Author’s response to reviews

Title: Detection of Large Rearrangements in a Hereditary Pan-Cancer Panel using Next-Generation Sequencing

Authors:

Debora Mancini-Dinardo (dmancini@myriad.com)
Thaddeus Judkins (tjudkins@myriad.com)
John Kidd (jkidd@myriad.com)
Ryan Bernhisel (rbernhis@myriad.com)
Courtney Daniels (cdaniels@myriad.com)
Krystal Brown (kbrown@myriad.com)
Kirsten Meek (Kirsten.Meek@biofiredx.com)
Jonathan Craft (jcraft@myriad.com)
Jayson Holladay (jhollada@myriad.com)
Brian Morris (bmorris@myriad.com)
Benjamin Roa (broa@myriad.com)

Version: 2 Date: 28 May

Author’s response to reviews:

May 28, 2019

Matteo Pasini, PhD
Editor, BMC Medical Genomics
BioMed Central
The Campus, 4 Crinan Street
London N1 9XW
Dear Dr. Pasini,

Thank you for your continued review of our manuscript, entitled “Detection of Large Rearrangements in a Hereditary Pan-Cancer Panel using Next-Generation Sequencing,” which we submitted for consideration as a research article in BMC Medical Genomics.

We have revised and are resubmitting the manuscript (redline and clean versions) in response to the Editor’s and Reviewer’s comments, and we provide a point-by-point response below.

We appreciate your consideration of the manuscript and look forward to hearing from you.

Sincerely,

Debora Mancini-DiNardo, PhD, FACMG
Laboratory Director
Myriad Genetic Laboratories Inc.
320 Wakara Way
Salt Lake City, UT 84108
+1 (801) 505-5121
dmancini@myriad.com

Editor Comments:
1. Please confirm whether your study was submitted to and approved by your institutional ethics committee and include a statement to this effect in your Methods and Ethics approval and consent to participate sections. Please also ensure that the full name of your ethics committee is
included in this statement. If the need for ethics approval was waived by an IRB or is deemed unnecessary according to national regulations, please clearly state this, including the name of the IRB or a reference to the relevant legislation.

Response: The analysis described in this manuscript was not reviewed by an institutional review board. It was performed using de-identified data obtained during the course of routine healthcare operations. In addition, only aggregate data are presented in the manuscript. As such, this does not meet the Human Health Services (HHS) definition of research on human subjects (HHS 46.102). We have revised the Methods to indicate that no additional information was obtained from patients or healthcare providers for this analysis.

2. Please confirm whether informed consent, written or verbal, was obtained from all participants and clearly state this in your Methods and Ethics approval and consent to participate sections. If verbal, please state the reason and whether the ethics committee approved this procedure. If the need for consent was waived by an IRB or is deemed unnecessary according to national regulations, please clearly state this, including the name of the IRB or a reference to the relevant legislation.

Response: All individuals provided consent for clinical testing; this is stated in the Methods. As this analysis of aggregate, de-identified data obtained through the course of routine healthcare operations does not meet the HHS definition of research on human subjects (HHS 46.102), a requirement for written informed consent for study participation does not apply.

3. The Availability of data and materials section refers to the raw data used in your study and presenting tables and figures is not sufficient to state that all data is contained within the manuscript and additional files. Please only use this statement if you have indeed provided all raw data on which your study is based. We strongly encourage all authors to share their raw data, either by providing it in a supplementary file or depositing it in a public repository and providing the details on how to access it in this section. If you do not wish to share your data, please clearly state this in this section along with a justification. Data availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

- The datasets generated and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]

- The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.
• All data generated or analysed during this study are included in this published article [and its supplementary information files].

• The datasets generated and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

• The data that support the findings of this study are available from [third party name] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [third party name].

Please note that if you do wish to share your raw data and do not have consent from all patients to publish this data it will need to be de-identified.

Please also note that if you include your raw data as a supplementary file you will need to provide, after the References, a section titled “Additional files” where you list the following information about each of your supplementary files: * File name (e.g. Additional file 1), * Title of data, * Description of data. All additional files will also need to have been cited in the main manuscript.

Response: All data used for the analysis were collected in the course of clinical testing. The manuscript presents aggregate data; however, raw data would include patient-level information. This is not appropriate to make publicly available, as these tested individuals were not consented for research. Accordingly, we have revised the availability statement in the manuscript to read, “The datasets analyzed during the current study are not publicly available due to patient privacy.”

4. Please represent authors’ names using their initials, not their full name, in the Authors’ Contributions section. If there are any duplicated initials, please differentiate them to make it clear that the initials refer to separate authors.

Response: We have made this revision.

Reviewer 2 comments:
GENERAL COMMENTS: The authors have responded to the comments but rely on their previous validation paper. This is not a true check on sensitivity. They tested 212 samples with known status but give no details about the number with known CNVs. This is a very small validation set. There were only 48 positive CNVs evaluated with no record of how many were in the really important genes. This is inadequate for any true test of sensitivity given that at most this may be 10 each for BRCA1 and BRCA2. A much larger fully blinded sample set with no 'known' number of positive CNVs should be assessed.

REQUESTED REVISIONS:

They still need to carry out a proper validation study for BRCA1 and BRCA2 CNVs.

ADDITIONAL REQUESTS/SUGGESTIONS:

No, just a proper validation.


The most recently published CAP validation standards, Guidelines for Validation of Next-Generation Sequencing-Based Oncology Panels (Jennings et al., 2017) summarize sample requirements as follows:

“We recommend that the validation samples include previously characterized clinical samples of the specimen type intended for the assay (FFPE, blood, bone marrow); previously characterized clinical samples with each type of pathogenic alteration that the assay is intended to detect (eg, SNVs, indels, CNAs, SVs); samples with most common mutations relevant to the intended clinical use of the panel; two or more samples for which a consensus sequence has been previously established (eg, National Institute of Standards and Technology reference material).
for all regions covered by the panel; and a minimum of 59 samples to assess quality metrics and performance characteristics.”

CAP recommends a minimum of 59 samples for NGS panel validation based on tolerance interval calculations. The CAP standards specify no minimum sample number for validating specific variant types in individual panel genes. Judkins et al. (2015) validated deletion and duplication analyses for 23 genes on 212 anonymized samples, along with validating on the 100 non-LR-containing samples that were used to validate sequence variants. Of the 212 anonymized samples, 51 were genomic positive controls. Eight were previously characterized LRs in BRCA1, and a ninth was in BRCA2 (Table 3). The authors also confirmed large rearrangement positives in APC (2), EPCAM (1), MLH1 (4), MSH2 (8), MSH6 (1) and MUTYH (5). The study evaluated three LR detection methods: microarray CGH, NGS dosage analysis, and MLPA. Microarray CGH correctly identified 51/51 positive controls among the 212 samples. In a subset of 110/212 containing 49 LR positive controls, NGS dosage analysis identified 48/49. The 49th fell victim to incomplete lab processing, as the report describes. MLPA was validated for PMS2 and CHEK2 in 110 anonymized samples. All LR results were concordant with microarray data on the same samples as well as with MLPA data where applicable. Based on these guidelines, the Judkins et al. (2015) study far exceeded minimum CAP validation standards for NGS panel tests in oncology.

