Supplemental Data

Authors: **Hauber et al.**

Title : **Identification of cellular deoxyhypusine synthase as a novel target for antiretroviral therapy.**

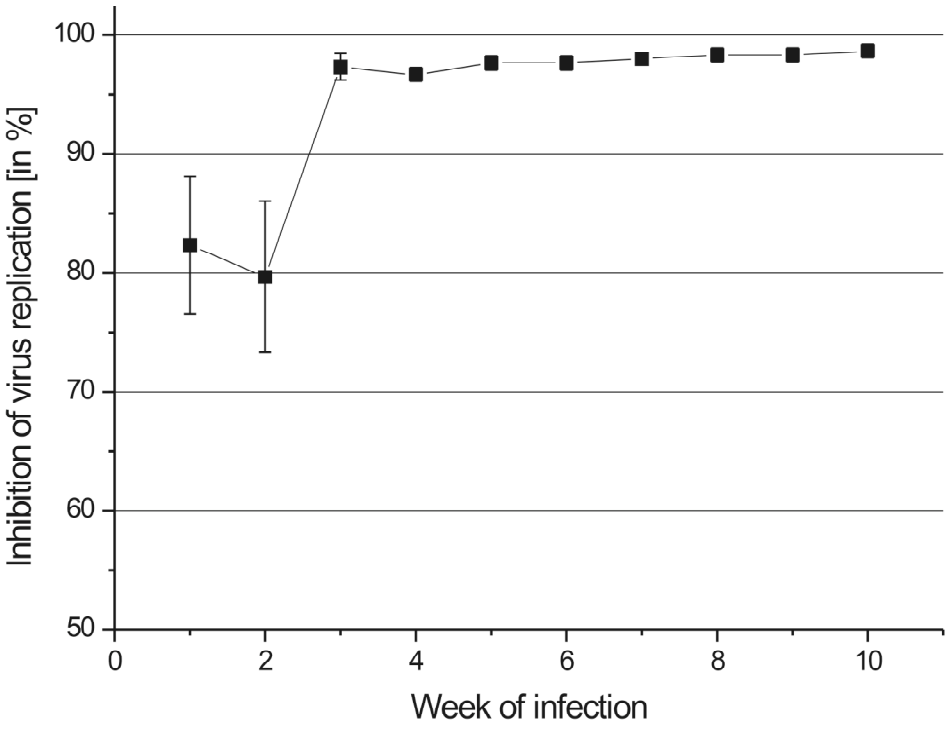


Figure 1. Long-term inhibition of a clinical HIV-1 isolate. PBMCs from an AIDS patient (infected with a multidrug-resistant virus; viral load of 22,000 copies/ml) were cultured in the presence of 1.0 µM CNI-1493 as described in the manuscript in Figure 3. Every week, cell viability, cell counts and p24 levels were determined (cell counts ranged from 0.9 – 1.1x10^6/ml;

75 - 80% viable cells were present in all cultures tested). The percentage of inhibition of virus replication as compared to replication in the respective untreated patient cells is shown.

