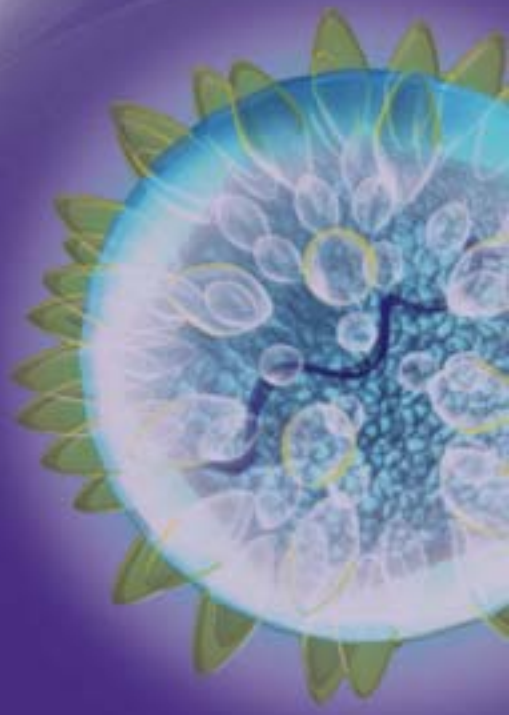
# Searchable HCV Resistance Literature Repository

Jim Sullivan, HCV DRAG 2009

24 March 2009

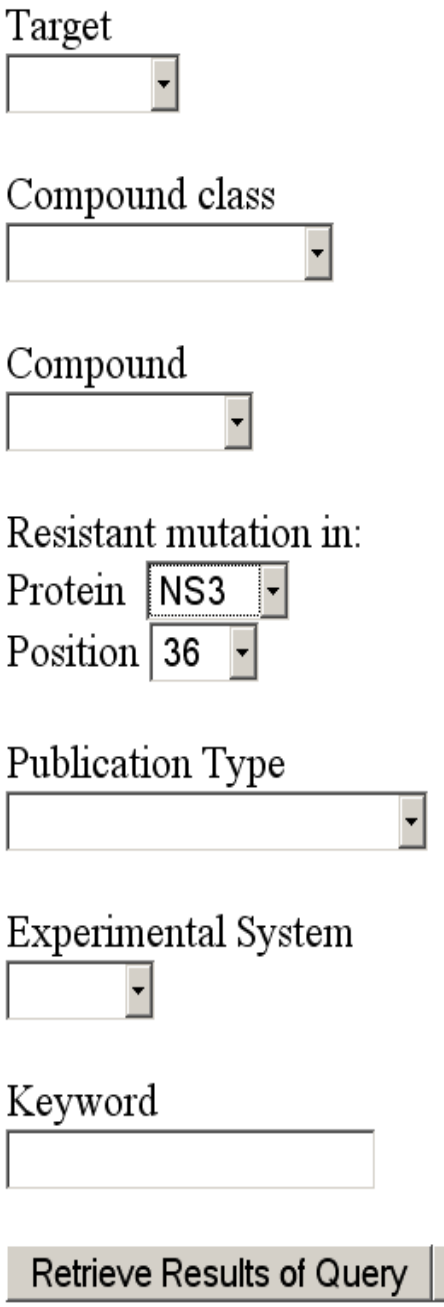


# The need for an HCV resistance literature database

- Within the past few years, numerous papers or abstracts on resistance to STAT-Cs have been presented at various scientific meetings
- Peer-reviewed publications can be retrieved through literature database searches, but…
  - **There is no single comprehensive repository dedicated to indexing and retrieving these papers, and**
  - **Data presented at conferences that is not later published is extremely difficult to identify, retrieve, and cite**

# Bird eye's view of the solution

- We propose the development of a searchable literature repository
  - Database and software development to be shared between HCV DRAG and HIV Forum (*beta version has been completed*)
- Proposed Roles
  - HIV Forum: *Host database and software; maintain server*
  - Research Community: *Submit presentations and publications for inclusion in the database*
  - HCV DRAG Working Group: *Periodically review status of the database and suggest improvements for consideration by the developers*
- Intended audience:
  - Researchers
  - Clinicians
  - Regulatory agencies




Search modalities allow results to be filtered based on one or more of the following criteria:

