# Abstract 21 Development of Recombinant Replicon Report Assays for Characterization of HCV NS5A and NS3/4A Protease Inhibitors

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## BACKGROUND

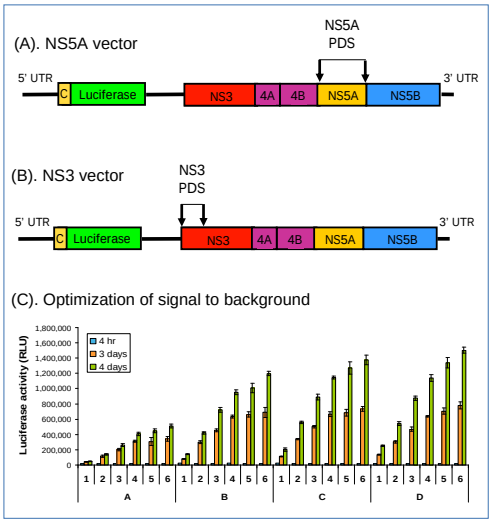
• Small molecule inhibitors of HCV NS5A and NS3/4A protease hold significant promise for the development of curative treatments of HCV infection. In the absence of robust infectious cell-based assays, the pre-clinical and clinical evaluation of resistance to these direct acting antiviral (DAA) drug candidates is facilitated by the use of sub-genomic replicon assays. To date, technical obstacles have largely limited the application of these assays to the evaluation of select molecular clones and laboratory engineered sequences.

• In this study, we are pursuing the development of robust replicon assay systems that will enable routine, high throughput evaluation of site-directed mutations and virus populations derived from HCV infected patient plasma.

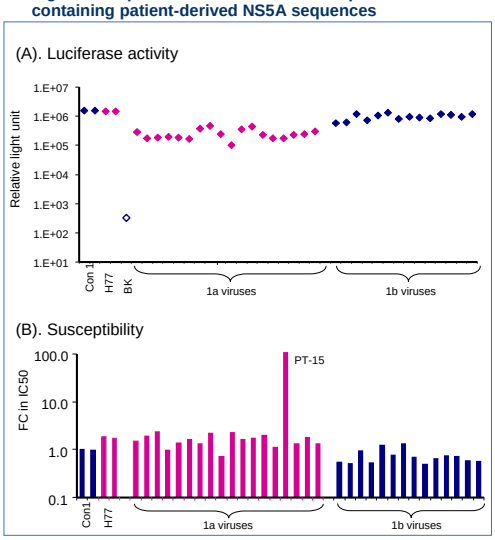
## METHODS

• Luciferase reporter replicons were engineered to accommodate the efficient insertion of patient derived NS5A and NS3 sequences that are derived by RT-PCR from HCV infected patient plasma. Modifications to replicon sequences, target cells and cell assay conditions were developed and evaluated to maximize luciferase signal to background ratios. Assay performance was evaluated using reference replicons engineered to contain NS3 protease and NS5A mutations that are associated with reductions in drug susceptibility, as well as replicons containing patient derived NS5A and NS3 protease amplification products.

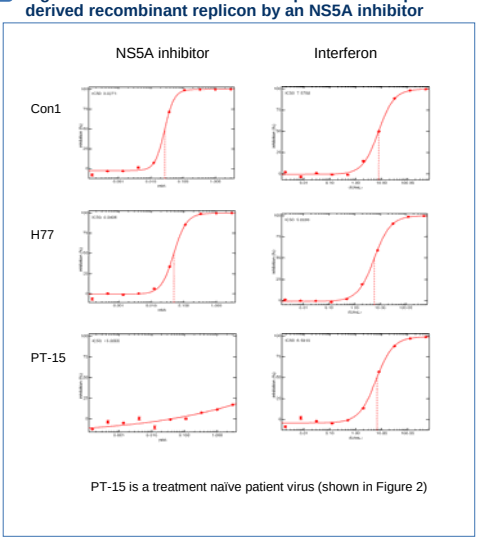
### Figure 1: Optimization of HCV replicon assays for the assessment of NS5A and NS3 inhibitors




### Figure 2: Replication and inhibition of replicons containing patient-derived NS5A sequences

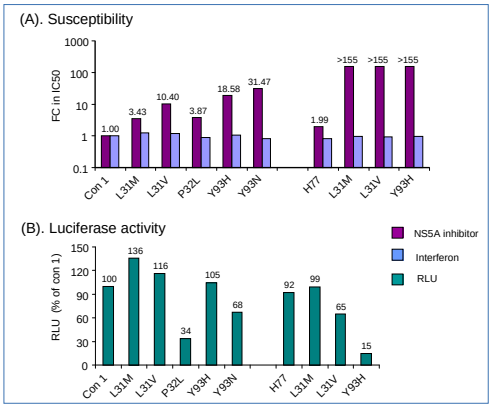



### Figure 3: Inhibition of reference replicons and a patient derived recombinant replicon by an NS5A inhibitor




PT-15 is a treatment naïve patient virus (shown in Figure 2)

