



January 17, 2019

**LETTER OF SUPPORT**

Dr. David W. Salzman, CEO  
sRNAlytics, Inc.  
35 Gatehouse Drive  
Waltham, MA 02451

**SUBJECT: Biomarker Letter of Support**

Dear Dr. Salzman,

We are issuing this Letter of Support to sRNAlytics, Inc., to encourage further identification and study of isomiRs (microRNA variants) as exploratory monitoring biomarkers in patients who have symptomatic Huntington's disease (HD) or in people who are at risk of developing symptomatic HD (have the Huntingtin gene mutation).

HD is a genetic, progressive fatal disorder that results in neurodegeneration before the symptoms appear in patients. Presently, there are no qualified biomarkers capable of detecting and monitoring HD prior to the onset of symptoms. Disease staging is evaluated through various clinical assessments (e.g., the Unified Huntington's Disease Rating Scale (UHDRS) and the total functional capacity (TFC) score). These assessments, like all symptomatic assessments, provide limited information about disease progression in the pre-symptomatic phase of HD. Development of a valid and sensitive biomarker for HD progression during both the pre-symptomatic and symptomatic phases of HD could aid in assessing novel drugs for the treatment of HD.

We support sRNAlytics, Inc.'s, proposal to identify certain HD-specific isomiRs and demonstrate that they may be useful in monitoring disease in symptomatic HD patients and in people who are at risk of developing symptomatic HD. MicroRNA (miRNA) functions in RNA silencing and post-transcriptional regulation of gene expression. IsomiRs and their concentrations in blood may aid in better understanding the underlying pathological process in clinical studies of HD drugs.

More experience with the identification of HD-specific isomiRs as biomarkers for HD would be useful to more accurately determine their utility for monitoring patients who have symptomatic HD and in people who are at risk of developing symptomatic HD. In addition, it would be helpful to have a well-defined, validated approach to HD staging with validated cut points for the relevant concentrations of isomiRs.

The data you provided indicate that HD-specific isomiRs were detected in cerebrospinal fluid (CSF) and post-mortem brain tissue. Based upon the biological function/role of an individual isomiR, you determined that the implicated biomarker may be involved in the pathogenesis of HD. The absence of the implicated isomiRs in a limited non-Huntington's population provided limited validation that these isomiRs appeared to only be present in the HD brain tissue samples. Exploration of certain isomiRs for HD staging appeared to demonstrate a trend of increasing concentration with later stage of HD based on grading of post-mortem brain tissue. You identified two HD-specific isomiRs in CSF, HDB-1 and HDB-17 (hsa-miR-10b-5p [+1A|0] and hsa-miR-196a-5p [0|+2TT]<sup>1</sup>, respectively), which appeared to increase in concentration with increased HD progression. While interesting, the findings from these exploratory studies are preliminary and represent a very limited sample.

---

<sup>1</sup> Gene Expression Omnibus Database available at <https://www.ncbi.nlm.nih.gov/geo/>, accessed on 1/17/2019.



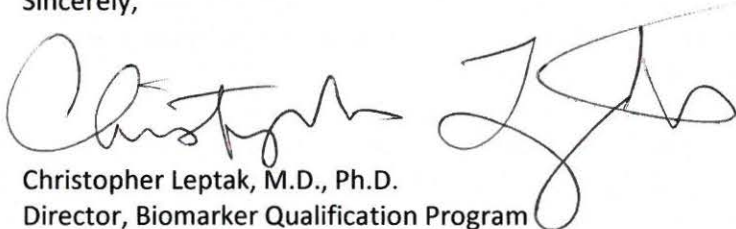
We agree with your plans for future work continuing the identification and development of isomiRs as staging biomarkers for HD, including further exploration of isomiRs related to HD, validation of these as relevant markers of HD, correlation to HD staging methods, and methods for identification of isomiRs in new matrices. In your future work, you should address the following considerations: sample collection and testing procedures, citations or support for proposed roles or biologic plausibility/implication of isomiRs in HD, correlation between levels of isomiRNAs in blood/CSF and HD stage, explain any apparent conflicting findings in studies (e.g., RT-qPCR and sRNA FIND results), explain the approach to validation of the Custom TaqMan assay, and validate the HD staging approaches. Note that imaging information may be helpful in staging or bridging with HD standards.

Strong emphasis on applying rigorous, scientific, and statistically and rationally sound laboratory and software development practices for quality control and validation of isomiRs is imperative. We have the following comments on the analytics you propose to use in your studies: 1) explain how RT-qPCR data ( $\Delta Ct$ ) will be normalized for miRNA quantification across patient samples, and what factors will be considered in the normalization. Level of miRNA can be affected by cellular, pathophysiological and metabolic processes, body compartment shifts, and physiological states including dehydration, amongst others, for the specific matrices used in staging HD; 2) the accuracy of the assay will need to be tested comparing performance to a reference or comparator method, using pre-defined acceptance criteria; and 3) multi-center studies are needed to further evaluate the utility of the test.

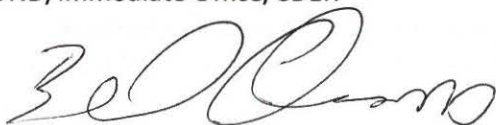
We encourage continued exploration of isomiRs for HD staging. We will consider data collection on this biomarker exploratory in nature. We believe data sharing and integrating data across trials can foster an accelerated path for staging HD in drug development programs. If sponsors intend to include analyses of this biomarker to support regulatory decision making for a given IND drug development program, they should prospectively discuss the approach to these analyses with the appropriate review divisions in CDER and/or CBER.

Any groups (e.g., academia, industry, government) that would like to join in this effort or have information or data that may be useful can contact David Salzman (david.salzman@srnalytics.com), the sRNAlytics, Inc., point of contact for this project.

Sincerely,



Christopher Leptak, M.D., Ph.D.  
Director, Biomarker Qualification Program  
OND/Immediate Office/CDER



Billy Dunn, M.D.  
Director, Division of Neurology Products  
Office of Drug Evaluation I/OND/CDER