if selected gp41 mutational clusters correlate with viro-immunological outcome. One hundred ninety five sequences of HIV-1 gp41 and clinical follow-up from 77 T-20 treated pts were analyzed from baseline (BL) up to week (wk) 48. Covariation analysis was based on the binomial correlation coefficient and hierarchical clustering. Nine mutations (A30T/L54M/E119Q/S129D/G/N126K/N140I/D239H/T268A) were positively associated with T-20 treatment and correlated with known T-20 resistance mutations. In particular, strong correlation was observed for N140I with V38A and for D239H with Q40H and L45M. Our analysis revealed the existence of 4 clusters of mutations: (1) V38A with N140I, S129G and A30T; (2) N43D with S138A; (3) G36V with N126K; (4) Q40H, L45M with the L54M, E119Q, S129D, T286A and D239H. Co-presence of N140I with V38A was associated ($P < .05$) with a CD4 increase from BL (40 c/μl) of 2-fold (210 c/μl) at week 24 and 4.7-fold (249 c/μl) at week 48 compared with V38A alone, without significant changes in VL. In contrast, the presence of D329H duplicated CD4 loss from BL (124 c/μl) to week 48 (35 c/μl) given by Q40H + L45M ($P = .05$), without significant changes in VL. Moreover, specific polymorphisms at BL were correlated ($P < .05$) with the on treatment development of T-20 resistance mutations. In particular, P213Q and R236Q at BL correlated with development of V38A and N43D, respectively. Our study shows that gp41-mutational patterns under T-20 pressure are more complex than currently known, suggesting that an ordered network of mutations, regulated by natural polymorphisms present before T-20 treatment, modulates positively and negatively the HIV ability to damage the immune system. Their knowledge is important for a correct use of T-20 and for innovative therapeutic strategies.

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6

Drug Resistance to Tipranavir (TPV) or Darunavir (DRV) According to New Interpretation Algorithms in PI-naïve HIV-1 Infected Patients

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Background: Genotypic interpretation algorithms to newly approved anti-HIV drugs are mainly derived from respective phase II and phase III trials. However, study conditions and patients included might not reflect the situation in routine clinical circumstances. Therefore, interpretation algorithms might be biased depended on patient populations used for evaluation and validation. Current algorithms for interpretation of TPV and DRV include mutations, which are present in naïve patients, especially in patients infected with non-B subtypes. The aim of this study was to analyze the performance of these algorithms in PI-naïve patients.

Methods: The Frankfurt HIV Cohort was searched for genotypes from PI-naïve patients; 1002 samples were analyzed. A score ≥5 out of 10V, 13V, 20MRV, 33F, 35G, 36I, 43T, 46L, 47V, 54AMV, 58E, 69K, 74P, 82LT, 83D, 84V and ≥3 out of 11I, 32I, 33F, 47V, 50V, 54LM, 73S, 76V, 84V, 89V was associated with intermediate resistance to TPV and DRV, respectively. Subtypes were analyzed based on the respective pol-region (not available for all samples).

Results: 510/1002 (50.9%) and 993/1002 (99.1%) samples had a score of 0 for TPV and DRV, respectively. 491/1002 (49.0%) samples had a score of 1–4 for TPV, and 9/1002 (0.9%) samples a score of 1 for DRV. Only 1/1002 (0.1%) showed intermediate resistance to TPV (score of 5), and none to DRV. The proportion of non-B subtypes significantly increased from 0% to 4.5%, 64.6%, 97.2%, 100% and 100% with an increasing TPV score ($p < 0.001$; Kruskal-Wallis Test).

Conclusions: Intermediate resistance to TPV and DRV in PI-naïve patients was detected very rarely, if at all. Based on our data, both algorithms seemed to be practical for the interpretation of TPV and DRV at least in PI-naïve patients. However, an increase in the TPV score was significantly associated with a non-B subtype, which might reflect a bias in patient populations used for evaluation and validation of the TPV algorithm. More clinical data from TPV and/or DRV failing patients not included in studies are necessary to validate current algorithms.

