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A Role for Nitazoxanide in Combination with STAT-C Agents for Inhibition of HCV Replication and the Potential for the Prevention of Viral Resistance

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Nitazoxanide (NTZ, Alinia®) is a thialozide anti-infective currently in development for the treatment of chronic hepatitis C virus (HCV) infection. Recent clinical trials of NTZ plus peg-IFN/ribavirin demonstrated SVR rates of approximately 80% with no enhancement of adverse side effects. Current data indicate that tizoxanide (TIZ), the active metabolite of NTZ, targets host cell pathways rather than directly interfering with viral functions. NTZ and TIZ are potent inhibitors of HCV replication (genotypes 1a [H77], 1b [CON1] replicons; genotype 2a [JFH-1, infectious virus] in cell culture (EC50 ca. 0.2 μM; EC90, ca. 0.9 μM). Combinations of TIZ with IFN alfa-2b produced synergistic interactions against HCV replication. Combinations of TIZ and compounds targeting anti-HCV proteins: 2′C-methyl cytidine (2′CmeC, nucleoside), HCV-796 (non-nucleoside polymerase inhibitor), and the protease inhibitors telepravir (VX-950) and BILN-2061 also displayed synergistic interactions. Synergistic interactions were strongest with HCV-796 and VX-950, while interactions with 2′CmeC were the least favorable. The addition of ribavirin did not affect the relative potency of combinations of TIZ and interferon, indicating no interference between ribavirin and TIZ. TIZ monotherapy was equally effective in inhibiting several clinically relevant drug-resistant mutants (NS5b S282T, genotypes 1a and 1b; NS3 A156V/T, genotype 1b; NS3 R155K, genotype 1a). It was possible to select HCV replicon-containing cell lines that were resistant to 10 μM NTZ or TIZ (10X EC90). However, it was not possible to transfer the TIZ-resistant phenotype to naive Huh7.5 cells by transfection of HCV RNA from these lines indicating that primary resistance was conferred by changes in the host, not the virus. The favorable interactions of NTZ with NS5B and NS3 inhibitors, coupled with broad activity against relevant drug-resistant variants and an apparent high barrier for direct viral resistance, makes NTZ a strong candidate for combination therapies with STAT-C agents in the absence of interferon and/or ribavirin.

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Discovery and In vitro Characterization of a Novel, Chain-terminating Tricyclic Nucleoside, GL60630, with Potent Anti-HCV Activity

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During the course of our ongoing search for nucleoside analogs directed against the hepatitis C virus, we discovered a class of tricyclic nucleosides with potent antiviral activity. From this class, the nucleoside GL60630 was ultimately identified as possessing the most promising profile for further evaluation. Nucleoside GL60630 inhibited the sub-genomic HCV replicon with an EC_{50} = 500 nM and did not display cytotoxicity (CC_{50} > 50 μM) in either of two liver cell lines (Huh7 or HepG2) or in a T-cell line (MT-4). The synthetic NTP inhibited the purified NS5B enzyme with an IC_{50} = 325 nM and was kinetically competitive with ATP. The NTP functioned as an adenosine surrogate, being incorporated opposite uridine and causing complete chain termination following incorporation. Furthermore, the NTP did not inhibit purified human DNA polymerases α, β or γ or human RNA polymerase II. Following treatment of replicon cells with the parent nucleoside, a significant quantity of intracellular NTP was detected. A replicon cell line containing the NS5B S282T mutation demonstrated reduced susceptibility to GL60630. Taken together, these data support the anti-HCV mechanism of replicon anabolism to NTP, incorporation by HCV NS5B and subsequent chain termination. Treatment of replicon cells for 20 days resulted in a >4 $\log_{10}$ drop of HCV RNA levels with no cytotoxicity. Finally, in vitro combination studies of GL60630 with other inhibitor classes displayed additive anti-HCV activity in vitro, suggesting that novel tricyclic nucleoside GL60630 may possess future utility in the treatment of chronic HCV infection.

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In vitro Anti-hepatitis C Virus (HCV) Activities and Resistance Profile of Debio 025, A Non-immunosuppressive Cyclophilin Binding Molecule

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Debio 025 (D25) is a potent inhibitor of HCV replication both *in vitro* as *in vivo* [Hepatology 43:761–770; Hepatology 47:817–826]. Here we elaborate on the particular *in vitro* anti-HCV characteristics of D25. Combining D25 with either interferon-α, ribavirin or STAT-C inhibitors [protease or (non)-nucleoside polymerase HCV inhibitors] resulted in an additive to slightly synergistic antiviral activity in a 3-day antiviral assay. D25 has the unique ability to rapidly clear hepatoma cells from their HCV replicon when used alone or in combination with interferon-α and STAT-C inhibitors. Moreover D25 was able to delay the development of escape variants against several STAT-C inhibitors in colony formation assays. D25 proved as potent against HCV replicons that are resistant to various STAT-C inhibitors as against wild-type HCV. Subgenomic replicons were selected that are resistant to D25 (3 independent selections). Mutations were identified in domain II of the NS5A gene. Reintroduction of these mutations in a WT background partially recovered resistance. D25 resistant ($D25^r$) replicons remained fully susceptible to interferon and several STAT-C inhibitors. Transfection of Huh Lunet cells with $D25^r$ replicon RNA, resulted in a partial transfer of the resistance suggesting that also host cell factors are involved in the antiviral activity of D25. D25 forms, given the particular anti-HCV activity and the unique resistance profile, an attractive candidate for the treatment of HCV infections in combination with standard of care and/or STAT-C.

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