cent) were successfully genotyped: HIV-1 subtype C was the most prevalent (97 per cent), followed by subtype A1 (1.2 per cent), mosaic recombinant forms (1.2 per cent), subtype G (0.5 per cent), and subtype D (0.2 per cent). Over 60 per cent of the children had at least one mutation associated to drug resistance to NRTI/NNRTI. As expected a high frequency of mutations to NNRT (52.9 per cent) in comparison to NRTIs (11.4 per cent) due to the low genetic barrier of this class of drugs was observed. For NNRTI, K103N, Y181C, E138A, and G190A were most common whereas for the NRTI, M184V was the most common. Maternal prophylaxis with HAART was significantly (P < 0.05, OR 2.41) associated with the emergence of drug resistance mutation to NRTI in HIV infected children. In conclusion, the high level of transmitted-resistant viruses observed in this study suggests that political strategies to enhance the level of adherence in maternal HAART should be taken into account. This is an important consideration due to the fact that Mozambique recently implemented the integrated PMTCT option B+ approach. Low adherence in such a treatment context may undermine de efficacy of first-line option in the country. Continuing surveillance studies among HIV infected children remains critical to monitor the impact of PMTCT strategies. Furthermore, current results highlight the need to reinforce adherence counseling with appropriate follow up.

A17 Transmitted drug-resistant mutations among recently infected HIV-1 patients in Israel, 2000–2014

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Transmitted drug-resistant mutations (TDRM) may hamper successful anti-HIV-1 therapy and impact future control of the HIV-1 epidemic. Recently infected, therapy-naïve individuals are best suited for surveillance of TDRM. Here, we investigated the prevalence of HIV-1 mutations in a sample of recently infected HIV-1 patients diagnosed between 2000 and 2014 in Israel. Historical samples from eighty recently infected patients were subjected to ABI-based sequencing (ABS) and MiSeq next-generation sequencing (NGS). DeepChek-HIV software was used to analyze the results. Most patients were males (80 per cent) and men who have sex with men were the major risk group (58.8 per cent). Overall, TDRM was detected in 8.8 per cent of patients by ABS and 31.3 per cent by NGS, ranging from 2.7 or 24.3 per cent, respectively, in 2000–2007 to 13.9 or 37.2 per cent, respectively, in 2008–2014. All ABS-detected TDRM were identified by NGS. The prevalence of TDRM impacting protease inhibitors, nucleoside reverse transcriptase inhibitors and nonnucleoside reverse transcriptase inhibitors was 0, 3.8, and 5 per cent, respectively, for ABS analyses and 11.3, 26.2, 7.5 per cent, respectively, for NGS analyses. Patients with NGS detected TDRM had significantly lower viral load (4.9 vs. 5.7 median log copies/ml, P < 0.05) and higher number of low-prevalence non-synonymous reverse transcriptase (RT) mutations compared to those without NGS-detected TDRM. None had integrase resistance mutations. The most abundant, albeit, minor-frequency RT TDRM were the K65R and D67N, while K103N, M184V, and T215S were observed at high frequency. Minor TDRM did not persist in later samples and did not hinder successful treatment. NGS can substitute ABS for surveillance of TDRM. Although rates of HIV-1 protease and RT TDRM in Israel are high and continue to increase from 2000 to 2014, minor TDRM do not become major

species or interfere with therapy. The need for ongoing surveillance of low-frequency TDRM should be revisited in a larger study.

A18 HIV-1 genetic diversity and drug resistance in Haiti

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HIV-1 subtype B appears to be the most genetically diverse AIDS virus in Haiti. However, there have been few phylogenetic analyses on circulating drug-resistant HIV-1 strains. The primary objective of this study is to analyze the patterns of genetic diversity of HIV-1 in Haiti and describe the molecular epidemiology of HIV-1 drug resistance (HIV-DR) in the population. HIV-1 genotyping was performed for HIV-positive patients who were either initiating or failing antiretroviral therapy from 2005 to 2010. The Stanford University Database (http://hivdb.stanford.edu/hiv) was used to analyze the protease-RT sequences for mutations associated with resistance to antiretroviral drugs. In the context of this course, the pol gene sequences obtained from these patients will be pooled in a database and used to perform the phylogenetic analyses. This study will provide a global view of HIV-1 genetic diversity and drug resistance strains circulating and transmitted within the country. Phylogenetic analysis of HIV-DR strains is absolutely necessary to monitor HIV-DR strain evolution, and will also contribute to strengthening the implementation of public health strategies to prevent and address the emergence of HIV-DR in Haiti.

A19 The impact of HIV-1 on the evolution of *Mycobacterium tuberculosis*

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HIV significantly affects the immunological environment during tuberculosis co-infection, and therefore may influence the selective landscape upon which *M. tuberculosis* evolves. To test this hypothesis, whole-genome sequences were determined for 169 South African *M. tuberculosis* strains from HIV-1 co-infected and uninfected individuals and analysed using two Bayesian codon-model based selection analysis approaches: FUBAR which was used to detect persistent positive and negative selection (selection respectively favouring and disfavouring nonsynonymous substitutions); and MEDS which was used to detect episodic directional selection specifically favouring

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

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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 barcoded, 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

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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

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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