3

The CXCR4 Antagonist POL3026 is a Potent Inhibitor of Human Immunodeficiency Virus

Gemma Moncunill [1,*], Imma Clotet-Codina [1], Anuska LLano [1], Mercedes Armand-Ugón [1], Bonaventura Clotet [1], Jan Willem Vrijbloed [2], José A. Esté [1]

[1] Retrovirology Laboratory IrsiCaixa and AIDS Unit, Hospital Germans Trias i Pujol, 08916 Badalona, Spain; [2] Polyphor Ltd., Basel, Switzerland

The HIV-1 coreceptors are an important target for the design of new antiviral compounds for the multidrug therapy of the HIV infection. Here, we show the potent anti-HIV activity of novel specific b-hairpin mimetic CXCR4 antagonist POL3026 and its mechanism of action. POL3026 consistently blocked the replication of X4 and dualtropic HIV strains in different stable cell lines, peripheral blood lymphocytes, macrophages and ex vivo lymphoid tissue. Our results show that POL3026 was active with 50% effective concentrations (EC_{50}) at the nanomolar range (25–0.1 ng/ml) against laboratory adapted and clinical isolates of HIV-1 including virus strains that are resistant to current antiretroviral agents (nucleoside and non-nucleoside reverse transcriptase inhibitors, protease inhibitors and the fusion inhibitor enfuvirtide). However, AMD3100-resistant and SDF-1 resistant HIV-1 strains presented cross-resistance to POL3026, suggesting a similar mode of anti-HIV activity. POL3026 specifically blocked the binding of anti-CXCR4 monoclonal antibody 12G5 (IC_{50}: 0.0005 mg/ml) and the intracellular Ca^{2+} signal induced by CXCL12 but did not inhibit anti-CCR5, -CD4 or -CD45 monoclonal binding or signaling induced by CCR5 ligands, suggesting its specificity for CXCR4. In a cell culture model of the evolution of HIV-1 coreceptor use, POL3026 prevented the emergence of the X4 variants from a R5 HIV-1 strain. POL3026 and analogues, have shown excellent plasma stability, high selectivity for CXCR4 and favorable pharmacokinetics. These agents have the potential to become a therapeutic option for application in the treatment of HIV infections.

doi:10.1016/j.antiviral.2007.01.011

4

Mutations in the RNaseH Region Observed in the HIV-1 of Antiretroviral Treatment (ART) Experienced Patients

J. Waters [1,*], W. O'Neal [1], K. White [2], E. Lansdon [2], M. Miller [2], J. Harris [1], K. Borroto-Esoda [1]

[1] Gilead Sciences, Durham, NC, USA; [2] Gilead Sciences, Foster City, CA, USA

Background: ART that targets the HIV-1 protease and reverse transcriptase (RT) has been effective in the management of HIV-1 disease. As new drugs are being developed to target RNaseH activity, it is imperative to evaluate if previous ART, specifically RT inhibitors, may induce mutations in RNaseH that could lessen the efficacy of novel RNaseH inhibitors.

Methods: Genotypic analysis was performed on HIV polymerase from 100 ART-naive and 110 ART-experienced patients with subtype B HIV-1. Sequences were divided into four regions: RT (amino acid (AA) 1–240), RT C-terminus (AA 241–318), RT connection domain (AA 319–426) and RNaseH (AA 427–560). Patient sequences were compared to a reference and the percent conserved at each AA position was calculated for each group separately and then compared to the other using a Fisher's exact test. Changes between the two groups within the RT region were used as a control to establish significance. An unadjusted cutoff for significance was defined as a p-value ≤ 0.01 at conserved AA positions ($\geq$95% homology in naive or 100% conserved in either group).

Results: Within the RT domain, there were 21 positions at known resistance sites with significant AA changes between the groups. Within the RNaseH domain, there were two AA positions, 451 and 463, with significant changes between the groups. The consensus K451 was replaced by arginine in 7.3% of experienced patients but remained 100% conserved among naive patients. This position appears to interact with the phosphate backbone of the primer in HIV-1 polymerase models. By contrast, the consensus R463 was 100% conserved in experienced patients while 8% of naive patients had lysine at this position. Differences between the two groups were also observed at position 284 ($p = 0.007$) in the RT C-terminus and at position 348 ($p < 0.001$) in the RT connection domain. At each of these positions there was only one AA change.

Conclusions: Significant AA differences between ART-naive and experienced patients were observed at positions outside of HIV-RT that appear to be the result of ART with RT inhibitors. These changes may impact the development of new compounds that target other functions of the HIV polymerase such as RNaseH.

doi:10.1016/j.antiviral.2007.01.012

5

Involvement of New Mutational Pattern in HIV-1 gp41 in T-20 Treatment

Stefano Aquaro [1,2,*], Valentina Svicher [2], Roberta D'Arrigo [3], Mario Santoro [2], Giovanni Di Perri [4], Sergio Lo Caputo [5], Ubaldo Visco-Comandini [3], Pasquale Narciso [3], Andrea Antinori [3], Carlo Federico Perno [3]

[1] University of Calabria, Rende (CS), Italy; [2] University "Tor Vergata", Rome, Italy; [3] "L Spallanzani" Institute, Rome, Italy; [4] University of Turin, Italy; [5] "SM Annunziata" Hospital, Florence, Italy

Recently, V38A and Q40H + L45M have been correlated with a gain and with a loss of CD4 count, respectively, in HIV-infected patients (pts) receiving T-20. The aim of this study is to investigate the long-term association of such mutations with the viro-immunological parameters, and

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.

doi:10.1016/j.antiviral.2007.01.013

6

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

M. Stuermer [1,*], B. Dauer [1], A. Haberl [1], A. Mueller [1], P. Gute [2], S. Klauke [3], S. Staszewski [1], H.W. Doerr [1]

[1] JWG-University Hospital, Frankfurt, Germany; [2] HIV Specialty Practice, Frankfurt, Germany; [3] IFS, Frankfurt, Germany

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.

doi:10.1016/j.antiviral.2007.01.014

7

HIV Interactions With Other Viruses Determine Pathogenesis in Human Lymphoid Tissues

Leonid Margolis

National Institute of Child Health and Human Development

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,