HCV

***D**rug*

***R**esistance*

***A**dvisory*

***G**roup*

HCV DRAG: Today's Goals

- Identify the questions:
  - Genotype
  - Phenotype
  - Clinical
- Form working groups
- Define timetables for action items

HCV DRAG: Working Groups

Sequence Analysis   **Phenotype**   **Clinical**

Ann Kwong   **Neil Parkin**   **Chip Schooley**

Database?

Representatives from 3 main databases
Under umbrella of this group

HCV DRAG: Considerations

Question categories

1. Immediate recommendation needed to guide process in right direction

    *   Genotype vs genetic resistance?
    *   Chip: "harmonization/coordination" activities

2. Capable of recommendation in working group

    *   Biological standards for distribution, databases

3. Multiple possibilities to be described by working group with pro/con and context

    *   Population vs clonal sequencing

4. Not enough data/methodology at this time, noted as issue

    *   Clinical cutoffs

HCV DRAG: Genotype questions

- Nomenclature: Genotype vs genotypic resistance
- What is best format for representing mutations?
    - To regulatory agencies
    - Can we standardize?
- How do we "simplify" data to make accessible to physicians?
- Why standardize? How do we standardize?
    - Compare data
    - Compare assays
    - Develop control reagents
    - Assure basic questions addressed
    - Can we share reagents?
    - Timing for standardization: after methodology advanced?
    - Standardization during publication?
    - **Joint effort of geno and pheno working groups**
- **What is a drug-associated mutation and what is a SNP?**
    - **Are existing databases sufficiently comprehensive?**
    - **Stanford database method – Bob Shafer**
- Databases:
    - How to support financially?
    - How to implement: Los Alamos? Other?

HCV DRAG: Genotype questions

- What should be sequenced?
  - Consensus for each drug class
- At what viral load do you try to obtain a sequence?
- **What is most important information to gather during clinical development (before and after registration)?**
  - **Data-driven regulatory pathway**
  - How does this impact "resistance-based marketing"?
  - Balance of information vs cost

HCV DRAG: Genotype questions

Clonal vs population sequencing?

- Population sequencing vs highly sensitive methods?
  - When best used?
    - Upon rebound, upon retreatment
- Clonal analysis:
  - Recommended number of clones to be sequenced?
    - Relationship to viral load
  - Mutation linkage
  - Replicaton fitness?
- Provide guidelines for sensitivity of minority species detection?
- **Clonal vs population sequencing: are they in competition or complementary?**
  - **Standardization in both methodologies**
    - **Population sequencing:**
      - **What do you call a mixture?**
      - **How quantitate?**
  - **Effect of viral load**
  - **In what development context: phase 2, 3 etc.?**
  - **In what clinical context: rebound or retreatment?**

HCV DRAG: Genotype questions

- **Do we compare isolates to baseline or to consensus sequences?**
- Measure in vivo fitness by looking at re-emergence of wt vs mutants
  - Impacts sample collection in protocol
  - What threshold should it return to
  - Impacts length of monotherapy recommendations
    - Longer therapy may cause evolution of more fit viruses that do not decay upon cessation of therapy
- **What is the definition of a resistance mutation?**
  - **>10% in rebounders**
  - **<1-5% in naives (need databases)**
  - **Make a "working definition"?**
  - **Specific amino acid substitutions at a particular difference**

HCV DRAG: Genotype questions

Communication of resistance findings:

- How do we share information?
- Statements about resistance be accompanied by sensitivity of method
- Data management/organization

HCV DRAG: Phenotype questions

- How do we standardize assays?
  - IP issue
  - **Availability of standards for sharing, to compare assays?**
    - **Viral standards?**
    - **Cured cell standards?**
- Which methods:
  - Replicon systems
    - How transfer pt viral population?
    - Stable vs transient transfection
    - Relevance of adapted constructs that produce high RNA levels
    - What is "representative clone" for transfection
  - Chimeric cell-based systems
  - Enzyme (cell-free system)
    - What is "representative clone" for transfection
  - Is there an advantage in having a phenotypic assay that is "closer" to patient?
- Replicon vs enzyme analysis
  - Effect of backbone

HCV DRAG: Phenotype questions

- Replicon method:
  - What is the reference WT for use in phenotypic assays?
  - Is population method sufficient or also require clonal introduction into replicon?
- Chimera method:
  - Appropriate sequence to introduce into chimera?

