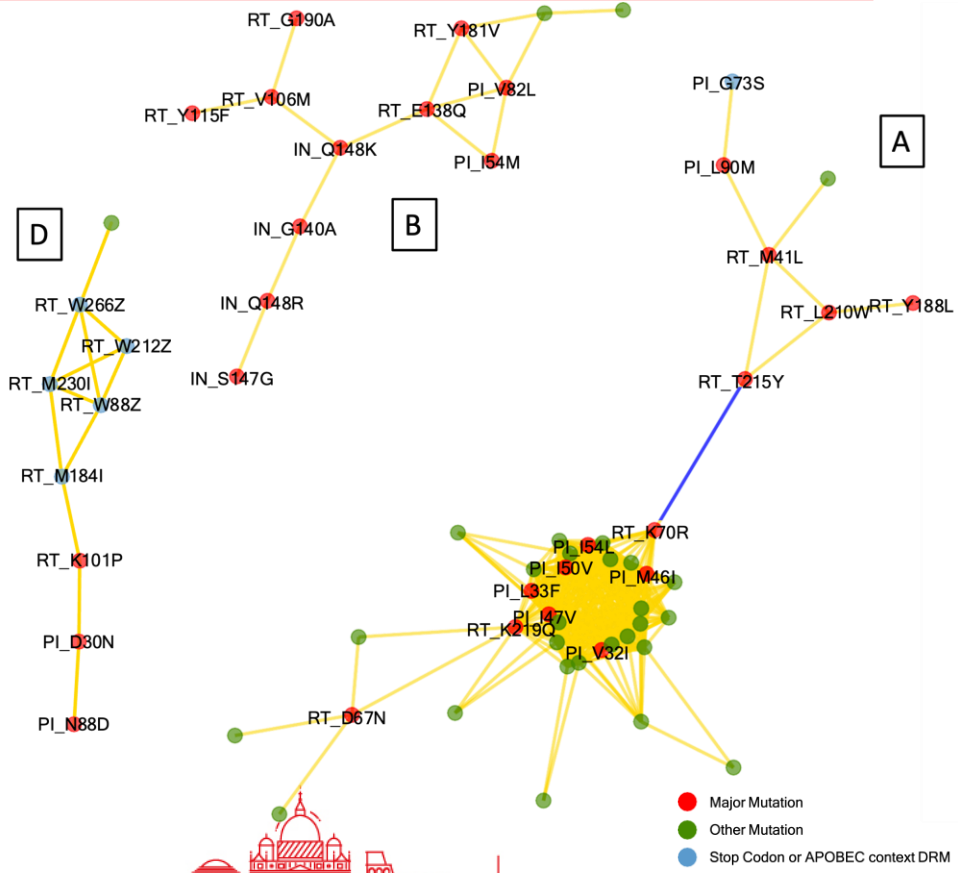


368 Spearman correlations with $p_{adjusted}$<0.001 among 202 mutations



Correlation network of major and other mutations





Conclusions

- The present study showed that Network Analysis is a valid alternative approach to evaluate the relationships between HIV-1 mutations potentially involved in resistance.
- In virologically suppressed PWH-4DR, our analysis allowed to identify patterns of major mutations across different drug classes, reflecting their complex therapeutic history.
- Links between several stop codons and APOBEC-related context mutations were also identified.

Next step will be to define the added value of this new methodology in predicting viral rebound in PWH and MDR virus.

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