Author’s response to reviews

Title: Treatment recommendations to cancer patients in the context of FDA guidance for next generation sequencing

Authors:
Grace Dy (Grace.Dy@RoswellPark.org)
Mary Nesline (Mary.Nesline@omniseq.com)
Antonios Papanicolau-Sengos (Antonios.Papanicolau-Sengos@omniseq.com)
Paul DePietro (Paul.DePietro@omniseq.com)
Charles LeVea (Charles.LeVea@RoswellPark.org)
Amy Early (amy.early@roswellpark.org)
Hongbin Chen (hongbin.chen@roswellpark.org)
Anne Grand’Maison (Anne.Grand’Maison@RoswellPark.org)
Patrick Boland (Patrick.boland@roswellpark.org)
Marc Ernstoff (marc.ernstoff@roswellpark.org)
Stephen Edge (Stephen.Edge@RoswellPark.org)
Stacey Akers (stacey.akers@roswellpark.org)
Mateusz Opyrchal (Mateusz.Opyrchal@RoswellPark.org)
Gurkamal Chatta (Gurkamal.chatta@roswellpark.org)
Kunle Odunsi (Kunle.Odunsi@RoswellPark.org)
Sarabjot Pabla (Sarabjot.Pabla@omniseq.com)
Jeffrey Conroy (jeffrey.conroy@omniseq.com)
Sean Glenn (sean.Glenn@omniseq.com)
Hanchun DeFedericis (Hanchun.DeFedericis@omniseq.com)
Blake Burgher (Blake.Burgher@OmniSeq.com)
Dear Dr. Krüger,

Thank you again for your consideration of our manuscript, “Treatment recommendations to cancer patients in the context of FDA guidelines for next generation sequencing,” for publication in BMC Medical Informatics and Decision Making.

To address the remaining minor revisions requested by reviewers, we have made the following changes to the manuscript:

Alessandro Lagana (Reviewer 1):

I have only one minor concern: why aren't fusion partners reported in Tables 1, 2 and 3?

Thank you for noting this. We added this information to Table 4 where the detected mutations are displayed and clarified the title of Table 1 to state it is a list of Gene-variant types tested, not the specific alterations detected. Similarly, table 2 lists therapeutic associations tested. Table 3 is Patient
Characteristics, and Table 4, as previously noted, is what mutations were identified.

Takahiko Koyama (Reviewer 3):

1. What is the coverage of sequencing?
   We clarified in METHODS that the assay requires a minimum of 457 reads to detect single nucleotide variants and indels (page 6).

2. Did you use matching normal sample to remove germline mutations?
   We clarified in METHODS that the assay does not sequence normal tissue for germline mutations, but does report variants detected in ACMG genes as potentially hereditary (page 6).

3. In table 4, BRCA1, BRCA2, NF1 have very vague definitions of actionable mutations. Do you consider any mutations in these genes actionable or just inactivating such as stop gain, splicing, and frameshift? How do you evaluate SNVs with no evidence of low activity?
   We clarified in METHODS that variant actionability is defined by our knowledgebase, which can match therapeutic associations to detected variants at the nucleotide, codon, exon, or gene or fusion level based on clinic evidence. The proprietary bioinformatics pipeline filters and noted that variants in tumor suppressor genes must either be pathogenic by ClinVar or predicted to be deleterious by both SIFT and PolyPhen to be reportable (page 6).

Requested technical comments were addressed in the prior submission so we did not replicate them here.

Thank you again for the opportunity to submit a revision. We very much appreciate your time, and look forward to your reply.

Carl Morrison, MD, DVM  
CMO, President, & Founder  
OmniSeq, Inc.  
700 Ellicott Street  
Buffalo NY, 14203