## Oral Session I: Retrovirus I

### 1

### Identification of a Novel Small Molecule Inhibitor that Targets HIV-1 Envelope Maturation

Judith Jimenez*, Joan Cao, Lynn Jackson, Qinghai Peng, Hua Wu, Jason Isaacson, Scott Butler, Amy K. Patick, Wade S. Blair

Pfizer Global Research and Development, La Jolla, CA, USA

**Background:** Therapeutic options for treatment-experienced patients are often limited due in part to drug resistance. One way to address the problem of drug resistance is to identify HIV-1 inhibitors directed against new targets in the HIV-1 replication cycle. As part of an effort to search for inhibitors targeting new mechanisms, a high throughput HIV-1 full-replication screen (HIV Rep) was executed resulting in the identification of a novel HIV-1 envelope (Env) maturation inhibitor.

**Methods:** Antiviral activity was determined after infection of MT-2 cells or HeLa CD4 LTR/beta-Gal indicator cells with HIV-1 NL4-3 or NL4-3 variants. Resistant viruses were selected in vitro using HIV-1 NL4-3 and MT-2 cells and characterized in susceptibility assays using HeLa CD4 LTR/beta-Gal indicator cells.

**Results:** A small molecule inhibitor of HIV-1 Env maturation (UK-201844) was discovered via a high throughput antiviral screen. UK-201844 exhibited antiviral activity against HIV-1 NL4-3 with an $EC_{50}$ value of 1.3 μM and $CC_{50}$ of 53 μM, yielding a therapeutic index of 41. However, the compound was not active against 15 clinical isolates tested, suggesting that antiviral spectrum may be an issue. UK-201844 specifically inhibited the production of infectious virions in an HIV-1 Env dependent manner. In addition, amino acid substitutions in HIV-1 Env were identified in in vitro resistant virus studies that were necessary and sufficient to confer resistance to UK-201844. These results demonstrate that UK-201844 acts during the late stages of HIV replication and suggest that HIV-1 Env is the target. Additional mechanism-of-action studies showed that UK-201844 interferes with HIV-1 gp160 processing in infected cells, resulting in the production of non-infectious virions.

**Conclusions:** UK-201844 represents the prototype for a unique HIV-1 inhibitor class that either directly or indirectly inhibits HIV-1 gp160 processing. Additional studies are warranted to determine if broad-spectrum antiviral activity is achievable with compounds targeting this mechanism.

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### 2

### GS-8374, a Novel Phosphonate HIV Protease Inhibitor with Potent In Vitro Antiretroviral Activity, Low Metabolic Toxicity, and Favorable Resistance Profile

Christian Callebaut[1,*], Kirsten Stray[1], Luong Tsai[1], Lianhong Xu[1], Gong-Xin He[1], Andrew Mulato[1], Tina Priskich[2], Neil Parkin[2], William Lee[1], Tomas Cihlar[1]

[1] Gilead Sciences, Foster City, CA, USA; [2] Monogram Biosciences, S. San Francisco, CA, USA

**Background:** GS-8374 is a novel bis-tetrahydrofuran-based HIV protease (Pr) inhibitor (PI) with a unique diethylphosphonate motif. This study characterizes its in vitro activity, resistance and toxicity profile in comparison with clinically used PIs.

**Methods:** Enzyme inhibition was determined using synthetic fluorescent substrates. p24 ELISA and/or XTT-based assays were used for antiretroviral activity and cytotoxicity. Effect on lipid accumulation and glucose uptake was assessed in human and mouse adipocytes, respectively. Resistance was characterized using the PhenoSense assay against a panel of 24 PI-resistant viruses with an average of 10 mutations in Pr.

**Results:** GS-8374 is a potent inhibitor of Pr ($K_i = 8$ pM) with a minimal effect on host aspartic proteases. It is active against HIV-1 in T-cell lines, primary lymphocytes and macrophages with potency comparable to that of darunavir and atazanavir. In contrast to ritonavir, lopinavir and several other PIs, GS-8374 showed little effect on the lipid accumulation and insulin-stimulated glucose uptake in adipocytes. In resistance profiling, GS-8374 exhibited a mean $EC_{50}$ fold change (FC) of 6.2 (range 0.6–26) relative to the wild-type control virus, whereas darunavir and brecanavir showed a mean FC of 29.8 (1.0–157) and 23.6 (1.2–121), respectively. Although tipranavir had similar FC as GS-8374 (5.9, range 0.5–27), its mean $EC_{50}$ was 90-fold higher than that of GS-8374. All the other marketed PIs showed a mean FC > 60. These results are consistent with the unique interaction of GS-8374 with mutant forms of Pr (Cihlar et al., J. Mol. Biol., 2006).

**Conclusions:** GS-8374 exhibits a favorable in vitro pharmacological profile with potent antiretroviral activity and low toxicity. Its resistance profile is superior to all tested PIs including darunavir. These data demonstrate the unique role of the phosphonate moiety in the resistance profile of GS-8374 and support further evaluation of this novel PI.

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