Characterization of a Replication-Competent, Integrase-Defective Human Immunodeficiency Virus (HIV)/Simian Virus 40 Chimera as a Powerful Tool for the Discovery and Validation of HIV Integrase Inhibitors†,‡

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Integrase is actively studied as an antiviral target, but many inhibitors selected from biochemical screens fail to inhibit human immunodeficiency virus (HIV) replication or primarily affect off-site targets. Here we develop and validate a replication-competent, simian virus 40-HIV integrase mutant chimera as a novel tool to classify the mechanism of action of potential integrase inhibitors. Whereas the mutant was more susceptible than the wild type to entry, reverse transcriptase, and protease inhibitors, it specifically resisted the action of integrase inhibitor L-870,810. We furthermore demonstrate inhibition of integration by GS-9137 and GS-9160 and off-site targeting by the 6-aminoquinolone antibiotic WM-5.

Integration of reverse-transcribed DNA is essential for productive human immunodeficiency virus type 1 (HIV-1) replication (14, 21), defining integrase (IN) as a target for antiretroviral therapy. Intense effort has been dedicated to developing IN inhibitors, and a variety of chemical classes have been described. Many of these, however, fail to function in cell culture or predominantly inhibit off-site targets (26). Zintevir, for example, potently inhibits HIV-1 replication and IN activity in vitro (22) yet primarily blocks virus entry (5, 8). L-Chicoric acid and derivatives also counteract IN activity, but virus entry can again be blocked (25, 28). Diketo compounds, in contrast, specifically inhibit IN activity and HIV-1 integration in cell culture (9–11). Two selective strand transfer inhibitors, S-1360 (Shionogi & Co.) (2) and L-870,810 (Merck & Company, Inc.) (9) (Fig. 1A), entered into clinical trials, though their development ceased due to unfavorable pharmacokinetics (29). Compounds GS-9137 (Gilead Sciences, Inc.) and MK-0518 (Merck & Company, Inc.) are in active development (7, 16).

GS-9137, a quinolone antibiotic (Fig. 1B), potently inhibits IN activity and HIV-1 replication (31), though the step in the viral life cycle inhibited by GS-9137 remains to be determined. It is important to note that the related 6-aminoquinolone antibiotic WM-5 (Fig. 1D), which likewise inhibits IN in vitro, can inhibit Tat-dependent transcription from the HIV-1 promoter (4, 23).

A time-of-addition experiment was performed to examine the replication step(s) affected by GS-9137 and WM-5, as well as the novel diketo compound GS-9160 (Fig. 1C). This design determines how long the addition of a compound can be postponed before it loses its antiviral function. Replication was determined by p24 content in MT-4 cell supernatants at 31 h following infection with HIV-1MN (provided by R. C. Gallo and M. Popovic) (27) at a multiplicity of infection of 0.5 (method adapted from reference 24). Control compounds included the entry inhibitor dextran sulfate (DS5000) (Sigma, Bornem, Belgium) (1, 18), reverse transcriptase inhibitors 3′-azido-3′-deoxythymidine (12, 19) and nevirapine (17) (obtained from Boehringer Ingelheim, Ridgefield, CT), IN inhibitor L-870,810 (a kind gift from D. Hazuda, Merck & Co., West Point, PA), and the protease inhibitor ritonavir (13) (obtained from Abbott Laboratories, Abbott Park, IL). As expected, DS5000 required addition along with the virus; a delay of even 1 h counteracted antiviral activity because virus adsorption had already occurred (Fig. 2). Protease inhibitor addition, in contrast, could be delayed for more than 12 h. Reverse transcriptase inhibitor addition could be delayed for 4 to 5 h, whereas L-870,810 could be delayed an additional 2 h. The addition of GS-9137 or GS-9160 (kindly provided by Gilead Sciences, Foster City, CA) could also be postponed for 7 h from the start of the experiment before potency losses were observed (Fig. 2). These results are indistinguishable from that obtained with the naphthyridine carboxamide IN inhibitor L-870,810 (9), consistent with the notion that GS-9137 or GS-9160 each inhibit integration in cell culture. In contrast, the addition of WM-5 (kindly supplied by O. Tabarrini and A. Fravolini, Perugia, Italy) could be delayed for 9 h, which is consistent with the potential to inhibit postintegration transcription (32).

