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Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane
Room 1061
Rockville, MD 20852

Subject: Docket No2004D-0484

Comments on the Draft Guidance for Industry Role of HIV Drug Resistance Testing in Antiretroviral Drug Development

Dear Sir or Madam:

Thank you for the opportunity to provide comments on the *Draft Guidance for Industry on Role of HIV Drug Resistance Testing in Antiretroviral Drug Development*. Listed below for your consideration are the comments of Virco, a provider of HIV-1 resistance testing services and analyses to the patient market and pharmaceutical sponsors developing and marketing antiretroviral drugs with facilities in Mechelen, Belgium, Raritan, New Jersey, and Durham, North Carolina,

Lines 74-76: **In addition, this guidance does not address the use of virtual phenotype data in drug development. Sponsors should discuss with the Division in advance any plans to incorporate virtual phenotype into trials**

The document should clarify that the Division is not advising against or prohibiting the use of virtual phenotyping (virco@TYPE HIV-1) as a resistance analysis methodology during drug development. VircoTYPE HIV-1 analysis is currently being used in clinical trials during drug development, frequently as a means to optimize background therapy. We note that vircoTYPE HIV-1 analysis is provided only for drugs that have received regulatory approval, and therefore cannot substitute for conventional drug susceptibility assays for an investigational compound. As recognized by the Division, virtual phenotyping can provide similar information in drug development to the current genotypic resistance testing. In discussions with Regulatory Professionals, the statement "Sponsors should discuss with the Division in advance any plans to incorporate virtual phenotype into trials" is interpreted as a requirement for the Sponsor to discuss use of virtual phenotyping in the drug development prior to its use. This is viewed as an additional burden or task needed that is not demanded for genotyping with other forms of interpretation and can result in a preference to these forms of genotyping for drug development instead of genotyping with virtual phenotype

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analysis. We recommend that the Division remove the perceived requirement that Sponsors **should discuss with the Division in advance of any plans to incorporate virtual phenotype into trials**. We believe that this guidance can be revised to include virtual phenotyping. Virco would like to work with the Division to make these changes and provide additional information on virtual phenotyping.

Line 117 However, only one HIV resistance assay has been FDA approved, and performance characteristics (e.g., sensitivity, specificity, and reproducibility) for many of the assays in investigational use have not been fully established

Line 117 (modified) – only two HIV resistance assays have been FDA cleared, Rationale for change: two HIV resistance assays were FDA-cleared for product code NHS, (both were 510(k)s, not PMAs, therefore the dossiers were ‘cleared’ and not ‘approved’). The two currently cleared assays are the Visible Genetics (Bayer HealthCare) TRUGENE HIV-1 Genotyping Kit and OpenGene DNA Sequencing System (BK000032) cleared on 4/24/2002 and the Celera Diagnostics Viroseq HIV-1 Genotyping System with the ABI 3700 Genetic Analyzer (BK030033). The Division should clarify that the use of an FDA cleared resistance test is not required. Currently, most phenotyping assays, are performed in centralized laboratories regulated by CLIA, CAP, etc, and not by the FDA.

Line 183-184 A well-characterized wild-type HIV laboratory strain grown in peripheral blood mononuclear cells (PBMCs) should serve as a reference standard.

While it is appropriate to consider data generated utilizing human PBMCs, use of such a cell system as a reference standard for resistance testing is impractical. PBMC cultures are notoriously variable in their ability to support HIV replication, leading to wide variability of test results. In addition, the relevance of growing a laboratory strain in PBMCs is unclear. We suggest instead consistently using growth of a wild type laboratory strain in an established cell line as a reference, with comparison in a more limited set of test results to growth of laboratory strains, clinical isolates, or recombinant viruses in PBMCs, monocyte/macrophage cultures, and other cell types.

Line 238-241 Drug susceptibility (IC₅₀ values) for resistant variants and the fold change in IC₅₀ values relative to the parent virus should be determined (see section IV.E Characterization of Phenotypic and Genotypic Assays). Drug resistant variants exhibit a statistically significant increase in the IC₅₀

From the Divison’s experience, how many phenotypes have to be run to determine this "statistically significant difference"? What is the difference deemed



relevant? Smaller differences became statistically significant if more experiments are done.

Line 268-278 **The performance characteristics of genotypic assays should be described, including elaboration of the following characteristics:**

- **minimum plasma viral RNA level**
- **purification methodology for viral nucleic acids**
- **amplification methodology and primers**
- **PCR controls**
- **clade differences**
- **nucleic acid sequencing methodology**
- **description of sequencing primers**
- **range of mutant/wild-type ratios detectable**
- **interpretation criteria for mutant scoring**

The vircoTYPE analysis of viral genotype, resulting in a prediction of drug susceptibility phenotype, is based on a large, proprietary database of HIV genotype and drug susceptibility phenotype test results. The resistance analysis is based on extensive analysis of this database to identify key mutations associated with resistance to individual antiretrovirals. Is the Division suggesting that use of vircoTYPE analysis by a Pharmaceutical sponsor would require disclosure of Virco company confidential information? See also Lines 364-366. **Information about the specific assays and mutational algorithms used in protocols should also be provided in advance to the Division.**

Lines 286-287 (current) – **The sponsor should identify sequencing primers and state how many bases from them can be read accurately.**

Line 286-287 (modified) – **If the sponsor is not using an FDA-cleared assay as a sequencing methodology, then the sponsor should identify sequencing primers and state how many bases from them can be read accurately.**

Rationale for addition: Primer sequences may be proprietary for FDA-cleared assays. Only 'home brew' primer sequences will be known to the sponsor.

