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Unusual Resistance Profile of Conocurvone, an Early-Stage Inhibitor of HIV-1 Replication

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Conocurvone, a trimeric naphthoquinone derivative isolated from extracts of the Australian shrub Conospermum incurvum, is a potent inhibitor of HIV-1-induced cell killing (EC_{50} < 20 μM) that is relatively strain specific (HIV-1_{RF} > HIV-1_{NL4-3} >>> HIV-1_{IIIB}). Recent *in vitro* mechanism of action studies reveal that conocurvone possesses dual inhibitory activity against both HIV-1 integrase and HIV-mediated cell fusion. To determine whether either of these mechanisms is a biologically relevant viral target for conocurvone, a drug-resistant HIV-1_{RF} virus (cono-R) was generated in CEM-SS cells using a standard dose-escalating selection protocol. Genotypic analysis of this cono-R virus revealed four amino acid changes in gp120, four in gp41, and none in integrase. One of the HIV Env mutations introduces a premature stop codon at position W766 and, as a result, truncated 100 amino acids from the cytoplasmic domain of gp41. Interestingly when an analogous proviral clone (HIV-1_{NL4-3} W766*) was constructed, the resultant virus was replication-defective yet able to fully induce syncitia formation in CEM-SS cells. Since studies elsewhere have described compensatory Gag matrix mutations in replication-competent viruses with similar HIV-1 Env truncations, we undertook a series of studies to identify compensatory Gag matrix mutations in the cono-R virus. To date, construction and characterization of numerous cono-R/HIV-1_{NL4-3} Gag matrix chimeric viruses has not identified any specific compensatory matrix mutations. As such, our data suggests the existence of as-yet-unidentified compensatory mutations in the conocurvone-resistant genome and provides evidence for the existence of potentially novel viral targets that may be exploited for future drug development.

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Genotypic and Phenotypic Analysis of HIV-1 from Patients Experiencing Virological Failure While on Combination Therapy Containing Two Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and the NNRTI, Emivirine (EMV, MKC-442): 48 Week Follow Up.

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Emivirine is a potent non-nucleoside reverse transcriptase inhibitor (NNRTI) with convenient twice daily dosing that has been well tolerated in Phase II/III trials. Therapy-naive patients were enrolled in two randomized, double blind, placebo-controlled studies to examine the addition of EMV in patients initiating therapy with d4T/3TC (MKC-301) or d4T/ddI (MKC-302). To determine if antiviral drug resistance was associated with therapy failure, all patients who experienced a protocol-defined virological failure had DNA sequence analysis performed for the polymerase gene of HIV isolated from plasma specimens from baseline and failure visits. Of 138 patients in both studies who were randomized to EMV, mutations associated with resistance to NNRTIs were observed for 32/138 (23.2%). The mutations observed were K103N (13/32), E138K (4/32), G190A (3/32), Y181C (2/32), K101E (2/32), E138A (1/32), E138Q (1/32), K103T (1/32), V108I,E138K (1/32), K101E,K103N (1/32), K103N,Y188H (1/32), K101E,V106M,138K (1/32), and K101E,K103N,E138K (1/32). Recombinant viruses containing the mutant RT genes from the patients' plasma HIV were assayed *in vitro* for susceptibility to EMV, delavirdine, efavirenz, and nevirapine. Viruses containing a E138K mutation remained sensitive to inhibition by all four NNRTI's. Viruses containing a K103N or K101E mutation were resistant to EMV and were also resistant to efavirenz, delavirdine, and nevirapine. Viruses containing mutations at V108I, E138A/Q, Y181C, or G190A, showed varying levels of resistance to EMV but remained sensitive to one or more of the other NNRTIs. The results of these studies indicate that viruses containing NNRTI mutations that arise during therapy with EMV remain sensitive *in vitro* to at least one other approved NNRTI in 13/32 cases (40.6%).

