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## DEVELOPMENT OF A RAPID PHENOTYPIC SUSCEPTIBILITY ASSAY FOR HCV POLYMERASE INHIBITORS

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E Penuel, D Han, K Favero, E Lam, Y Liu, NT Parkin

*Monogram Biosciences, South San Francisco, CA USA*

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**BACKGROUND:** The HCV NS5B polymerase is a promising target for new anti-HCV drugs. The propensity of RNA viruses to evolve in the face of changing environmental factors makes the likelihood of the development of HCV drug resistance high. Yet, no validated assays for measuring HCV drug resistance in patient samples have been described.

**METHODS:** The NS5B polymerase gene from 98 HCV-infected individuals was amplified by RT-PCR and transferred without cloning to a luciferase containing, HCV subtype 1b replicon (con1) vector optimized for transient transfection replication assays. RNA derived from these resistance test vectors (RTVs) was transfected into "cured" Huh7 cells, and luciferase activity was measured 4 (input) and 72 hours later. Susceptibility to various polymerase inhibitors (both nucleoside and non-nucleoside) was determined by quantitation of luciferase activity at various drug concentrations.

**RESULTS:** Several known NS5B resistance mutations (e.g. S282T, M414T, C451R), introduced into the replicon vector by site-directed mutagenesis, produced the expected shifts in susceptibility to HCV polymerase inhibitors. Approximately 65% of RTVs harbouring patient-derived NS5B sequences replicated above background levels (greater than 20% of input relative to the replicon control); 11% replicated more efficiently than control, con1 vector. Chimeras constructed from subtype 1b replicated with greater efficiency than 1a: 19% of subtype 1a samples achieved 50% of control replication levels versus 43% for subtype 1b. Specific sequence differences present in subtype 1a samples involved in the "kissing loop" may account for this reduced activity. Approximately 50% of samples produced sufficient luciferase activity to allow drug susceptibility determination. Sequence analysis revealed considerable diversity including a very low

but detectable frequency of known resistance mutations in these patient samples, which demonstrated the expected shifts in susceptibility.

**CONCLUSIONS:** We have developed a prototype transient transfection, luciferase-based phenotypic susceptibility assay for patient-derived NS5B polymerase. Approximately 50% of patient samples can be assayed for drug susceptibility. Several reported resistance mutations previously identified by *in vitro* selection also occur as natural polymorphisms in patient samples with reductions in susceptibility to NS5B polymerase inhibitors. We expect that the use of phenotypic susceptibility assays will be crucial for complete understanding of HCV drug resistance.

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