Line 302-305 **To detect clinically relevant breakpoints, drugs for which the plasma levels are close to the IC₅₀ value can call for an assay with greater sensitivity than would be sufficient for drugs maintaining plasma levels far in excess of the IC₅₀ value**



How should sponsors respond to this guidance? Is sensitivity of the assay the only allowable selection criterion? What about reproducibility, biological relevance, logistical issues? In addition, what type of virus strain is considered in assessing whether the plasma level is maintained far in excess of the (serum adjusted) IC_{50} . Drug levels far in excess of the IC_{50} for a wild type virus may not be far in excess of the IC_{50} for highly resistant strains.

Line 332-333 the Division strongly recommends that samples for baseline resistance testing (preferably for both genotype and phenotype) be collected on all HIV-infected participants in multiple-dose studies.

The Division should clarify whether they are requiring testing and reporting of baseline genotype and/or phenotype results **on all HIV-infected participants in multiple-dose studies** or merely collection of samples for potential retrospective analysis in individual cases?

Line 364-365 – Information about the specific assays and mutational algorithms used in protocols should also be provided in advance to the Division. Also see Lines 75-76 re. ‘virtual phenotype’.

For genotypic interpretation of mutational patterns CBER accepts interpretive data (based on published literature). The virtual phenotype is similarly interpretive data (based on real clinical cases). The difference is not clear to the commenter as to why one method is acceptable while the other is not. It is suggested that virtual phenotype be included as an option in the ‘genotypic’ analysis.

Line 404-406 To facilitate pooling data, sponsors should attempt to use similar, if not identical, assays throughout the course of drug development

Clinical development of a new drug can extend over many years, during which time new, improved assays are likely to become available. Particularly in the area of HIV Drug Resistance testing, assays evolve rapidly. To mandate use of a single version of an assay over the entire course of a clinical development program is likely to be impractical, and runs the risk of denying study participants access to the current standard of clinical care. Retrospective analysis of stored samples from crucial earlier studies may be a more effective way to develop a comprehensive, consistent resistance dataset.

In addition, commercial providers of resistance testing gain experience with new drugs by providing support to pharmaceutical sponsors during the course of drug development. If only a single provider, utilizing a single test, is allowed during clinical development, patients who may be required to utilize other resistance test providers could experience significant delays in the provision of relevant resistance analysis from their mandated provider once the drug is approved.



Lines 445-447: **For some drugs, defining specific mutational patterns that best correlate with a reduction in treatment response is difficult. In these cases, another approach is to investigate the number of baseline mutations that affects overall response.**

Analyses focusing merely on number of mutations, even from a select list, are likely to oversimplify the relationship between resistance mutations and treatment response. Some indication of the prevalence of the specific mutational patterns present in the specific dataset analyzed would be beneficial, if only to clarify which mutational patterns have not have been included.

Line 486-488 **The proportion of subjects who develop any NRTI (nucleoside analogue reverse transcriptase inhibitor), NNRTI (non-nucleoside reverse transcriptase inhibitor), or PI-associated mutation and the time to development of these mutations should be presented**Request that the Division clarify---Does “time to development of these mutations” mean time on study drug, or time after reaching a study defined virologic failure?

It is important to evaluate potential susceptibility break-points for which no response or reduced response rates are anticipated. In addition, determination of baseline susceptibility to other drugs within the investigational drug’s class is important. Agreement on susceptibility breakpoints for most antiretroviral agents is limited; therefore, the median-fold change in susceptibility can be used as a breakpoint.

What is meant by reduced response i.e. how much does the reduction have to be?

While dividing a specific study population on the basis of median fold change in susceptibility at baseline is one convenient way to create subgroups whose response to treatment can be described, there is no reason to believe that the median fold change can function as a susceptibility break-point delimiting phenotypic resistance levels at which no or reduced response rates are anticipated. The median fold change in baseline susceptibility is a characteristic of the specific study population at entry, not a reflection of response to treatment with the drug. One could take a naive population, determine a median FC and describe this as a breakpoint. On the other hand, the more resistant the population, the higher the breakpoint would be according to the proposed median baseline FC definition. Virologic response should be involved in the definition of a susceptibility breakpoint.

The results of the proposed analysis will depend highly on other parameters such as the activity of the background regimen and the baseline viral load. Even in a



controlled trial, these parameters may not be comparable, especially since there are often a limited number of observations in certain resistance classes.

Lines 724-727. Genotypic Data: (for baseline isolates of all patients and endpoint isolates from virologic failures and discontinuations): Clade

Is a clade assignment based on the nucleic acid sequence of the portion of the viral genome sequenced for resistance studies (often protease and RT only) sufficient?

Line 776 Protease cleavage sites: FOR PROTEASE INHIBITORS ONLY

We would recommend that sequences around the listed protease cleavage sites be determined without requiring complete sequence from p2/NC through protease and RT?

Lines 867-870 Sponsors should assess the development of resistance in vitro over the concentration range spanning the anticipated in vivo concentration

The concentration range explored should take into consideration differences in sequestration by protein binding between *in vitro* cell culture medium and plasma proteins *in vivo*.

We believe that overall the draft guidance will be helpful in providing information on HIV drug resistance testing in antiretroviral drug development. Virco appreciates this opportunity to comment on the Draft Guidance for Industry Role of HIV Drug Resistance Testing in Antiretroviral Drug Development. Please contact me at (919) 313-2664 should you have any questions regarding this letter.

Cordially,

Lee Bacheler, Ph.D
Vice President, Clinical Virology
