DEPARTMENT OF HEALTH & HUMAN SERVICES **Public Health Service**

Food and Drug Administration
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TO: Stephen F. Kingsmore, Director
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FROM: E. David Litwack, PhD
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RE: Q140271
DEVICE: Illumina HiSeq 2000/2500, NextSeq 500
DATED: March 4, 2014
RECEIVED: March 6, 2014

DATE: April 28, 2014

Dear Dr. Kingsmore,

Thank you for submitting this premarket protocol for our review. This pre-submission seeks FDA input regarding the use of next generation sequencing in your NIH-funded investigation, Statseq.

This is an informal communication that represents the best judgment of the Office of In Vitro Diagnostics and Radiological Health staff and consultants who reviewed the protocol. It does not constitute an advisory opinion and does not bind or otherwise obligate or commit the agency to the views expressed, as per 21 CFR 10.85(k).

Based on the information you have submitted, we have provided an evaluation of your proposed protocols**.**

Summary of the study protocol provided, excerpted from the pre-submission:

The purpose of this study is to evaluate the clinical use of whole genome sequencing (WGS) in the care of acutely ill neonates. This study will enroll acutely ill neonates and infants with potential diagnoses, and randomize them and their families to standard of care or WGS in addition to standard testing, if one of the following inclusion criteria are met: 1) clinical genetic testing or a genetic consult is ordered by the neonatologist, 2) the subject has either one major structural anomaly or three or more minor anomalies, 3) patient has a clinical feature or laboratory test value suggestive of a genetic disease, or 4) patient has abnormal response to standard therapy for the major underlying condition. The sponsors expect to enroll 1000 neonates and 2500 family members.

For the study, 1-3 ml blood will be collected and genomic DNA will be isolated (additional specimens (including tissue) may be collected for unspecified future purposes). WGS will be performed, and variants will be called, annotated, and clinically interpreted. Results will be reported to physicians after confirmation by Sanger sequencing, except in cases where variants of unknown significance are identified or a case involves identification of a life-threatening and treatable condition. In those cases, results will be returned prior to confirmation by Sanger sequencing.

The study is composed of the following aims:

- *Aim 1. 1 Generate rapid WGS sequencing data on 500 acutely ill neonates and their families.*
- *Aim 1.2 Assess the molecular diagnostic rate and time to diagnosis amongst those receiving standard of care including expanded newborn screening to those who received the same care plus rapid whole genome sequencing.*
- *Aim 1.3 Assess the impact WGS had on clinical and ethical/social outcomes of acutely ill neonates over a time period of at least one year.*

The proposed whole genome sequencing uses the following reagents, equipment, and software:

- DNA isolation: Chemagen MSM1 (Perkin Elmer) robotics system
- Library preparation: TruSeq (PCR-free, Illumina), NGS Express workstation (Perkin Elmer), QPCR standard curve (Kapa Biosystems), Viia7 qPCR machine (Life Tech)
- QC: Agilent Bioanalyzer 2100 (Agilent)
- Next generation sequencing: HiSeq 2000 or 2500, version 3 or 4 chemistry. NextSeq 500, MiSeq version 2 (Illumina)
- Alignment and variant calling-Pipeline 1: iSAAC Genome Alignment and iSAAC Variant Caller software (Illumina)
- Alignment and variant calling-Pipeline 2: GSNAP version 2012.07.12, GATK version 1.6.13

- Variant annotation: RUNES (CPGM)
- Clinical interpretation: Integrated Variant analysis (Qiagen), VIKING, ALAMUT HD (Interactive Biosciences)

Specific questions for FDA:

CMHC requests feedback on the following questions in our pre-submission teleconference:

1) *Will our proposed study require an IDE? Please highlight the specific areas of concern that determined the IDE designation.*

FDA response: The return of results from an investigational device to patients is exempt from the IDE regulation when those results are confirmed by a reference method or an FDA cleared or approved assay, and if the sampling is noninvasive and does not present added risk to study subjects. We do not believe that your study meets these criteria. While your protocol states that confirmation of results by Sanger sequencing will be performed in most cases, it does allow for disclosure of results to clinicians *prior to* Sanger sequencing in cases that involve "…identification of a life-threatening, treatable condition [and] novel variants of uncertain clinical significance" (p13). The protocol also appears to leave open the possibility that return of results without confirmation may occur in other, undefined situations. Finally, the protocol states that for negative study results a statement about the testing will be placed in patients' medical records. We are uncertain what kinds of results would be considered "negative" for this purpose.

Furthermore, we cannot make a determination that the blood collection does not pose added risk to study subjects. To make this determination, we will require information on volume when encountering conditions such as anemia. Moreover, your protocol also provides for the possibility of collection of blood, urine, and tissue for future unspecified purposes, and it is unclear whether this would include invasive sampling outside of standard of care.

Non-exempt studies require the approval of an IDE submission when specimen collection poses significant risk to patients. Because results may be returned without confirmation, and because it is not possible to anticipate in advance the relevant conditions and treatment decisions that will be encountered in this investigation, we cannot make a confident finding at this time that this is a nonsignficant risk study. As such, your study appears to be significant risk, requiring the approval of an IDE submission unless you are able to provide further clarifying information or modifications to the protocol that allow for confirmation of all results with Sanger sequencing or an FDA cleared or approved test and, if necessary, allow for alternatives to any sample collection that is determined to pose a significant risk to subjects.

2) *What modifications/details in the protocol are recommended by the FDA prior to IDE submission if such submission is deemed necessary?*

FDA Response: The FDA does not have specific modifications to suggest. However, we note that you suggest that unspecified changes to the test system may be made during the course of the study. FDA generally recommends that the sequencing test system is "locked down", including the alignment and variant calling software components, prior to the study. Because you have not provided specific changes that might be made, FDA cannot assess how certain performance

criteria, such analytical sensitivity and specificity, would change if the test system is changed during the course of the study. We recommend that you assess the potential for changed performance, including changes to software that might alter the variants reported, prior to using the changed test to report results. If modifications are made, both the principle investigator and the IRB should review the modifications and determine if the changes result in either: an exempt or non-significant risk study becoming a significant risk study (therefore requiring an IDE) or a need for an IDE supplement (for modifications to an existing IDE) prior to proceeding If you have any questions you may also submit a supplement to this pre-submission to obtain FDA's feedback.

We look forward to our teleconference on Thursday May 1, 2014.

Note that any revisions that you would like to submit in response to this letter (after the meeting) should be submitted in triplicate to the address below and should reference the pre-submission number above (Q140271) in the cover letter to facilitate processing.

U.S. Food and Drug Administration
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If you have any questions or comments regarding this review, please contact David Litwack at (301) 796-6697 or at ernest.litwack@fda.hhs.gov.