ViroLogic

February 23, 2005

Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, rm. 1061
Rockville, MD 20852

RE: ***Comments on "Draft Guidance: Role of HIV Drug Resistance Testing in Antiretroviral Drug Development"***

Docket No.: ***2004D-0484, CDER 2003157***

Dear Sir/Madam:

ViroLogic, Inc. is providing the Food and Drug Administration comments regarding the *Draft Guidance: Role of HIV Drug Resistance Testing in Antiretroviral Drug Development.*

The information enclosed includes two copies of the following: (1) Cover Letter and (2) Comments on the Draft Guidance. If you have any questions regarding the comments submitted, please contact me at (650) 866-7458, or Kathy Hibbs, Vice President and General Counsel.

Sincerely,

Shannon L. Utter

Shannon L. Utter
Director, Quality Assurance

2004D-0484

ViroLogic, Inc.
345 Oyster Point Blvd.
South San Francisco, CA 94080
Telephone: 650.635.1100
Facsimile: 650.635.1111
http://www.virologic com

C2

Comment Number	Section	Line Number	Suggestion/Comment
1	I. Introduction	49 - 82	It should be noted that although co-receptor tropism is discussed, the guidance focuses on resistance testing for NRTI, NNRTI and PI drugs and does not address the susceptibility testing of new drug classes such as entry inhibitors and integrase inhibitors. However, some of the general approaches to resistance testing may apply to the newer classes of drugs and the accompanying assays as they become available. It should also be noted that the measurement of replication capacity is not addressed in the guidance, as it is a unique method for evaluating virus pathogenicity that is not necessarily related to drug resistance.
2	III. HIV Resistance Testing - General	117 - 119	It should be noted that although only one HIV resistance assay has been FDA approved, numerous validated assays are available through clinical reference labs that are regulated under CLIA. In addition, FDA approved or CLIA validated assays may not always be necessary to support drug development and characterization. In certain cases, research-use-only technologies may be sufficient to meet the needs for resistance testing, provided appropriate quality control procedures are practiced.
3	III. HIV Resistance Testing - General	134 - 140	Although it is critical to gather resistance data in early (phase 1/2) and late (phase 3) clinical trials, it is not always possible to characterize a drug as being "active" or a virus "resistant". Terminology that is less subject to misinterpretation (e.g. reduced susceptibility or less susceptible than wild-type) should be encouraged.
4	IV. Nonclinical Studies	150 - 163	Although in vitro studies may be predictive of the situation in vivo, it is important to clearly highlight that all possible pathways for the development of resistance are not captured in vitro. In other words, mutations selected in vitro may well also be observed in vivo, but in vivo the baseline variability that is typically encountered is so much greater that the virus may evolve to be come resistant by developing completely different sets of mutations. Early on after drug approval, phenotypic assays may be used to monitor for the emergence of these resistant strains.
5	IV. Nonclinical Studies	188 - 189	The "broad spectrum" of viruses to be tested should contain resistant as well as wild-type (i.e. lacking known resistant mutations) samples. The inclusion of wild-type samples is not explicit.
6	IV. Nonclinical Studies	191	Commercial (standardized) phenotype assays are not applicable to HIV-2. Therefore, it should be expected that data from assay to assay would be variable and not comparable across assays.
7	IV. Nonclinical Studies C. In Vitro Selection of Drug Resistant HIV-1 Variants	207 - 209	Not only should selection experiments be repeated multiple times, they should also be performed using multiple different starting strains. This will guard against the results being specific to an unusual lab strain and at least increase the chances of observing more than one genetic pathway to resistance.

