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Development and Validation of a High Throughput Screen for Inhibitors of Respiratory Syncytial Virus

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Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis and pneumonia among infants and children under 1 year of age, but severe lower respiratory tract disease may occur at any age, especially among the elderly or among those with compromised cardiac, pulmonary, or immune systems. The existing therapies for the acute infection are ribavirin, which has inconsistent clinical results, and the prophylactic humanized monoclonal antibody (Synagis® from MedImmune) that is expensive and limited to use in high risk pediatric patients. Thus, there is a critical need to discover novel antiviral drugs to supplement existing chemotherapeutics. To meet this need, we have developed a high-throughput screen (HTS) that allows for the identification of potential inhibitors of RSV from large compound libraries. This cell-based assay measures the inhibition of RSV strain long-induced cytopathic effect (CPE) in HEp-2 cells 72 h post-infection using the CellTiter-Glo Luminescent Cell Viability Assay (Promega). The assay is sensitive and robust, with Z values > 0.8, signal to background, S/B > 22, and signal to noise, S/N > 5. Various parameters were optimized and validated including cell density, viral concentration, DMSO tolerance for compound dilution, incubation time for virus-induced CPE and effective control drug concentration. Additional parameters, such as day-to-day assay variability, reagent and read stability, edge effects, and IC50 stability were also examined during validation. We are using this assay to screen chemical libraries and report here our findings from these screens.

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Development of Intergenotypic Chimeric Replicons for Broad-spectrum Antiviral Activity Characterization of Hepatitis C Virus Polymerase Inhibitors

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The identification of hepatitis C virus (HCV) inhibitors with broad-spectrum activity has been hampered by the limited number of HCV replicons that replicate efficiently in cell culture. To date, only genotype (gt) 1a, 1b, and 2a subgenomic replicons are available. To address the need for broad-spectrum activity characterization of HCV NS5B polymerase inhibitors, we created a panel of intergenotypic chimeric replicons containing NS5B sequences from gt 2b, 3a, 4a, 5a, and 6a HCV isolates.

Viral RNA extracted from non-gt1 HCV patient plasma was subjected to reverse transcription. The NS5B region was amplified by nested PCR and cloned into the corresponding region of the gt 1b (Con-1) subgenomic reporter replicon by SOEing PCR. Replication fitness was determined based on reporter activity and colony formation efficiency following electroporation of chimeric replicon RNAs into Huh7.5 cells. Stable replicon cell lines were generated for in vitro antiviral activity determination of HCV inhibitors. Inhibition of HCV RNA replication was measured, after a 3-day incubation with compounds, using the reporter activity of the stable cell lines as an endpoint.

Replication fitness of the gt 4a NS5B chimeric replicon was comparable to that of the gt 1b replicon, whereas introduction of the gt 2b, 3a, 5a, and 6a NS5B sequences reduced replication efficiency by 6–100-fold compared to the gt 1b replicon. In antiviral assays, nonnucleoside polymerase inhibitors (NNPI) that bind to either the thumb base or the primer grip regions of NS5B displayed a 2–3 log decrease in antiviral activity against the gt 2b, 3a, 4a, 5a, and 6a NS5B chimeric replicons when compared to the gt 1a and 1b replicons. Evaluation of the antiviral activity for an NNPI that binds to the thumb tip site demonstrated a comparable reduction in activity against the gt 2b chimeric replicon, and smaller reductions against gt 3a, 4a, and 5a chimeras when compared to the gt 1a and 1b replicons.

In conclusion, evaluation of HCV polymerase inhibitors against intergenotypic chimeric replicons showed differences in activity spectrum for inhibitors that target different regions of the enzyme. Our study demonstrates the utility of chimeric replicons for broad-spectrum activity determination of HCV inhibitors.

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shRNAs Targeting Hepatitis C: Effects of Sequence and Structural Features, and Comparison with siRNA

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Hepatitis C virus (HCV) is a leading cause of liver cirrhosis and hepatocellular carcinoma worldwide. Currently available treatment options are of limited efficacy, and there is an urgent need for development of alternative therapies. RNA interference (RNAi) is a natural mechanism by which small interfering RNA (siRNA) or short hairpin RNA (shRNA) can mediate degradation of a target RNA molecule in a sequence-specific manner. In this study, we screened in vitro-transcribed 25-bp shRNAs targeting the internal ribosome entry site (IRES) of HCV for the ability to inhibit IRES-driven gene expression in cultured cells. We identified a 44-nt region at the 3′-end of the IRES within which all shRNAs efficiently inhibited expression of an IRES-linked reporter gene. Subsequent scans within this region with 19 bp shRNAs identified even more potent molecules, providing effec-