EXTERNAL QUALITY ASSESSMENT FOR HIV PROVIRAL-DNA TROPISM TESTING FOR AN INTERNATIONAL CLINICAL STUDY - THE MARAVIROC SWITCH COLLABORATIVE STUDY (MARCH)

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Introduction:

Results in an External Quality Assessment Scheme (EQAS) were harnessed to assure that multiple laboratories testing HIV co-receptor tropism for an international clinical study – MARCH – were performing to an agreed level of competency and reporting consistent test outcomes. MARCH is a maraviroc switch study in virologically suppressed subjects on stable protease inhibitor-based therapy. HIV-1 tropism is determined using population-based sequencing of proviral-DNA in aviraemic patients. Before initiating the clinical trial, a 3-phase proviral-DNA tropism EQAS was implemented across the candidate international testing laboratories.

Methods:

The EQAS for MARCH was conceived to involve two pre-study phases [Phases 1 and 2] and an on-study phase [Phase 3]. Phase 1 required chromatogram interpretation (n=10); samples deemed to be of poor sequence quality were included (n=2); tropism was determined by reference laboratory testing; competency was defined as 100% concordance with reference laboratory output. Phase 2 required triplicate testing of 20 DNA samples from HIV-positive volunteers [VL<50cp/mL (n=18); at least ten were CXCR4-tropic on prior phenotypic testing] where the lowest FPR [false positive rate] of any replicate defined tropism of a sample as determined by online algorithm Geno2Pheno [http://coreceptor.bioinf.mpi-inf.mpg.de/index.php]. The Geno2Pheno algorithm derives the FPR from the V3 sequence of each replicate. In Phase 3 two samples were derived from clones (and therefore did not contain viral quasispecies) and eight were randomly selected from those that had been used in Phase 2.

Results:

In Phase 1 the reference tropism was reported by all laboratories for all samples of acceptable sequence quality however most laboratories also reported results from sequence of substandard quality. The Phase 2 results highlighted the potential for variability between laboratories’ test outcome using samples derived from clinical material. Follow-up of Phase 2 results showed automated sequence analysis can increase concordance between laboratories. As outcomes of the Phase 2 results the FPR was reviewed from 20% to 10%; competency criteria when testing clinical material were adjusted to allow one CXCR4 sample and two CCR5 samples to be misclassified; and a requirement to amplify a PCR product from at least 80% of clinically derived samples. For Phase 3, 11/12 laboratories were competent; one laboratory miscalled two CXCR4 samples as CCR5. Their processes are undergoing further investigation.

Conclusion:

This international EQAS revealed concordance when laboratories tested chromatograms but inter-laboratory variability in tropism determination from proviral-DNA. This variability would have been missed had a single or duplicate sequencing approach been used. These outcomes highlight the necessity to quality assure laboratory groups testing for clinical studies to ensure competency and consistency across test outcomes and further, the need to implement EQAS prior to commencement of the study. Parameters of this EQAS were flexible in response to outcomes; any suboptimal performance was addressed and rectified by supplemental testing and coaching by an expert panel. In the end consistent outcomes were achieved, competent testing promoted, and inter-laboratory networks were strengthened.