Working Group 2: Retrospective Analysis of RAVs for Approved Drugs

DRAG Meeting 15
Barcelona
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Who should be included in the Resistance Analysis Population?

- All subjects with baseline sequences => prevalence and impact of baseline RAVs on SVR
  - Include subjects with baseline sequences and SVR information but no post-baseline sequences
- All subjects who have both baseline and post-baseline sequences => RAVs in virologic failures
  - SVR12 failures vs. any failure (i.e. including late relapse)
- Should we also include subjects who received sub-optimal treatment e.g. Non-compliant or those with dose or treatment duration reduction/addition?
  - If so, distinguish early discontinuation with 4-6 wks vs. 1-2 wks?
- Should we include subjects who were lost to follow-up? If so, criteria?
  - Undetectable RNA vs. detectable RNA at last visit

Which regimen, treatment duration, patient characteristics should be included in the Analysis?

- As per Label recommended indications only?
- All Phase 2/3 studies?
  - Separate analysis by study arm or dose/duration to evaluate impact on label recommended vs. not recommended regimens
- “Real World” studies to be analyzed separately?

Which variants should be included in the analysis (1)?

- How to define drug-specific resistance loci?
  - Advantages/disadvantages of drug-specific vs. class- or subclass-level resistance loci
- How to define drug-specific substitutions within each resistance loci
  - Replicon EC50 fold-shift. Define cut-offs.
  - How to analyze mixtures with multiple RAVs with low levels of resistance if only EC50s for single RAV are available?
  - Utility of PK(Cmin):replicon EC50 ratio? (low utility: plasma conc. Of NS3 and nuc is not representative of liver concentration. Bioavailability varies)
  - Utility of enzyme IC50s for NS3 and NS5B?
  - Utility of reporter cell assay e.g. SEAP assay for NS3?

Which variants should be included in the analysis (2)?

- EC50 fold-shifts other than the standard background e.g. Y93H mutations in the background of GT2b (M31)?
- How to use EC50 data generated from clinical isolates which include multiple substitutions and “non-standard” background?
- When there are differences in WT EC50 between different GTs/subtypes, how to compare the EC50 fold-shifts between different GTs/subtypes to be meaningful?
- Composite of resistance information from baseline analyses, treatment-emergent analyses across trials (including monotherapy), cell culture selection and phenotype resistance data) -

Sequencing/Genotyping and Analysis Methods

- Is cross-lab validation necessary. If so, How? What?
  - Sanger Population Sequencing (RECALL)
  - Next Generation Sequencing (assays & pipelines)
- For studies that only have NGS data, which cut-offs (e.g. 10%, 15% or 20%) should be used to generate consensus sequences for comparison?
- Which genotypes/subtypes should be used if results differ between LiPA and phylogentic analysis?
- How to assign novel subtypes or recombinant HCVs?
- Full length or domain per target gene e.g. first 180 a.a. for NS3, first 100 a.a. for NS5A etc

Receiver Operator Characteristic

- Threshold for variant calls
- RAVs selected for analysis
- EC50 fold-shifts etc.

