

## Abstract: 5

*Mechanism of HIV Resistance*

# New HIV-1 gp120 V3 signatures modulate co-receptor usage and the interaction with CCR5 N-terminus

*V. Svicher*<sup>1</sup>, *C. Alteri*<sup>1</sup>, *A. Artese*<sup>2</sup>, *R. D'Arrigo*<sup>3</sup>, *R. Cammarota*<sup>1</sup>, *S. Alcaro*<sup>2</sup>, *F. Ceccherini-Silberstein*<sup>1</sup>, *P. Bagnarelli*<sup>4</sup>, *G. Antonelli*<sup>5</sup>, *V. Micheli*<sup>6</sup>, *V. Ghisetti*<sup>7</sup>, *W. Gennari*<sup>8</sup>, *F. Baldanti*<sup>9</sup>, *A.P. Callegaro*<sup>10</sup>, *B. Bruzzone*<sup>11</sup>, *G. Fadda*<sup>12</sup>, *M. Re*<sup>13</sup>, *A. De Luca*<sup>12</sup>, *M. Andreoni*<sup>1</sup>, *A. Antinori*<sup>2</sup>, *M. Zazzi*<sup>14</sup>, *M. Clementi*<sup>15</sup>, *G. Angarano*<sup>16</sup>, *S. Parisi*<sup>17</sup>, *A. Lazzarin*<sup>15</sup>, *C. Perno*<sup>1</sup>, on behalf of OSCAR group.

<sup>1</sup>University of Rome Tor Vergata, Department of Experimental Medicine, Rome, Italy; <sup>2</sup>University of Catanzaro "Magna Graecia", Department of Pharmacobiological Sciences, Roccelletta di Borgia (CZ), Italy; <sup>3</sup>I.N.M.I. "L. Spallanzani", Sequencing and Antiviral Drug Monitoring Unit, Rome, Italy; <sup>4</sup>Marche Polytechnic University Medical School, Institute of Microbiology, Ancona, Italy; <sup>5</sup>"Sapienza" University of Rome, Department of Experimental Medicine Virology Section, Rome, Italy; <sup>6</sup>"L. Sacco Hospital", Infectious Disease Division, Milan, Italy; <sup>7</sup>Amedeo di Savoia Hospital University of Turin, - Department of Infectious Diseases, Turin, Italy; <sup>8</sup>Modena University Hospital, Department of Diagnostic and Laboratory Services and Legal Medicine, Modena, Italy; <sup>9</sup>Foundation IRCCS Policlinico "S. Matteo", Virology Unit, Pavia, Italy; <sup>10</sup>Ospedali Riuniti, Department of Infectious Diseases, Bergamo, Italy; <sup>11</sup>San Martino Hospital, Microbiology and Virology Laboratory, Genoa, Italy; <sup>12</sup>Catholic University of Sacred Heart, Institute of Clinical Infectious Diseases, Rome, Italy; <sup>13</sup>University of Bologna, Section of Microbiology of the Department of Hematology and Oncologic Science, Bologna, Italy; <sup>14</sup>University of Siena, Department of Molecular Biology, Siena, Italy; <sup>15</sup>"S. Raffaele" Scientific Institute, Department of Infectious Diseases, Milan, Italy; <sup>16</sup>University of Foggia, Clinic of Infectious Diseases, Foggia, Italy; <sup>17</sup>University of Padua, Department of Histology, Microbiology and Medical Biotechnology, Padua, Italy

**Background.** The interaction between gp120 and CCR5 N-terminus is critical for R5 virus entry and affects CCR5-antagonist activity. Knowledge on how different genetic signatures of the gp120 V3 domain impact on the strength of this interaction is limited.

**Methods.** HIV-1 co-receptor usage was assessed in 348 patients using Enhanced Sensitivity Trofile assay (ESTA) and V3-sequencing. Genotypic determination of HIV-1 tropism was performed by Geno2pheno (set at a False Positive Rate [FPR] of 10% and 5%) and PSSM. The association of mutations with different co-receptor usage was assessed by Fisher's exact test. Covariation-analysis was based on the binomial correlation coefficient ( $\phi$ ) and hierarchical clustering. Benjamini-Hochberg method was used to correct for multiple hypothesis testing. The impact of mutational patterns on CCR5 N-terminus interaction was evaluated by docking analysis and molecular modeling.

**Results.** ESTA reports were obtained for 319 samples indicating R5 in 68.3%, and DM/X4 in 31.7% of samples (30.1% DM and 1.6% X4). Genotypic testing successfully assesses viral tropism for all 348 samples, including the 29 with undetermined result by ESTA.

Correlation between genotypic and phenotypic data let us identify new V3 genetic determinants (other than the classical positions 11 and 25) associated with CCR5- and CXCR4-usage. Among them, K10Q, T22A, and R18Q (the latter completely absent in X4-viruses) occur in 2.9%, 72.5% and 16.7% of R5-viruses, and correlate with negatively charged amino acids at position 25 (E/D) ( $P < 0.001$ ).

Conversely, the acquisition of positively charged amino acids in V3 correlates with CXCR4-usage. This is the case of Q32K/R and N7K (the latter completely absent in R5-viruses) occurring in 45.9% and 6.6% of X4-viruses ( $P < 0.03$ ), and correlating with positively charged aa (K/R) at position 11 and 25 ( $P < 0.01$ ). The co-presence of positive charges at position 25+32 is also associated with a decreased g2p score (25K/R:3.2%, 25K/R+32K:1.1%,  $P = 0.006$ ) supporting an increased CXCR4-usage potential. Docking analysis reveals that positive charge loss in V3 stem (as in presence of K10Q) increases gp120 affinity toward CCR5 N-terminus in terms of estimated free energy of complexation (K10Q:-6.8Kcal/mol, WT:-6.5Kcal/mol). Such affinity is not affected by positive charge loss in V3 crown.

By contrast, N7K and Q32K determine a reduction of gp120 binding affinity with CCR5 N-terminus even stronger than that observed when positive charges are present at the classical positions 11 and 25 (N7K:-5.7Kcal/mol, Q32K:-5.2Kcal/mol, E25R=S11R:-6.4Kcal/mol, WT:-6.5Kcal/mol). Interestingly the presence of positive charge in Q32K involves a shift of the sulphotyrosine at CCR5 position 14 from the crevice between V3 and the bridging sheet of gp120.

**Conclusions.** New genetic determinants of tropism within the V3 domain are detected and confirmed by phenotypic and structural analysis. This supports the use of genotype for a finer tuning of potential efficacy of CCR5 antagonists.

No conflict of interest