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Estimation of Phenotypic Clinical Cutoffs for *Virtual*Phenotype™ through Meta Analyses of Clinical Trial and Cohort Data

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Background

The clinical utility of phenotypic resistance data can be enhanced by evaluation of the levels of resistance associated with reduced treatment response. To define clinical cutoffs (CCO) for multiple drugs in a consistent manner, we collected resistance, treatment, and virologic response data for patients treated with combination regimens. Quantitative phenotypic resistance levels were predicted by *Virtual*Phenotype™ analysis of viral genotype.

Goals for Clinical Cutoffs on a Resistance Report

- Provide relevant guidance to clinician as to whether a drug is likely to be active for a specific patient
- Treat all drugs consistently
- Provide guidance that is applicable to diverse patient populations.

Four Step Process for Defining Clinical Cutoffs

Step 1: Setup of clinical database and creation of analysis data set

Minimum Criteria for inclusion in the analysis dataset

- Regimen change after resistance test
- Baseline sequence and viral load within 3 months of starting new regimen
- New regimen stable for >4 weeks
- No experimental ARV treatments for which FC cannot be predicted by *Virtual*Phenotype™
- Viral load assessment 8 and/or 24 weeks after beginning new regimen

Clinical Datasets utilized

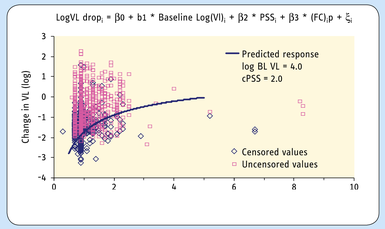
- Clinical Cohorts
  - Chelsea and Westminster Healthcare NHS Trust, London, UK
  - BC Centre for Excellence in HIV/AIDS, Vancouver, Canada
  - 10,674 patient records reviewed
  - 2,249 treatment regimens in analysis dataset
- Clinical Trials
  - Reafvirphen
  - Gilead 907
  - CREST
  - 2NN
  - GART
  - VIRA 3001
  - CERT
  - 2352 patient records reviewed
  - 907 treatment regimens in analysis dataset

Characteristics of Analysis Datasets (8 week outcome) for Individual Drugs

	Range (Drug)
Median Baseline Viral Load (Log)	3.32 (TDF) – 4.71 (boosted IDV)
Median background cPSS	1.34 (ddC) – 2.58 (LPV/r)
# regimens	24 (unboosted APV) - 1551 (3TC)
% cohort data	21% (unboosted APV) - 83% (ddI – EC)
% with no resistance mutations	14.5% (boosted APV) - 75% (EFV)

Example: Analysis Dataset for d4T

- The viral load response data includes censored (<400 copies/ml) values. Parameters of the underlying uncensored distribution were estimated by maximum likelihood estimation in SAS (proc lifereg)
- The viral load response data shown are corrected for baseline viral load and cPSS

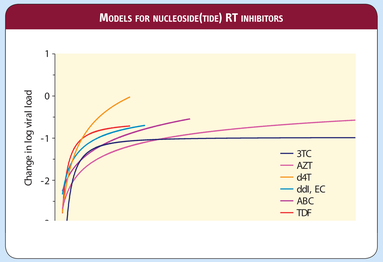


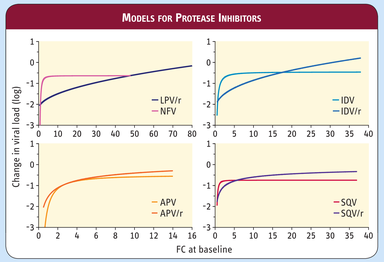
Step 2: Modeling clinical outcome as a function of baseline resistance and other variables

- 3 approaches explored
  - Linear Regression (Drop in viral load)

    general equation:
    $\text{LogVL drop}_i = \beta 0 + \beta 1 * \text{Baseline Log(Vl)}_i + \beta 2 * \text{PSS}_i + \beta 3 * (\text{FC})_i p + \xi_i$
  - Logistic Regression (Response rate)
  - Classification Trees (Response rate)
- Covariates considered
  - "Simple models"
    - Baseline VL
    - Background regimen
    - Categorical PSS in first round
    - Continuous PSS by CCO in subsequent rounds
  - "Complex models"
    - Add Treatment history
    - Evaluate Interactions