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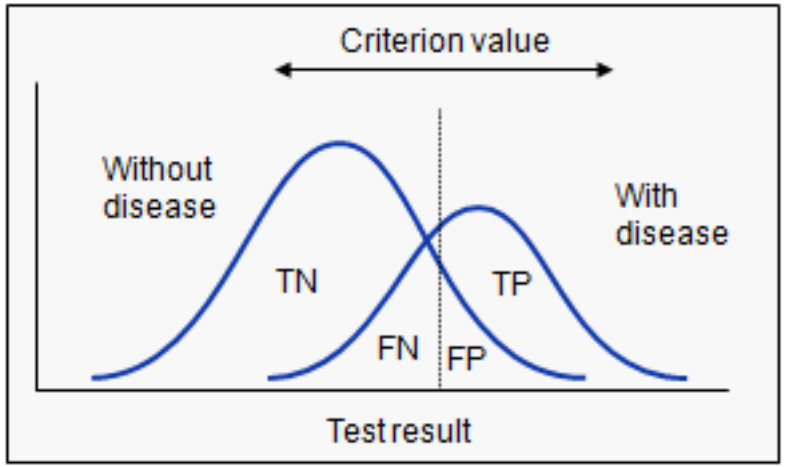
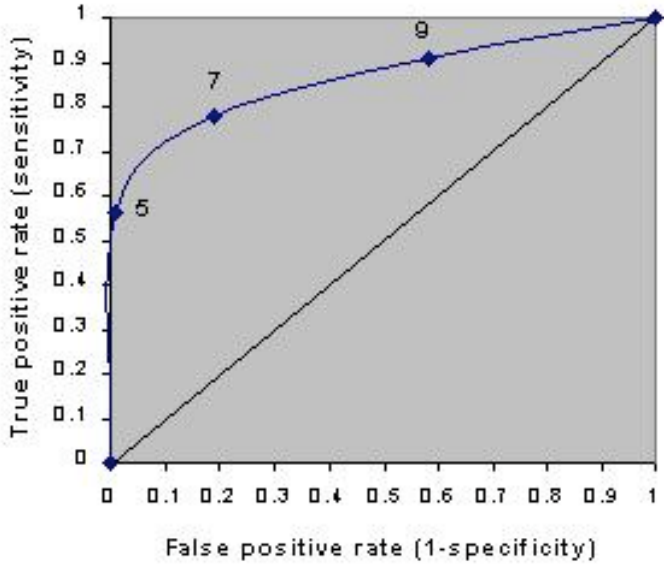
Optimal Conditions for RAV Analysis

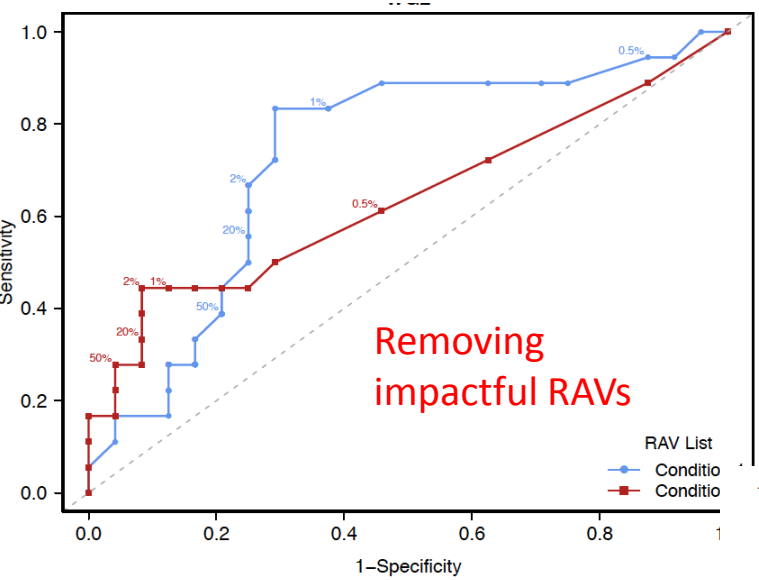
Red – disease (e.g. non-SVR)
Blue – without disease (e.g. SVR)