- Fitness:
  - Role of specific mutations?
    - In context of site-directed mutants
    - In context of clinical isolates
  - How measure:
    - In vitro (enzyme, replicon)?
    - In vivo (replacement by WT in monotherapy studies)?
  - Is this best measured by genotype or phenotype?
  - Don't put this in—research issue—no guidance?
  - Belongs in "genotype

HCV DRAG: Phenotype questions

- Minority species:
  - What are limits of phenotypic assays?
  - Amplification of minority species within assay?
    - Effect of fitness within the selection process?
    - What is to prevent the selection of additional mutations within amplification process?
- Interpretation:
  - Fold change most appropriate output?
    - How account for natural variation?
    - Compare to baseline virus or compare to standard wt virus?
      - Clinical trials vs real world
      - **What reference virus to use?**
        - » **Same genotype as sample?**
        - » **One genotype (e.g. 1b)**
- Definition of resistance (cutoffs)
  - Clinical vs biological vs technical?
  - Prior to cutoffs: "reduced susceptibility" vs "activity" and "resistant"

HCV DRAG: Phenotype questions

- Influence of adaptive mutations?
- Cutoffs:
- Patient sequence/vector compatibility?
  - Genotype and subtype mismatch
- What are appropriate boundaries for section of pt virus to amplify?
- How deal with differences in transfection efficiency?
  - Does this affect phenotype readout?
- Feasibility of full-length infectious system?
  - Does it matter to have envelope for inhibitors of viral NS proteins?
  - Restricted to genotype 2?
  - Use in in vitro selection?

HCV DRAG: Phenotype questions

- What will be the role of phenotype in clinical practice?
    - How much need by physicians?
    - Who does phenotyping? Access?

- How define cutoffs?

- How do we define threshold value for resistance?
- What is best response factor for studying resistance (RVR, EVR, SVR)?

HCV DRAG: Clinical questions

- Database
  - Include database people in working group?
  - Create database working group?
  - Propose/create global database?
  - Just baseline (wt) or response data?
- Standard clinical strain library
  - How do we create this library? Is it feasible?
    - Who creates it?
  - What assay is used?
  - Where is the repository? Should it be created?
  - **How do we promote deposition of compounds?**
    - Restrictions on use for virology
    - Restrictions on amount of compound

HCV DRAG: Clinical questions

- Doug: double mutants preexist; how define genetic barrier?
  - Importance of combination therapy?
  - Implications of fitness cost?
  - Do clinical viruses back revert in the absence of drug?
  - Importance of "archiving" in absence of cure?
- Doug: what are essential elements of preclinical evaluation?
  - What are essential elements of clinical development?
- Jules: resistance is a safety issue
  - How do we minimize risk to pts:
    - Naïve: loss of an opportunity, cross resistance to an approved agent
    - PEG-IFN non responders
  - Potential for retreatment with same agent
    - How long to return to background levels?
    - Is there an acceptable level?
  - How complete the analysis?
  - How do we identify the population that will benefit from drug?
    - Genotypic predictors of success
    - Cross-resistance from prior drugs in same class
  - How does host genotype affect efficacy?

HCV DRAG: Clinical questions

- Are there ways to promote the combination of investigational agents?
- Correlation between baseline prevalence of variant or baseline phenotype and outcome (SVR)?

- Can we produce recommendations for clinical trial design to answer questions of resistance development early in clinical development program?
  - Monotherapy duration
  - Specimen timepoints on and off therapy
    - Access to pts off therapy for longer duration (consent)
  - Combination therapy

- How do we promote combination of investigational agents?
  - Especially across companies?

HCV DRAG: Clinical questions

Multiple vs central labs:

- Standardization :
  - Sample pool
  - Iterative refinement
- How do we achieve standardization with multiple labs, especially drug sponsored?

- How promote central lab (market driven)?
  - What do we do if central lab services are not available?
    - Effect on usefulness of phenotype vs genetic resistance?
  - Different issues for U.S. and Europe

HCV DRAG: Clinical questions

- Expanded access?
  - Need PK information
  - Does disease justify?

HCV DRAG: Other questions

- How do we create a "barrier" to resistance-based marketing?
  - Work to get endorsement from major scientific societies
    - EASL preferred to AASLD
    - Paris HCV/HBV resistance conference: target presentation
    - FDA/EMEA/Forum joint meeting
- Who is our target audience?
  - How do we educate the clinicians actually treating patients?
  - Movement in medicine to science-based care