Title: Clinically significant variant classification resulting from the use of RNA Sequencing: Experience at a high-volume cancer genetics center

Authors and Affiliations: Sydney Rudowski, Alexis Gallant, Ana Stupar, Ashley Reeves, Sarah Campian, Dana Zakalik; Nancy and James Grosfeld Cancer Genetics Center, Corewell Health, Royal Oak, MI; Ambry Genetics, Aliso Viejo; Nancy and James Grosfeld Cancer Genetics Center, Beaumont Health, Royal Oak, MI

Background:

Germline cancer genetic testing plays an important role in prevention, early detection, and targeted therapy1. The increasing use of multi-gene panel testing (MGPT) underscores the importance of accurate variant classification. We report on the significant contribution of RNA analysis to variant classification with impact on clinical care.

Methods:

A total of 6343 patients who presented to the Nancy & James Grosfeld Cancer Genetics Center between 2019 to 2023 and underwent MGPT at a laboratory with RNA sequencing were evaluated. Patients underwent MGPT using standard DNA technology with added RNA sequencing. We analyzed these patients for clinically significant variant reclassification which includes upgrades from non-actionable variants of uncertain significance (VUS) to pathogenic/likely pathogenic (P/LP) or P/LP to VUS as a significant downgrade.

Results:

A total of 177 patients (2.8%) had a variant classification significantly impacted by RNA sequencing. Of those 177 patients 45 (25.4%) had clinically relevant change due to their reclassification which resulted in a change in medical or surgical management. The majority of these 45 patients were upgraded from a non-actionable result to P/LP and 14 were downgraded to non-actionable. The clinically significant upgrades included the following genes: *BRCA1/2* (5), *MSH2* (4), *MSH6* (1), *PMS2* (1), *ATM* (5), *CHEK2* (5), *RAD51C* (5), *CDH1* (2), and *PALB2* (3), which lead to evidence-based early detection and prevention. The clinically significant downgrades included the following genes: *BRCA2* (5), *RAD50* (1), and *RAD51D* (8), which allowed for a reduction in medical burden. Of the 177 cases impacted by RNA sequencing, 61 uncertain variants (34.4%) were downgraded to benign polymorphisms, helping to reduce uncertainty regarding management.

Conclusions:

Our study demonstrates the importance and added benefit of RNA sequencing in variant classification for patients undergoing multigene panel testing for hereditary cancer. The addition of RNA sequencing improved identification of individuals at increased risk, while lowering the number of uncertain variants. This improved technology will allow for a more precise approach to cancer screening, treatment and prevention.