To address the important issue of IN inhibitor specificity, we have developed a simple and straightforward counterscreen based on the unique infectivity profile of a simian virus 40/HIV-1 chimera (N/N.Tag.oriT) with catalytically inactive IN (15) to determine whether compounds inhibit integration in cell culture. The chimera virus utilizes the simian virus 40 origin of DNA replication oriT and trans-acting T-antigen protein to drive the replication of a class I HIV-1 IN mutant carrying amino acid substitutions D64N and D116N in the enzyme active site (21). To validate this strategy, C8166 T cells

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infected with N/N.Tag.oriT in the presence of various concentrations of known inhibitors were monitored for p24 production in culture supernatants at 5 days postinfection. The CXCR4 coreceptor antagonist AMD3100 (6) (kindly provided by AnorMed, Langley, British Columbia, Canada), nevirapine, and ritonavir each elicited a strong reduction in viral replication compared to untreated controls, demonstrating that the coreceptor usage, reverse transcription, and protease functions of N/N.Tag.oriT are intact (Fig. 3A; Table 1). Furthermore, the calculated 50% effective concentrations (EC50s) for these compounds were approximately 5 to 10 times lower than the values obtained with wild-type HIV-1NL4-3 (Table 1). These results were not unexpected, considering that the chimera displayed a diminished capacity for replicative spread compared with wild-type HIV-1NL4-3 (15). The validated IN inhibitor L-870,810, in stark contrast, failed to inhibit N/N.Tag.oriT at...
concentrations up to 0.23 μM, while the EC_{50} for parental HIV-1_{NL4-3} was 0.013 ± 0.011 μM (Fig. 3B; Table 1). GS-9137 and GS-9160 also failed to inhibit N/N.Tag.orit at concentrations up to 2.23 and 0.24 μM, respectively, while their EC_{50}s for wild-type HIV-1_{NL4-3} were 0.00725 ± 0.00591 and 0.00849 ± 0.00534 μM, respectively (Fig. 3D and E; Table 1). WM-5 inhibited N/N.Tag.orit replication at a lower dose than that required for HIV-1_{NL4-3} (Table 1). We therefore conclude that GS-9137 and GS-9160 specifically target the integration step of the virus life cycle, whereas the main target of WM-5 action lies elsewhere.

We have utilized the unique replication profile of N/N.Tag.orit to establish a cell-based assay to validate the antiviral target of newly designed IN inhibitors. During the course of this project, a separate study based on the relatively low level (~0.2%) of transcription from IN active site mutant vectors was presented as a method to distinguish the mode of action of potential IN inhibitors in cell culture (3). Due to the naturally weakened ability of the chimera to replicate through multiple cycles of HIV-1 growth, an additional advantage of our assay is that N/N.Tag.orit is innately more sensitive than wild-type HIV-1_{NL4-3} to drugs that target steps in the life cycle other than integration. On the flip side, the chimera was ~18 to >308 times less susceptible to IN inhibitors (Table 1). In-

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FIG. 3. Concentration-dependent anti-HIV-1 effects of nevirapine (A), L-870,810 (B), WM-5 (C), GS-9137 (D), and GS-9160 (E). C8166 T cells infected with HIV-1_{NL4-3} or N/N.Tag.orit were treated with the indicated compound concentrations. One hundred percent was defined as the level of p24 attained in control, non-drug-treated samples.
version of the inhibitor susceptibility phenotype can significantly help to classify unknown inhibitors as to whether they target the integration step. Our method is simpler and more amenable to scale-up than previously described molecular techniques like real-time nested Alu-PCR to show that a potential inhibitor specifically interferes with integration in cell culture. The method is applicable to different classes of inhibitors that may differentially inhibit IN 3’ processing versus DNA strand transfer activity and will also be useful to screen inhibitors of potential IN-cell cofactor interactions. We conclude that the method is a valuable counter-screen to detect and validate inhibitors of IN catalytic function and HIV-1 integration in cell culture.

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