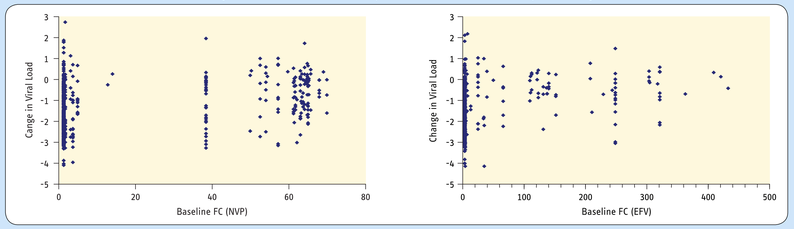
Examples of "simple" linear regression models of 8 week viral load response as a function of baseline phenotypic resistance. Curves shown are for subjects with a log baseline viral load = 4.0 and a cPSS score for the background regimen of 2.0





Issues for NNRTIs

- Polarization of FC values for nevirapine
- Extremely broad dispersal of FC values for efavirenz
- Fundamental Issue: Baseline FC for NNRTIs may have little effect on treatment response to current NNRTIs in NNRTI experienced subjects



Step 3: Determination of Clinical Cutoffs

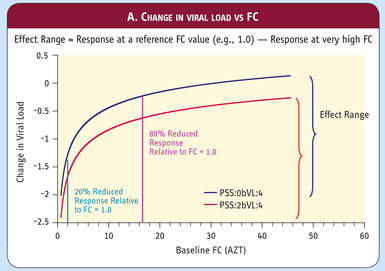
Approach is to Identify Two Cutoffs/Drug

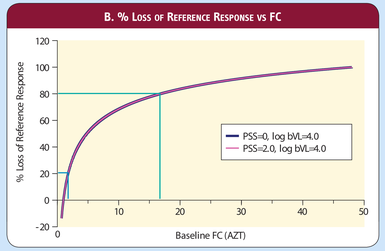
- "Lower" cutoff — FC at which response begins to be lost
- "Upper" cutoff — FC at which response is essentially gone

Several Definitions Possible: See Winters, et al. for Detailed Evaluation of Different Definitions

- FC associated with specific drops in viral load
- FC associated with fractions of a "reference response" at FC = 1.0
- FC that best separates "responders" from "non-responders"

Defining Clinical Cutoffs Based on Fractions of Effect Range

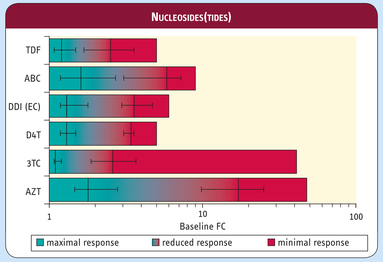


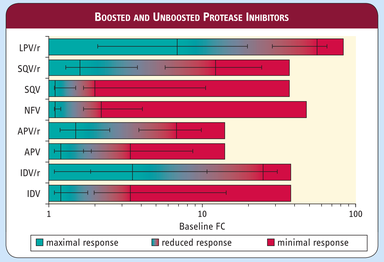


Preliminary VirtualPhenotype™ Clinical Cutoffs

Drug	*Virtual*Phenotype™ predicted FC of wild type clinical isolates	Virologic Response 20% reduction	Virologic Response 80% reduction
AZT	0.8	1.8	17
3TC	0.8	1.1	2.6
D4T	0.7	1.3	3.4
ddI	0.6	1.3	3.6
ABC	0.6	1.6	5.8
TDF	0.8	1.2	2.5
IDV	0.7	1.2	3.4
IVD/r		3.5	25
NFV	0.9	1.1	2.2
SQV	0.6	1.1	2.0
SQV/r		1.6	12
AMP	0.6	1.2	3.4
AMP/r		1.5	6.8
LPV/r	0.8	6.9	56

Step 4: Validation of Preliminary *Virtual*Phenotype™ Clinical Cutoffs by bootstrapping – 90% confidence interval of 100 samples





Conclusions

- The approach taken defines CCO in a uniform way, across multiple drugs, using response data from heterogeneous populations of patients treated with combination regimens.
  - CCO defined in reference to fractions of the effect range can be applied to diverse patient populations
  - The magnitude of the effect will vary (baseline VL, cPSS, Tx history)
- *Virtual*Phenotype™ prediction of phenotype allows the estimation of phenotypic CCO using outcome data for which only genotypic resistance data is available.
- These preliminary CCOs are specific for *Virtual*Phenotype™, which reduces the impact of individual phenotype assay variability
- CCO developed in this study should assist clinicians in the selection of optimal treatment regimens.

Many Thanks to…

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Presented at the XIII International HIV Resistance Workshop, Tenerife, Canary Islands, June 8-11, 2004