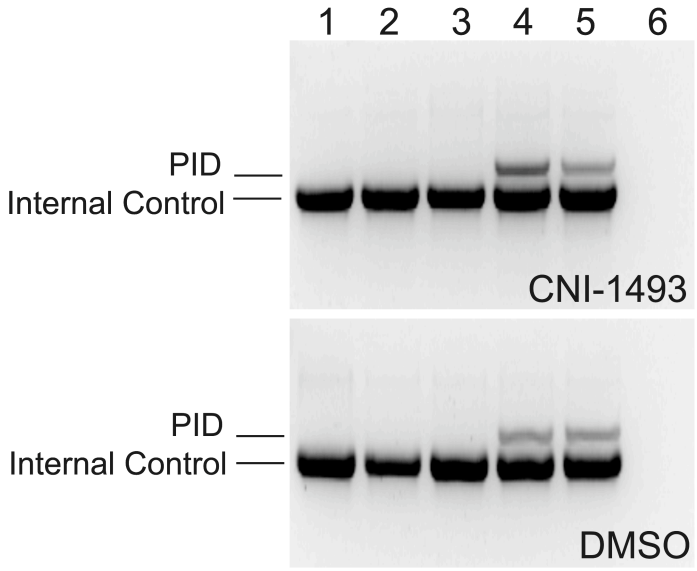
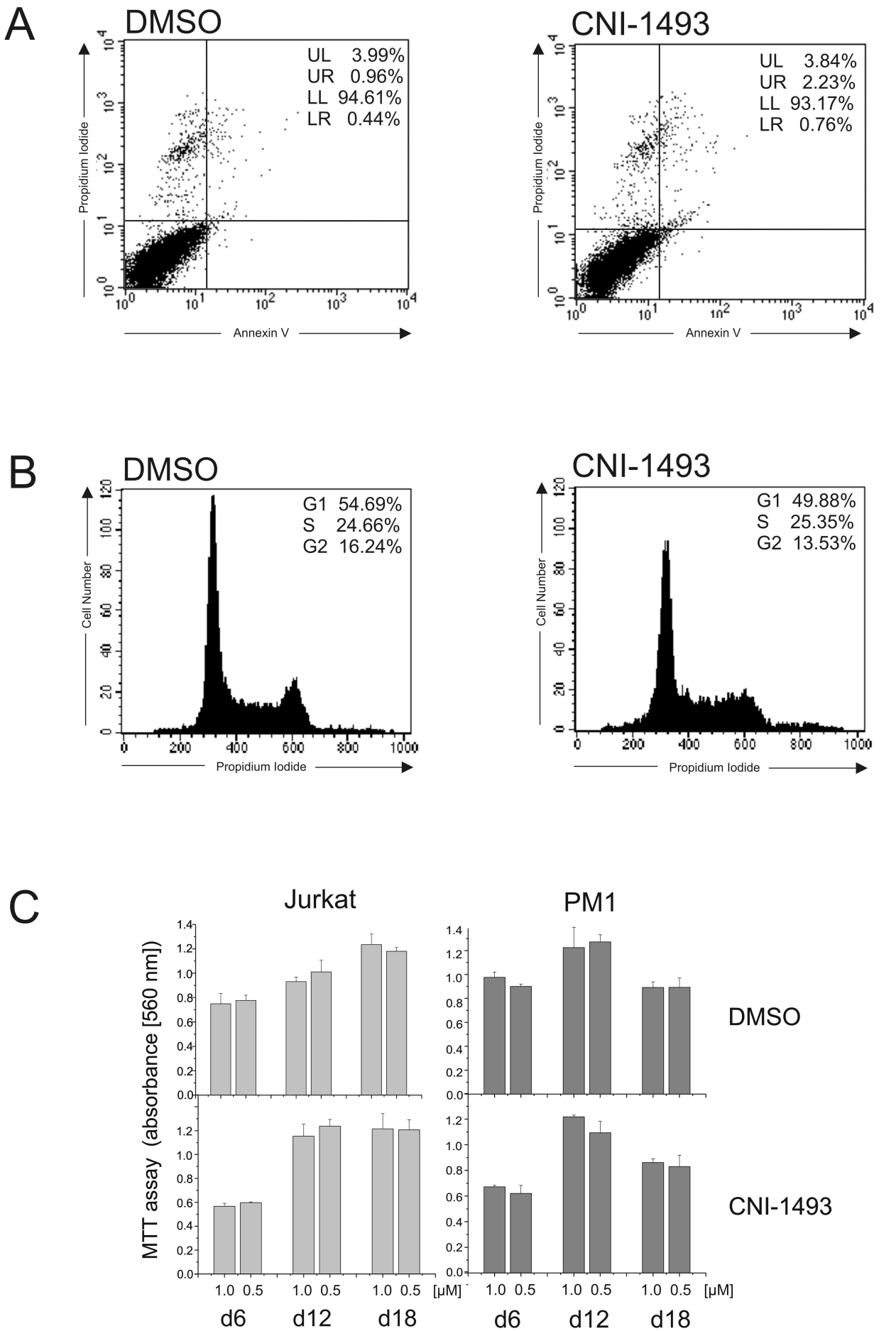