### Figure 4: NS5A inhibitor susceptibility and replication of HCV replicons containing NS5A site-directed mutations

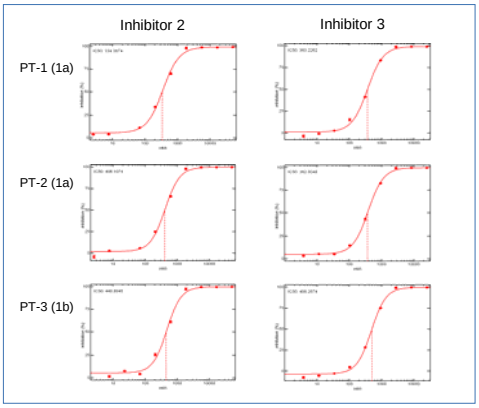



### Figure 5: NS3 protease inhibitor susceptibility and replication of HCV replicons containing NS3 site-directed mutations

| SDM | Inhibitor 1 | Inhibitor 2 | Inhibitor 3 | Interferon | RC (% Con1) |
|---|---|---|---|---|---|
| V36M | 1.93 | 4.61 | 2.25 | 0.97 | 80 |
| T54A | 0.83 | 4.06 | 3.11 | 0.96 | 68 |
| R155K | >350 | 4.01 | 2.89 | 1.02 | 66 |
| V36M R155K | >350 | 32.15 | 4.43 | 0.73 | 43 |
| A156S | 7.79 | 8.14 | 4.20 | 0.98 | 53 |
| A156T | 19.95 | 31.74 | 17.12 | 0.85 | 22 |
| A156V | 34.77 | >55 | 18.08 | 0.89 | 16 |
| D168A | 168.36 | 0.28 | 0.63 | 0.92 | 95 |
| V170A | 1.12 | 3.92 | 3.04 | 0.87 | 54 |

RLU (%): luciferase activity of site-directed mutants relative to the 1b reference Con1

### Figure 6: Inhibition of patient derived recombinant replicons by NS3 protease inhibitors




## RESULTS

• **Figure 1:** Restriction endonuclease cleavage sites were engineered into a luciferase reporter HCV replicon to accommodate the efficient incorporation of NS5A (panel A) and NS3 (panel B) sequences that are derived by RT-PCR amplification from HCV positive patient plasma (patient derived sequences, PDS). Modifications to replicon sequences, target cells and assay conditions were developed to optimize luciferase signal to background ratios (panel C) resulting in an assay dynamic range >4 $\log_{10}$.

• **Figure 2:** Current assay conditions enable the routine evaluation of HCV positive plasma samples for susceptibility to NS5A inhibitors (panel A). A single DAA-treatment naïve genotype 1a isolate exhibited a notable reduction in NS5A inhibitor susceptibility (panel B).

• **Figure 3**: Representative dose dependent NS5A inhibition profiles of HCV replicons containing genotype 1b (Con1) and 1a (H77) reference NS5A sequences are shown. A single recombinant replicon containing the NS5A sequences derived from a treatment naïve patient (PT-15) displayed a notable reduction in NS5A inhibitor susceptibility.

• **Figure 4:** NS5A mutations associated with reductions in NS5A inhibitor susceptibility were engineered into genotype 1a (L31M/V, Y93H) and genotype 1b (L31M/V, P32L, Y93H/N) NS5A reference sequences. Reductions in NS5A inhibitor susceptibility for reporter replicons containing these site directed mutations were consistent with previous reports (panel A). No alterations in interferon susceptibility were observed. Most of the NS5A mutations did not impair replicon replication (panel B).

• **Figure 5:** NS3 mutations associated with reductions in NS3 protease inhibitor susceptibility (V36M, T54A, R155K, A156S/T/V, D168A, V170A and V36M+R155K) were engineered into a genotype 1b NS3 reference sequence. Reductions in NS3 inhibitor susceptibility for reporter replicons containing these site directed mutations were consistent with previous reports. No alterations in interferon susceptibility were observed. The impact of NS3 protease inhibitor mutations on replicon replication varied by amino acid position and substitution.

•**Figure 6:** Current assay conditions enable the routine evaluation of HCV positive plasma samples for susceptibility to NS3 inhibitors. Representative dose dependent NS3 inhibition profiles of HCV replicons containing genotype 1a (PT-1, PT-2) and 1b (PT-3) patient derived NS3 sequences are shown.

## CONCLUSIONS

• We report progress on the development of robust replicon assays that will facilitate the pre-clinical and clinical evaluation of direct acting HCV NS3/4A protease and NS5A inhibitors. By incorporating several assay enhancements and optimizing assay conditions we have significantly improved the ability of this replicon reporter system to evaluate NS3, NS5A and NS5B sequences derived from HCV-infected patient plasma samples. The performance of the assay was demonstrated using a panel of reference replicons containing mutations associated with reduced susceptibility to NS3 and NS5A inhibitors.

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