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The evolution of HIV-1 resistance mutations in two French couples

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Replication rate, preexisting resistance mutations, and viral diversity of HIV can lead to rapid development of resistant viral strains during antiretroviral treatment. In this study, the effects of antiretroviral treatment on the evolution of resistance mutations were followed for eight years in two HIV-1 infected couples where transmission within each couple was confirmed. We monitored the emergence of resistance mutations in protease and RT genes arising from the initial infection event through individualized therapies for all four patients. Utilizing population-based sequencing, genotypic analysis of each patient's RT and protease genes was performed at sequential time points: 1) during primary infection; 2) following nucleoside and/or nonnucleoside treatment; 3) following protease treatment; and 4) during their current treatment regimen. Multiple phylogenetic analyses using different nucleotide substitution models were performed on the RT and protease sequences. The viral loads were also measured. The RT and protease gene mutations of each clinical isolate were consistent with the treatments received by the patients. Three of the four patients had undetectable viral loads at some time during their treatment. The genetic sequence of the RT and protease genes of a virus from a patient reflects the antiretroviral treatment and phylogenetic origin. Despite the genetic bottleneck of purifying selection caused by the effects of highly active antiretroviral therapy, the phylogenetic origin is apparent. Therefore, the phylogenetic imprint can persist even after years of therapy.

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Treatment Naïve HIV-1 Infected Patients Respond Immunologically when Administered HE2000; Results from a Phase I/II Clinical Trial. J. Frincke, N. Onizuka-Handa, D. Stickney, C. Ahlem and C. Reading, Hollis-Eden Pharmaceuticals, Inc., San Diego, CA, USA.

A human clinical trial is underway in South Africa where 36 treatment naïve HIV-1 infected patients with $CD4^+$ T-cells >200 and HIV RNA >5000 copies/mL are receiving 50, 100 or 200 mg doses of HE2000 (16*a*-bromoepiandrosterone, P. Prendergast, Colthurst, Ltd). Patients initially received 1 dose and then 5 sequential daily doses. With no further treatment HIV RNA, $CD4^+$ T-cells, cytokines (IL-2, 4, 6, 10, 12, TNF_α, IGF_1 and $_\gamma$IFN), intra-cytoplasmic cytokines and immune cell subsets are monitored for up to 7 weeks. Patients that show a reduction in virus below baseline are re-administered 5 sequential daily doses of drug for up to a total of 7 treatment courses. No serious adverse events have been observed. The only side effect was mild pain at the injection site. To date, patients have received up to three-5 day treatment courses of HE2000 and the average change in viral titer for all patients to date is –0.56 log (mean time 54 days). A virologic response (>0.5 log decrease) has been observed in 46% of patients with a mean –0.96 log change at 69 days on average. Viral production is observed in 10/13 patients and correlates with cytoplasmic IL-2 production in stimulated CD4+ T cells. After an initial increase in viral titer, patients generally show an anti-viral response. In 3/13 patients with no viral progression no IL-2 production was observed. The durability of the response from a single 5-day cycle of therapy has ranged from 2-5 weeks. An overall shift in the stimulated CD4+ cytoplasmic cytokine balance from T2 (IL-10) to T1 (γ-IFN) is seen in 11/13 patients with a change in the mean T1 response from 11% at baseline to 79% at on average day 62 (21 days post therapy). Cytokine changes are accompanied by significant increases ($p<0.001$) in circulating dendritic cell precursors (Lin- HLA-DR+ CD11c+ or CD123+, 13/13 ep), early activated CD8 T cells ($CD8^+CD69^+CD25^-$, 11/11 ep), LAK cell phenotype ($CD8^+CD16^+CD38^+$, 10/13 ep) and ADCC/NK cells ($CD8^-CD16^+$, $p<0.001$, 12/13 ep). The data set will be updated with additional patients and reported at the meeting. These immune cell phenotypes are important to the control of disease and therefore HIV infected patients may benefit from treatment with the novel drug HE2000.