- Target of compound
- Compound and/or Compound class
- Specific Residue
- Publication type
- Experimental System
- Keyword

HCV DRAG working group to define above

| Compound | Class | Target | Site | Mutation | In vitro data? | In vivo data? | Title |
|---|---|---|---|---|---|---|---|
| SCH6 | macrocyclic | NS3-4A | Catalytic Site | NS3 A156T | y | ND | Mutations conferring resistance to SCH6, a novel hepatitis C virus NS3/4A protease inhibitor. Reduced RNA replication fitness and partial rescue by second-site mutations |

Results from search allow retrieval of additional information through hyperlinked title

| | |
|---|---|
| **Reference Type** | Journal Article |
| **Authors** | M. Yi;X. Tong;A. Skelton;R. Chase;T. Chen;A. Prongay;S. L. Bogen;A. K. Saksena;F. G. Njoroge;R. L. Veselenak;R. B. Pyles;N. Bourne;B. A. Malcolm;S. M. Lemon |
| **Date** | Mar 24, 2006 |
| **Title** | Mutations conferring resistance to SCH6, a novel hepatitis C virus NS3/4A protease inhibitor. Reduced RNA replication fitness and partial rescue by second-site mutations |
| **Journal/Conference** | J Biol Chem |
| **Volume** | 281(12): 8205-15 |
| **URL** | http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16352601 |

URLs for published articles or links to PDFs may be provided

Abstract:

Drug resistance is a major issue in the development and use of specific antiviral therapies. Here we report the isolation and characterization of hepatitis C virus RNA replicons resistant to a novel ketoamide inhibitor of the NS3/4A protease, SCH6 (originally SCH446211). Resistant replicon RNAs were generated by G418 selection in the presence of SCH6 in a dose-dependent fashion, with the emergence of resistance reduced at higher SCH6 concentrations. Sequencing demonstrated remarkable consistency in the mutations conferring SCH6 resistance in genotype 1b replicons derived from two different strains of hepatitis C virus, A156T/A156V and R109K. R109K, a novel mutation not reported previously to cause resistance to NS3/4A inhibitors, conferred moderate resistance only to SCH6. Structural analysis indicated that this reflects unique interactions of SCH6 with P'-side residues in the protease active site. In contrast, A156T conferred high level resistance to SCH6 and a related ketoamide, SCH503034, as well as BILN 2061 and VX-950. Unlike R109K, which had minimal impact on NS3/4A enzymatic function, A156T significantly reduced NS3/4A catalytic efficiency, polyprotein processing, and replicon fitness. However, three separate second-site mutations, P89L, Q86R, and G162R, were capable of partially reversing A156T-associated defects in polyprotein processing and/or replicon fitness, without significantly reducing resistance to the protease inhibitor.

43EASL-PO_2556

43rd Annual Meeting for The European Association for the Study of the Liver
Milan, Italy, April 23-27, 2008

# Natural Prevalence of HCV Variants with Decreased Susceptibility to NS3•4A Protease and NS5B Polymerase Inhibitors in Treatment-Naive Subjects

Doug J. Bartels, Yi Zhou, Eileen Zhang, Michelle Marcial, Randal A. Byrn, Tom Pfeiffer, Bambang Adiwijaya, Chao Lin, Ann D. Kwong and Tara L. Kieffer
Vertex Pharmaceuticals Inc, Cambridge MA 02139, USA

## INTRODUCTION

- HCV NS3•4A protease & NS5B polymerase are essential enzymes for viral replication
- Inhibitors that target these enzymes comprise a new class of specifically targeted antiviral therapies for HCV (STAT-C) that are currently in clinical development
- HCV has high sequence diversity, resulting in a viral quasi-species that has the potential to select variants less sensitive to STAT-C treatment
- The prevalence of STAT-C-naïve patients whose quasispecies is predominantly a naturally-occurring variant less sensitive to STAT-Cs is described
- Clinical response to an HCV NS3 protease inhibitor, telaprevir (TVR), in combination with Peg-IFN (P) ± RBV (R) is shown for subjects with variants less sensitive to TVR predominant at baseline

## METHODS

**HCV RNA Levels:** Plasma HCV RNA levels were measured using the Roche COBAS TaqMan® HCV/HPS assay. The lower limit of quantitation (LLOQ) for the HCV RNA assay was 30 IU/mL and the limit of detection (LOD) was 10 IU/mL.

