Potential impact of differences in frequency of minor substitutions between HIV-1 subtypes on the genetic barrier for resistance to protease inhibitors

D.A.M.C. van de Vijver[1], A.M.J. Wensing[1,2], G. Angarano[3], B. Åsjö[4], C. Balotta5, E. Boeri[6], R. Camacho[7], M-L. Chaix[8], D. Costagliola[9], E.L.M. Op de Coul[10], A. de Luca[11], I. Maljkovic[12], C. de Mendoza[13], I. Derdelinckx[14], Z. Grossman[15], O. Hamouda[16], A. Hatzakis[17], I.M. Hoepelman[2], R. Hemmer[18] A. Horban[19], K. Korn[20], C. Kücherer[16], T. Leitner[21], C. Loveday[22], E. MacRae[22], L. Meyer[23], C. Nielsen[24], V. Ormaasen[25], L. Perrin[26], D. Paraskevis[17], E. Puchhammer-Stöckl[27], L. Ruiz[28], .M Salminen[29], J.C.C. Schmit[18], F. Schneider[18], R. Schuurman[1], V. Soriano[13], G. Stanczak[19], M. Stanojevic[30], A-M. Vandamme[14], K. Van Laethem[14], M. Violin[5], K. Wilbe[12], S. Yerly[26], M. Zazzi[31] and C.A.B. Boucher[1] on behalf of the SPREAD Programme.

1 Eijkman Winkler Institute, Dept of Virology, University Medical Center Utrecht, Utrecht, the Netherlands 2 Dept of Internal Medicine, University Medical Center Utrecht, Utrecht, the Netherlands 3. University of Foggia, Foggia, Italy 4. University of Bergen, Bergen, Norway 5. University of Milan, Milan, Italy 6. Diagnostica e Ricerca San Raffaele, Milan Italy 7. Hospital Egas Moniz, Lisbon, Portugal 8. Laboratoire de virologie, Hôpital Necker Paris, France 9. INSERM EMI 0214, CHU Pitié-Salpétrière, Paris, France 10. National Institute for Public Health and the Environment, Bilthoven, the Netherlands 11. Institute of Clinical Infectious Diseases, Catholic University, Italy, Rome 12. Swedish Institute for Infectious Disease Control, Solna, Sweden 13. Hospital Carlos III, Madrid, Spain. 14. Rega Institute, Katholieke Universiteit Leuven, Leuven, Belgium 15. Sheba Medical Center, Tel-Hashomer, Israel 16. Robert Koch Institute, Berlin, Germany 17. Athens University Medical School, Athens, Greece 18. Centre Hospitalier de Luxembourg, Luxembourg 19. Hospital for Infectious Diseases & AIDS Diagnosis and Therapy Center, Warsaw, Poland 20. University of Erlangen, Erlangen, Germany 21. Los Alamos National Laboratory, Los Alamos, USA 22. International Clinical Virology Centre, Buckinghamshire, England, United Kingdom 23. INSERM U569, Kremlin-Bicêtre, France 24. Statens Serum Institute, Copenhagen, Denmark 25. Ullevaal University Hospital, Oslo, Norway 26. Geneva University Hospital, Geneva, Switzerland 27. University of Vienna, Vienna, Austria 28. Retrovirology Laboratory IRSICAIXA Foundation, Badalona, Spain 29. National Public Health Institute, Helsinki, Finland 30. University of Belgrade, Belgrade, Serbia-Montenegro 31. University of Siena, Siena, Italy

Background – The number of minor protease substitutions in wild-type virus at positions targeted during antiretroviral drug treatment, varies considerably between HIV-1 subtypes. Minor protease substitutions by themselves do not impair drug susceptibility but might enhance resistance by improving the replicative capacity of the resistant virus. Therefore, the subtype naturally containing the largest number of minor protease substitutions could have a lower genetic barrier for drug resistance. In this study, the number of minor protease substitutions for every protease inhibitor (PI) was compared between subtypes using almost 2000 sequences from antiretroviral-naïve patients.

Methods – Identified were subtypes A to G, J, CRF01_AE and CRF02_AG. The most common clade was B (1299 sequences). Among the 556 sequences classified with a non-B subtype, C (n=209) and G (86) were the most frequent. The relevant minor substitutions were obtained for every PI from the IAS-USA list of 2004. The frequency of minor protease substitutions was compared between sequences of clade B and all individual non-B subtypes using Kruskal-Wallis and Mann-Whitney tests.

Results – The sequences of non-B subtype had 20% (subtypes C, D and CRF01_AE) to 70% (G, J, CRF02_AG) more minor protease substitutions as compared to clade B ($p<0.001$). All individual non-B subtypes contained on average more minor substitutions specific for indinavir, nelfinavir, atazanavir and ritonavir ($p<0.001$). Conversely, subtype B sequences generally harboured more minor protease substitutions relevant for amprenavir, saquinavir, lopinavir/ritonavir and tipranavir ($p<0.001$). Specifically, non-B sequences contained relatively more often the K20R (generally >15% for most non-B sequences versus 2% in B; $p<0.001$), and M36I substitutions (>85% in non-B and 17% in B; $p<0.001$). On the other hand, L63P (9-30% of non-B, 57% of B), and V77I (0% to 15% in individual non-B, 27% of B) were found more frequently in subtype B sequences ($p<0.001$).

Conclusion – Sequences of all individual non-B subtypes contain in general more minor protease substitutions. Whether these differences in frequency of minor substitutions between subtypes are associated with dissimilarities in the genetic barrier remains to be investigated. If confirmed, such differences should be taken into account in the initial choice of antiretroviral drug regimens.