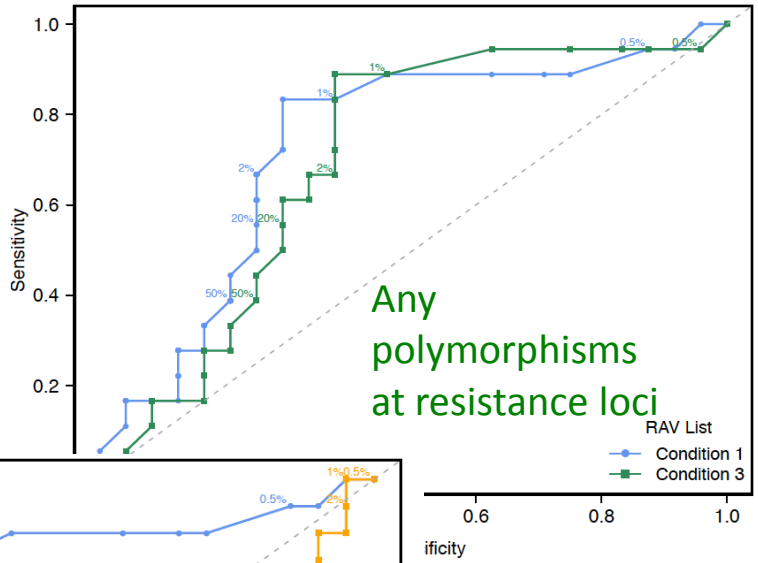


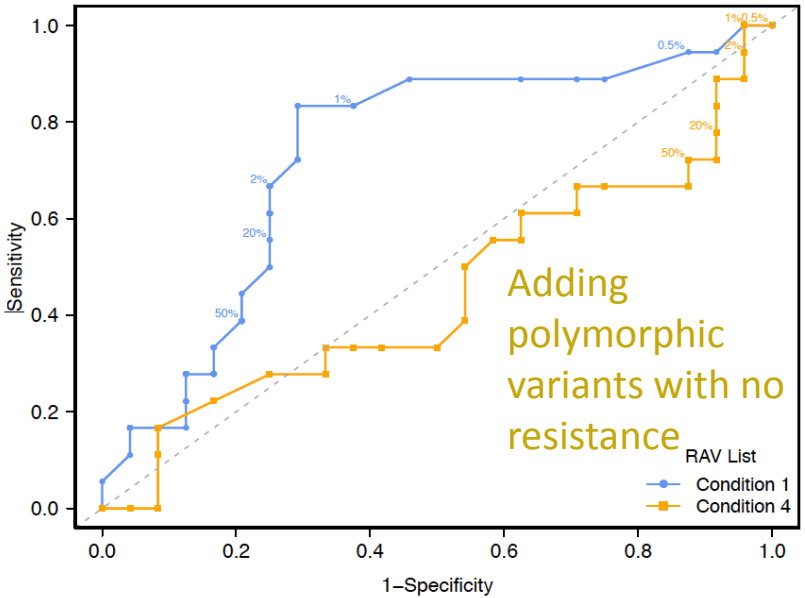
Test (threshold)

	Non-SVR	SVR
+RAV	**TP**	**FP**
-RAV	**FN**	**TN**









Condition 1: NS5A RAVs: M28A/G/T/V, Q30D/E/G/H/R/L, L31I/M/F/V, H58D and Y93any

Condition 2. as condition 1 but skip M28V, Q30H/L, L31M

Condition 3: any polymorphisms from GT1a_H77 i.e. M28Any, Q30any, L31any, H58any, Y93any

Condition 4: as condition 1 but add K24any, A92any, R44any and R78any

Analysis Methods

- Compare SVR/non-SVR in subjects with/without RAVs?
  - all RAVs and individual RAVs
- Assign sequence sensitivity score (similar to HIV)?
- Assign phenotypic sensitivity score (similar to HIV)?
- Statistics/modeling analysis

Analysis Methods

Sub-groups analyses (refer to WG1):

- By Genotype. Genotype subtypes?
- By Regimen
- By +/- RBV
- By Treatment Duration
- By Treatment History e.g. TN, TE (P/R Null vs. RL and IVR; DAA failures)
- By patient characteristics e.g.
  - Fibrosis/Cirrhosis Stage
  - Baseline viral load (cut-offs?)
  - IL28B CC vs. non-CC
  - Race
  - HIV co-infection
  - BMI
  - Others?

- Compare SVR/non-SVR in subjects with/without RAVs?
- Approaches to pool data across related subgroups to increase sample sizes
- Assign sequence sensitivity score (similar to HIV)?
- Assign phenotypic sensitivity score (similar to HIV)? Cut-offs derived from ROC?
- Statistics/modeling analysis

Reporting

- Reference sequences for reporting
  - Specific isolates vs. consensus sequences?
  - Subtype specific references for all genotypes?
- How to deal with references with uncommon amino acids e.g. NS5A in GT4, 5and 6?
- How to deal with M31 in genotype 4?
- How to deal with other resistance associated positions that are also polymorphic

Output of Analyses

Integrate with WG3

1. A list of (drug-specific) and a broader class-wide RAVs associated with virologic failure
2. In vitro EC50 and fold-shifts of RAVs against different drugs
3. An optimal NGS threshold for resistance analysis
4. Prevalence of baseline RAVs
5. Impact of baseline RAVs on SVR in different sub-groups per regimen
6. Clinical significance of individual RAV per drug (per regimen)
7. Impact on SVR rate based on EC50 fold-shifts of RAVs
8. Cross-resistance profile among drugs within the same class (in vitro)
9. Cross-resistance profile among regimens (clinical, based on output #5)
10. Data to be shared with WG#3 for database building
11. A summary of where data are lackng/insufficient
12. Others?