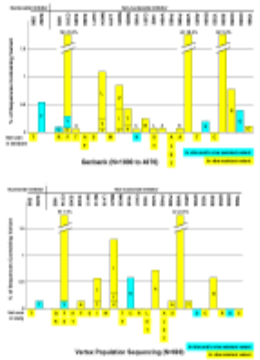
**Amplification and Sequencing of HCV from Subject Plasma:** Blood samples were collected from subjects before, during, and after the study drug dosing period. Sequence analysis of HCV was done by nested RT-PCR amplification of an approximately 9kb HCV RNA fragment spanning the HCV polyprotein coding region. The NS3, NS4A, and NS5B regions were then sequenced from the purified DNA (Agencourt® Biosciences, Beverly MA). The lower limit of detection (LOD) for the sequencing assay was 1000 IU/mL.

**Sequence Alignment and Analysis:** Sequences were aligned and analyzed for substitutions using the software Mutational Surveyor (SoftGenetics, State College, PA). Sequences obtained from GenBank were also included in this study.

**Replicon Assay:** The $IC_{50}$ values of HCV protease inhibitors (TVR, boceprevir, BILN 2061, and ITMN 191), were determined in a 48-hr assay using HCV replicon cells

## RESULTS: NS5B POLYMERASE

### Prevalence of HCV NS5B Polymerase Inhibitor-Resistant Variants

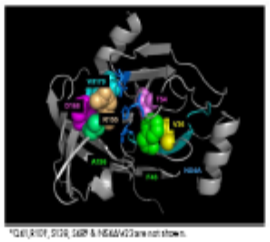


- Variants resistant to nucleoside inhibitors are <1% each
- Variants resistant to non-nucleoside inhibitors are more prevalent
  - C316N and V499A variants exist at higher levels (up to 22 and 36%, respectively)

Nucleoside inhibitors: MK-0608, NM283 (NM107), R1626 (R1479), R7128 (R5148)
Non-nucleoside inhibitors: A-782759, A-837093, AG-00518, GS-9190, HCV-371, HCV-796, TX-109

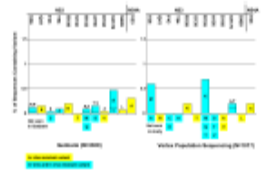
## RESULTS: N3•4A PROTEASE

### Location of HCV NS3•4A Protease Inhibitor-resistant Mutations* to BILN 2061, ITMN-191, SCH6, Boceprevir and Telaprevir



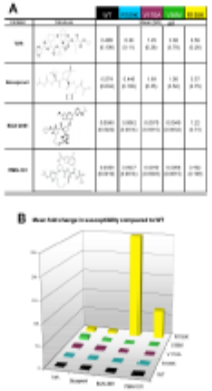
*Q41, R109, S139, D168 & N54A/V170 are not shown.

### Prevalence of HCV NS3•4A Protease Inhibitor-Resistant Variants



- Variants resistant to protease inhibitors are <1% each

### Sensitivity of WT and Variant Replicons to HCV Protease Inhibitors

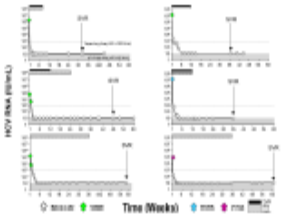


Variants resistant to protease inhibitors observed in Vertex Sequencing were tested for sensitivity against other protease inhibitors in vitro. The $IC_{50}$ of TVR, Boceprevir, BILN 2061 and ITMN-191 (panel B) and fold change from WT (panel A) for WT, V36M, R109K, R155K, and V170A HCV replicon cells lines are shown. Data shown are the mean from 3 independent experiments.

- V36M, R109K, and V170A variants confer low-level resistance (<7-fold decrease in sensitivity) to HCV protease inhibitors in replicon cells.
- The R155K variant confers low-level resistance to TVR and boceprevir and high-level (>70-fold) resistance to BILN 2061 and ITMN-191.

