Forum for Collaborative HIV Research

Recommendations from the Phenotypic Analysis Working Group

Isabel Nájera on behalf of the PAWG

DRAG meeting, Boston, November 2009

Definition of terms
(to agree across the 3 Working Groups)

- Biochemical assays: Used to quantify enzymatic activity in absence/presence of compounds (IC_{50})
- Cell based assays: Used to quantify viral replication and antiviral activity of compounds in cell culture HCV replication models (EC_{50})
- Clinical resistance: Demonstration that certain sequence and/or phenotypic property of the virus is associated with treatment failure (not PK, etc)
- Drug Susceptible: Preferred term for the ability of a virus to respond to treatment
- Genetic Barrier to Resistance: Ease with which a virus can escape drug pressure. At molecular level it is represented by the number of nucleotide changes that result in resistance; depends on drug exposure level, viral fitness, level of resistance conferred
- Quasispecies: Population of closely related but distinct viral variants
- Replication capacity: Capacity of a virus or replicon to replicate in cell based assays
- Viral Fitness: Capacity of a virus to reproduce and survive in a particular environment. Monitored in patients longitudinal studies
- Viral variant: Single molecular clone virus entity within a virus quasispecies
- Wild Type virus: Predominant virus population present before taking antivirals

Phenotypic Analysis

- "Critical for the association of specific mutation(s) with changes in drug susceptibility", complementary to Sequencing Analysis

- Uses of phenotypic assays (on target genes):
  - Drug susceptibility of laboratory references and of panels of DAA-treatment naive viruses (helpful for dose selection)
  - Characterization of resistance profiles: preclinical and clinical
  - Interpretation of complex resistance mutation patterns or not predictive of clinical response
  - Assessment of cross resistance
  - In the future: establishment of algorithms for prediction of treatment response (outside scope of this manuscript)

Recommendations on uses

- Preclinical
  - Assessment of drug susceptibility:
    - Reference strains: H77 for GT 1a and Con-1 for GT 1b
    - Panel of treatment naive clinical isolates
    - Drug-Drug combination studies
  - Resistance profile:
    - Level of resistance
    - Replication capacity
    - Cross resistance to same/different inhibitor class

Recommendations on uses

- Clinical: Preferably on <u>population</u> clinical isolates
  - Baseline:
    - On sustained failure (rebound) or partial response or on those patients with resistance mutations at baseline**
  - On treatment:
    - On sustained failure or partial response patients**.
    - Sampling close to viral load rebound to identify initial resistant virus. Later time points will identify fit resistant virus (if sustained rebound is due to resistance).
    - Clonal studies can be performed when complex mutation patterns/mixed populations or linkage (pure or hybrid)
  - Post-treatment:
    - Understand persistence of resistance mutations. Primarily population. Clonal can be performed when complete change at population level (<20 % variants)

* Learnings from Early phase trials will guide late stage procedures

**If resistance observed: can test IFN, RBV and other marketed drugs

Assays and Data reporting

- No standard assay to-date, different assays in use:
  - Cell based: population or clonal
    - Replicon based
    - Non replicon based
  - Biochemical: usually clonal, use of truncated enzymes
- Data needs to be reported in conjunction with:
  - Detailed procedures, system backbone and assay variability
  - Report data in reference to:
    - Same patient baseline, on treatment sample(s) and/or laboratory reference standards
    - Follow FDA and EMEA guidelines

Considerations and unanswered issues

- Low viral load samples (VL<10000 IU/ml): how representative of clinical population are amplicons?
- Replication capacity data and fitness: Correlation unclear
- Fitness: assessment through longitudinal studies in absence of drug

Moving forward

- Steps towards standarization of phenotypic assays:
  - Public compound repository
  - Public replicon/enzymes repository
  - Public key known resistance mutations