

nonsynonymous substitutions within HIV-1 infected individuals. Among the 25,251 polymorphic codon sites analysed, FUBAR revealed that 189-fold more were detectably evolving under persistent negative selection than were evolving under persistent positive selection. Three specific codon sites within the genes *celA2b*, *katG*, and *cyp138* were identified by MEDS as displaying significant evidence of evolving under directional selection influenced by HIV-1 co-infection. All three genes encode proteins that may indirectly interact with human proteins that, in turn, interact functionally with HIV proteins. Unexpectedly, epitope encoding regions were enriched for sites displaying weak evidence of directional selection influenced by HIV-1. Although the low degree of genetic diversity observed in our *M. tuberculosis* dataset means that these results should be interpreted carefully, the effects of HIV-1 on epitope evolution in *M. tuberculosis* may have implications for the design of *M. tuberculosis* vaccines that are intended for use in populations with high HIV-1 infection rates.

A20 Deep sequencing reveals viral evolution in GAG within protective HLA Alleles B*57: 02, B*58: 01, and B*7 supertype individuals acutely infected with HIV-1 subtype C in Durban, South Africa

K. Gounder,^{1,2} V. Naidoo,^{1,2} N. Padayachi,^{1,2} Q. Mthethwa,^{1,2} D. Dilemnia,³ E. Hunter,³ B. Walker,^{1,4} and T. Ndung'u^{1,2}

¹HIV Pathogenesis Programme, Doris Duke Medical Research Institute, University of KwaZulu-Natal, Durban 4013, South Africa, ²KwaZulu-Natal Research Institute for Tuberculosis and HIV (K-RITH), ³Emory Vaccine Center at Yerkes National Primate Research Center, Emory University, Atlanta, GA, USA and ⁴Ragon Institute of Massachusetts General Hospital, MIT and Harvard, Boston, Cambridge, MA, USA

Transmission of cytotoxic T cell escape variants and the timing and frequency of CTL-mediated viral escape following acute HIV-1 infection can profoundly influence disease course, but comprehensive analysis of CTL epitopes restricted by protective HLA class I alleles is lacking. We evaluated the transmission of CTL immune escape variants and immune selection over one-year following acute HIV-1 infection for epitopes restricted by the B*7 supertype and protective HLA-B*57 and HLA-B*58: 01 alleles. HIV-1 uninfected women were screened twice weekly for HIV-1 RNA by finger prick blood draw. Six females were identified possessing the HLA-B*7 supertype [HLA-B*07 ($n=2$), HLA-B*39: 01 ($n=2$), HLA-B*42: 01 ($n=1$), and HLA-B*42: 02 ($n=1$)] while six women possessed the protective HLA-B*57: 02 ($n=1$) and HLA-B*58: 01 ($n=5$) class I alleles. Plasma samples were available at baseline (Fiebig I-III) and at two to six subsequent time points thereafter over one year of infection. Deep sequencing of near full-length HIV-1 genomes was performed using the Illumina MiSeq platform. Amplicons were molecularly bar-coded, pooled, and sequenced resulting in >250-fold coverage. A subset of limiting-dilution full-length HIV-1 genome amplicons were also sequenced by PacBio at ~1,000-fold coverage. Sequence analysis was performed using Geneious v8.1.8 (Biomatters Ltd). In transmitted/founder viruses, the four known Gag CTL epitopes presented by HLA-B*57/B*58: 01 were wildtype in two of the six participants, with the remaining four participants showing evidence of CTL-mediated pre-adaptation in at least one epitope. By one-year post-infection, de novo CTL-mediated selection was observed in all six subjects, but never in all four epitopes. About five of six participants experienced escape within the immunodominant TW10 epitope. Of the five known Gag epitopes presented by the HLA-B*7 supertype, all six participants showed evidence of a CTL variant in at least three epitopes within the transmitted virus sequence. The TL9

epitope remained wild type in five of six participants with no evidence of CTL escape up to one-year post-infection. Transmitted escape variants remained virtually unchanged throughout the follow up period except in one participant who showed evidence of slow reversion in the HLA-B*57/58 ISW9 epitope. Deep sequencing reveals extensive transmission of pre-adapted CTL variants and slow immune selection within immunodominant epitopes restricted by protective HLA class I alleles.

A21 Evolvability of HIV-1 is influenced by codon pair usage

M. Nevot, M. Parera, G. Martrus and M. A. Martínez

Irsicaixa, Hospital Germans Trias i Pujol, Badalona, Spain

HIV-1 populations, like other RNA viruses, are established as a closely related mutant spectrum or cloud, which have an impact on virus evolvability, fitness, and virulence. The influence of codon choice in population diversity and evolvability of HIV-1 remains poorly explored. Here, we compared the development of HIV-1 resistance to protease inhibitors (PIs) of wild-type (WT) virus and a synthetic virus (MAX) carrying a codon-pair re-engineered protease sequence with thirty-eight (13 per cent) synonymous mutations. WT and MAX viruses replicated indistinguishably in MT-4 cells or PBMCs. To explore the evolvability of the codon pair re-coded protease, WT and MAX viruses were subjected to serial passages with the selective pressure of PIs [atazanavir (ATV) and darunavir (DRV)]. After the same number of successive passages in MT-4 cells in the presence of PIs, WT and MAX viruses developed phenotypic resistance to PIs (IC₅₀ 14.63 ± 5.39 nM and 21.26 ± 8.67 nM, for ATV; and IC₅₀ 5.69 ± 1.01 μM and 9.35 ± 1.89 for DRV, respectively). Sequence clonal analysis showed the presence, in both viruses, of previously described resistance mutations to ATV and DRV. However, a different resistance variant repertoire appeared in the MAX virus protease when compared to WT. The G16E substitution was only observed in the WT protease while the L10F, L33F, K45I, G48L, and L89I substitutions were only detected in the re-coded MAX protease population. The influence of the G48L mutation, which is extremely rare in vivo, on viral fitness was assessed by introducing this variant in the WT background. In the absence of drug, no differences on viral fitness were observed between the WT and MAX viruses carrying the G48L mutation. In order to detect minority variants in the quasispecies that could explain the emerge of G48L mutation, deep sequencing analysis using the Illumina MiSeq benchtop deep sequencer was performed. The implication of this mutation in the re-coded context is being explored considering that a particular sequence space can delineate the evolution of their mutant spectrum.

A22 Spatio-temporal history of the HIV-1 circulating recombinant form 35_AD in Afghanistan and Iran

S. Eybpoosh,^{1,2} E. Mostafavi,² K. Azadmanesh,³ and A.-A. Haghdoost^{2,4}

¹Research Centre for Emerging and Reemerging Infectious Diseases, Pasteur Institute of Iran, Tehran, IR Iran, ²HIV/STI Surveillance Research Center, and WHO Collaborating Center for HIV Surveillance, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran, ³Department of Virology, Pasteur Institute of Iran, Tehran, IR, Iran and ⁴HIV/STI Surveillance Research Center, and WHO Collaborating Center for HIV Surveillance, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran

Circulating recombinant form 35_AD (CRF35_AD) has an important role in the epidemiological profile of Afghanistan and Iran. Despite the presence of this clade in Afghanistan and Iran for