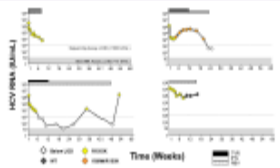
## CLINICAL RESPONSE

### Treatment Response to TVR/P±R or PR in Subjects with V36M, R109K, and V170A Variants Predominant at Baseline




- Subjects with V36M or R109K were treated with TVR/PR and the HCV RNA decline was similar to that observed in subjects with wild-type virus. All subjects achieved an SVR.

### Treatment Response to TVR/P±R or PR in Subjects with R155K Variants Predominant at Baseline




- TVR/PR provided greater antiviral activity than PR alone in subjects with the R155K variant.
- However, antiviral response was less than observed in subjects with wild-type virus.

| Variant | Treatment | N | Baseline HCV RNA: Median | Baseline HCV RNA: Mean | Baseline HCV RNA: Delta | Decline in Plasma HCV RNA (log10 IU/mL) Day 2: Median | Decline in Plasma HCV RNA (log10 IU/mL) Day 2: Mean | Decline in Plasma HCV RNA (log10 IU/mL) Day 2: Delta |
|---|---|---|---|---|---|---|---|---|
| WT | P/R | 327 | 6.83 | 6.83 | 0.04 | -3.74 | -3.81 | 0.07 |
| WT | TVR/PR | 314 | 6.89 | 6.89 | 0.03 | -3.93 | -3.97 | 0.03 |
| V36M | P/R | 1 | 5.06 | 5.06 | na | -2.28 | -1.35 | na |
| V36M | TVR/PR | 6 | 5.48 | 5.57 | 1.06 | -2.32 | -2.99 | 1.32 |
| R155K | P/R | 1 | 7.30 | 7.30 | na | -0.02 | 0.02 | na |
| R155K | TVR/PR | 3 | 6.07 | 6.15 | 0.56 | -2.16 | -1.92 | 0.21 |

## CONCLUSION

- The prevalence of STAT-C-naïve subjects with variants less sensitive to protease and nucleoside polymerase inhibitors is very uncommon (<1% for each mutation).
- However, the prevalence of subjects with certain variants less sensitive to non-nucleoside polymerase inhibitor is higher. Although the clinical implications of this is unknown, inhibitors that are affected by these prevalent variants may be optimally developed in combination with other STAT-C agents.
- TVR/PR efficiently inhibited V36M & R109K variants and patients with these variants predominant at baseline achieve an SVR.
- TVR contributed additional antiviral activity against R155K over PR alone, but based on the limited sample size this requires further investigation.
  - R155K confers low-level resistance to TVR and boceprevir and higher-level resistance to BILN-2061 and ITMN-191.
- Characterizing the effect of baseline variants on susceptibility is important for new HCV agents in development.

# Moving forward…

- Form the HCV DRAG ResistLit DB WG
  *(Resistance Literature DataBase Working Group)*
  - Optimize Vertex's beta version, launch database
  - Populate database with references available to date
  - Decide how the database will be updated
    - Working group to generate list of presentations wanted for submissions
    - Disseminate flyers to authors at posters requesting submission
    - HIV Forum to send email requesting authors of pertinent publications to submit their presentations to the database
  - Decide how to raise awareness of the database/service
    - Present a poster at relevant conferences announcing our presence to end-users (researchers, clinicians)
    - Announcement at 4th HCV Resistance Workshop (June, Boston, MA) and request data and/or comments
    - Publish a database announcement in an appropriate journal (Nucleic Acids Res, BMC Hepatology, others?)
    - Provide periodic updates / announcements using the HIV Forum's listserver
- Anything other issues/ideas?
- Please sign up!

# HCV Resistance Sequence DB

- Need for framework allowing collection of HCV drug resistance information in consistent, standardized format
- Meeting in Paris, Feb 2008 w Japanese, European and Los Alamos database representatives
- Mechanisms for integrating a new HCV resistance database into existing structures?

# HCV Resistance DB

- Protected section for HCV drug resistance sequences within a public warehouse that all three databases feed into
- A 4th entity: HCV drug resistance info, and all 4 feed into a public warehouse
- Restricted access:
  - Baseline vs follow up sequences

# Proposed Next Steps

- Needs assessment and interest on part of pharma
- Other possible funding sources: ANRS, NIH, EU
- Discuss governance structure and access