Comment Number	Section	Line Number	Suggestion/Comment
8	IV. Nonclinical Studies C. In Vitro Selection of Drug Resistant HIV-1 Variants 2. Phenotype	236 - 241	It is not correct to say that "Drug resistant variants exhibit a statistically significant increase in the IC50" because phenotypic testing results are quantitative and can be used to represent degrees of resistance, or partial resistance, which may not necessarily be characterized by a statistically significant different IC50 value.
9	IV. Nonclinical Studies C. In Vitro Selection of Drug Resistant HIV-1 Variants 2. Phenotype	240 - 241	The meaning of a "statistically significant increase in the IC50" should be clarified, and the use of the term "resistant" avoided (see above). Is the intent here to take into account assay variability? If assay variability is high, and the fold-change associated with true resistance is low, then a resistant virus would not have a "statistically significant increase in the IC50"; conversely, if the fold change required for true resistance is high, the lowest fold change that is "statistically significant" might not indicate "resistance".
10	IV. Nonclinical Studies D. Cross-Resistance	254 - 256	Laboratory strains containing mutations associated with resistance to other investigational compounds in the same class as the test compound are usually not available.
11	IV. Nonclinical Studies E. Characterization of Genotypic and Phenotypic Assays	281 - 282	Limiting the region sequenced to major mutations identified in early stages, implying in relatively small numbers of patients, will hamper efforts to refine interpretation algorithms once the drug is approved. For example a less common mutation outside a narrow region within which most mutations fall will prevent identification of this mutation and improvement of the genotype interpretation. Again, this would be a reason for encouraging the use of phenotypic testing (assuming the rare mutations are captured by the amplification steps of those assays).
12	IV. Nonclinical Studies E. Characterization of Genotypic and Phenotypic Assays	305 - 307	It should be clarified that using the fold change in IC50 relative to a reference virus to provide for an absolute comparison between assays applies to the comparison of results within the same assay, but not to results from different assays.
13	V. Clinical: Use of Resistance Testing in Clinical Phases of Drug Development A. General Considerations	330 - 346	It should be noted that ideally, it is best to collect both genotypic and phenotypic data on as many test samples as possible. Both methods offer advantages and when used together, they help to avoid complications of compensatory and/or accessory mutations, facilitate a better understanding of the true phenotypic implications of different mutation patterns and aid in the establishment of more predictive genotypic algorithms.
14	V. Clinical: Use of Resistance Testing in Clinical Phases of Drug Development B. Data Collection	383 - 384	Suggest collecting phenotype and genotype data for study participants who demonstrate a response (but still have detectable viral load) as controls for sporadic sequence variation, in comparison to variation seen in non-responders.

Comment Number	Section	Line Number	Suggestion/Comment
15	V. Clinical: Use of Resistance Testing in Clinical Phases of Drug Development C. Types of Analyses 1. Baseline Genotype and Virologic Response	424 - 426	An additional response parameter to consider would be proportion with at least 0.5 or 1.0 log drop in viral load; this may be particularly important in treatment-experienced patients where a completely suppressive regimen may not be available.
16	V. Clinical: Use of Resistance Testing in Clinical Phases of Drug Development C. Types of Analyses 1. Baseline Genotype and Virologic Response	465 – 469 732 - 734	Table 2B: note that although the table is intended only as an example, the mutations listed in the footnote do not agree with the list in Appendix A.
17	V. Clinical: Use of Resistance Testing in Clinical Phases of Drug Development C. Types of Analyses 4. Genotypic-Phenotypic Correlations: Changes in Susceptibility from Baseline	512 - 527	It is often found that the most clinically meaningful genotypic-phenotypic relationships are determined by combinations of mutations, rather than individual mutations. Therefore, it should be recommended that in addition to identifying genotypic-phenotypic correlations for individual mutations (as shown in Table 5), data should also be obtained for the phenotype susceptibility of the most common or predominant groupings of mutations relevant to the drug under study. The evaluation of specific mutations by the creation of site-directed mutations in wild-type backgrounds can be revealing, but some mutations will never be seen to exist in isolation in vivo. Rather, they exert their influence in collaboration with other "polymorphisms" that may compensate for the deleterious impact of the primary resistance-associated mutation on viral fitness. Therefore, an approach that includes the evaluation of early-stage therapeutics using numerous clinically derived viral isolates is more representative of drug efficacy.
18	Appendix A C. Genotypic Data: (for baseline isolates of all patients and endpoint isolates from virologic failures and discontinuations)	739 -743	Major mutations missing from the proposed list: V75A/M/S/T, Q151M, K219E/H/N/Q/R

Comment Number	Section	Line Number	Suggestion/Comment
19	Appendix A C. Genotypic Data: (for baseline isolates of all patients and endpoint isolates from virologic failures and discontinuations)	747 - 751	Major mutations missing from the proposed list: K10E/P, V179D, F227L
20	Appendix A C. Genotypic Data: (for baseline isolates of all patients and endpoint isolates from virologic failures and discontinuations)	756 - 770	Table A1: specify how insertions and deletions should be represented
21	Appendix A	774 - 779	The p2/NC cleavage site is not sequenced in any commercial genotype assay, and those assays that do sequence the other 2 cleavage sites do not routinely report these data. It is not clear that the standards applied for the validation of PR and RT mutation data are as stringent for the gag cleavage site mutations. Furthermore, numbering systems are variable and have not been standardized, due to the frequent occurrence of insertions and deletions in the p6 region. When requesting data for "cleavage sites", specify the length of sequence required e.g. 4 amino acids on either side of the cleavage site.
22	Appendix A	720 - 781	Note that the outline numbering for Appendix A skips from subpart C - Genotypic Data: (for baseline isolates of all patients and endpoint isolates from virologic failures and discontinuations) to subpart G - Phenotypic Data: (minimally for baseline isolates and endpoint isolates from virologic failures and discontinuation).
23	Appendix A	786 - 794	Note that IC50 data are less reproducible than fold-change data.
24	Appendix A	849 - 850	Clarify what is meant by "assay value", and clearly state that these values may or may not accurately represent the relative proportion of R5 and X4-tropic virus in the patient.