Figure 2. Exposure of cells to CNI-1493 does not block virus infection. Detection of de novo infection by analysis of extrachromosomal circular viral DNA. PM1 cells were cultured for 7 days in presence of 0.5 µM CNI-1493 (upper panel) or DMSO (lower panel) and subsequently infected with HIV-1 NL4/3. Total genomic DNA was isolated from uninfected cells (lane 1) and 15, 30, 45 and 60 min post-infection (lane 2 to 5, respectively). 3 µg of genomic DNA were directly amplified using HIV-1 specific primer pairs recognizing extrachromsomal 1-LTR and 2-LTR circular pre-integration DNA (PID) as described previously (Hauber et al. 2000 *AIDS* 14:2619-2621). Prior to amplification, 100 ng of a control plasmid were added to the reactions shown in lane 1 to 5. This plasmid contains heterologous sequences that are flanked by the same primer recognition sites that were used for the detection of the viral DNA circles, thereby

allowing the internal control and direct comparison of the reactions. A reaction in which template DNA was omitted, served as negative control (lane 6).

Figure 3. The antiviral activity of CNI-1493 is not caused by deleterious effects of the drug on the host cell. Analysis of cell death, cell-cycle progression and cell viability. (A) Apoptosis-assay. PBMCs from a healthy donor were cultured for 12 days in presence of 1.0 μM CNI-1493 or DMSO (control). Subsequently, apoptotic cells were assayed by FACS using FITC-coupled annexin V (Bender MedSystems). (B) Cell-cycle analysis. Jurkat T-cells were cultured for 12 days in presence of 1.0 μM CNI-1493 or DMSO. FACS-analysis was performed by DNA-staining with propidiumiodide (CycleTest^{TM} Plus; Becton Dickinson). (C) Analysis of cell viability. Jurkat T-cells and PM1 cells were cultured for 18 days in 0.5 or 1.0 μM of CNI-1493 or DMSO. MTT-assays (Roche Applied Science) were performed at day 6, 12 and 18 according to the manufacturer's protocol.


A
DMSO
UL 3.99%
UR 0.96%
LL 94.61%
LR 0.44%
Propidium Iodide
Annexin V
CNI-1493
UL 3.84%
UR 2.23%
LL 93.17%
LR 0.76%
Propidium Iodide
Annexin V
B
DMSO
G1 54.69%
S 24.66%
G2 16.24%
Cell Number
Propidium Iodide
CNI-1493
G1 49.88%
S 25.35%
G2 13.53%
Cell Number
Propidium Iodide
C
Jurkat
PM1
MTT assay (absorbance [560 nm])
DMSO
CNI-1493
1.0 0.5 1.0 0.5 1.0 0.5 [μM]
d6 d12 d18
1.0 0.5 1.0 0.5 1.0 0.5 [μM]
d6 d12 d18

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Genotypes of Multidrug-Resistant Viruses

Drug resistance conferring mutations in the indicated viruses were determined by DNA-Sequencing.

ABC, Abacavir; IDV, Indinavir; NFV, Nelfinavir; RTV, Ritonavir; SQV, Saquinavir.

PI-resistant virus (BN8): IDV/SQV/RTV/NFV (L10I, I54V, L63P, A71V, V77I, V82A, L90M)

NRTI-resistant virus (BJ1): AZT (M41L)
3TC/DDC/ABC (M184V)
Combination (L214F)
IDV/SQV/RTV/NFV (L10I, M36I, I54V, L63P, A71V, V82A, L90M)

NNRTI-resistant virus (BM2): NNRTI (K103N, Y188L)
Combinations (R211K, L214F)
IDV/NFV (L63P, V77V/I)

"Omni"-resistant virus (BE4): AZT (M41L, D67N, K70R, L210W, T215Y, K219E)
DDC/DDI/D4T (Q151M)
3TC (Q151M, E44D, V118I)
ABC (Q151M)
NNRTI (L100I, K103N, Y188L, K238T)
IDV/SQV/RTV/NFV (K20R, M36I, L63P, A71T, I84V, L90M)

"Omni"-resistant virus (FE9) : AZT (M41L, D67N, L210W, T215Y, K219R)
DDI (M41L, D67N, L74V, L210W,T215Y)
D4T (M41L, D67N, T215Y)
3TC (E44D, V118I)
ABC (L74V)