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7

HIV Interactions With Other Viruses Determine Pathogenesis in Human Lymphoid Tissues

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Critical events in HIV disease occur in lymphoid tissues, often coinfected with other microbes (co-pathogens). To investigate their interactions in these tissues, we studied viral pathogenesis in human tonsillar and gut tissues infected ex vivo with HIV-1 alone or in combination with other viruses, including human herpesviruses (HHV) 6, and 7, vaccinia virus (VV), measles (MV) and human cytomegalovirus (HCMV). As in vivo, both activated and non-activated T cells supported productive viral infection in blocks of human tonsillar tissue infected ex vivo with R5 or X4 HIV-1, although activated T cells determined the "viral load". Productive HIV infection correlated with CD25/HLA-DR expression but not with CD69 expression. HIV infection facilitates this pattern of activation creating new target cells that are efficient at replicating the virus, leading to cell apoptosis Upregulation of cytokines/chemokines in infected tissues is another sign of activation. This is typical of HIV-infected tonsillar tissue, whereas in infected rectosigmoid tissue these chemokines are not upregulated. The lack of such upregulation may contribute to the high vulnerability of the gut to HIV,

making this tissue a primary site of viral infection. Coinfection with human herpesviruses 6 and 7 or measles virus selectively modulates infection by R5 and X4 HIV by changing chemokine release and the expression of HIV receptors and coreceptors. In conclusion, we describe an HIV-triggered complex cycle of infection-activation-infection that creates new targets for HIV as well as for other viruses via cell activation, involving new cells in productive infection, upregulating cytokines and triggering apoptosis. This cycle is greatly affected by coinfecting pathogens, which by these means can determine the course of HIV disease progression. Intervention in the interactions between HIV and other pathogens may provide a new tool for antiviral therapy.

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Oral Session II: Respiratory and West Nile Viruses

8

Identification and Biochemical Characterization of Small Molecule Inhibitors of West Nile Virus Serine Protease by a High Throughput Screen

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West Nile Virus (WNV) and dengue virus (DV) are mosquito-borne members of *Flaviviridae* that cause widespread human disease for which there is no vaccine or chemotherapy. These viruses, like all flaviviruses, encode a serine protease (NS3-pro) that is essential for polyprotein processing, a required step in viral replication. In this study, we report the development and validation of an in vitro, high throughput screening (HTS) assay for WNV protease. Using this assay, more than 32,000 small molecule compounds were screened, from which three core chemical structures were identified among them that inhibit the protease. A secondary screen of seven compounds selected from the three core structure groups, identified two compounds (A and B) as strong WNV protease inhibitors with K_i values as low as ~3 μM. Based on molecular docking of compound B with the recently reported crystal structure of WNV protease, we propose that compound B binds in the vicinity or within the substrate-binding pocket involved in the interaction with the P1 residue of the substrate. Furthermore, we suggest a plausible mechanism of protease inhibition by this group of compounds. This assay will be useful to identify other potent inhibitors of the flaviviral protease and lead the way for development of antiviral therapeutics against WNV and related flaviviruses.

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9

Discovery of a New Class of Polycyclic RSV Inhibitors

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Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis and pneumonia in children under 1 year of age and is a leading cause of severe lower respiratory infections in infants and young children. Prophylactic antibodies such as Synagis® (palivizumab) effectively reduce the incidence and severity of RSV disease in high-risk pediatric populations but the only antiviral treatment available for patients with RSV disease is ribavirin, a nucleoside analog with suboptimal clinical efficacy and safety profile.

We have discovered a new class of imidazoisoindolone RSV inhibitors with general structure as depicted in Fig. 1. The synthesis of this novel series of compounds will be described with the identification of key features important for antiviral activity. Medicinal chemistry has been applied to develop highly active and specific small molecules species that inhibit RSV.

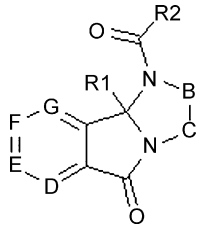

Fig. 1.

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Potent Inhibition of Viral Entry and Replication of SARS-CoV by siRNAs Targeting the Genes Encoding the Cellular ACE2 Receptor or the Viral Nucleocapsid Protein

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Severe acute respiratory syndrome (SARS), which is caused by a newly identified human coronavirus named SARS-associated