Title: Impact of RNA sequencing on variant classification: Experience at a high-volume cancer genetics center

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Background: Germline cancer genetic testing plays an important role in prevention, early detection, and targeted therapy. 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: Total of 6343 patients presented to the Nancy & James Grosfeld Cancer Genetics Center between 2019-2023 and underwent MGPT using standard DNA technology and RNA sequencing. We analyzed these patients for clinically significant variant reclassification which includes upgrades from variants of uncertain significance (VUS) to pathogenic (P) or downgrades from P to VUS as a downgrade.

Results: RNA sequencing significantly impacted variant classification for 177 patients (2.8%). Of those 45 (25.4%), had clinically relevant change due to their reclassification. 31 were upgraded from a VUS to P and 14 were downgraded from P to VUS. The clinically significant upgrades included: BRCA1/2 (5), MSH2 (4), MSH6 (1), PMS2 (1), ATM (5), CHEK2 (5), RAD51C (5), CDH1 (2), and PALB2 (3). Notably, RNA identified a deep intronic P variant in MSH2 (c.2458+985A>G) in a patient who previously tested negative without RNA. The clinically significant downgrades included: BRCA2 (5), RAD50 (1), and RAD51D (8). Of the 177 cases impacted by RNA sequencing, 61 VUS's (34.4%) were downgraded to benign polymorphisms.

Conclusions: Our study demonstrates the impact and added benefit of RNA sequencing in variant classification for patients undergoing MGPT for hereditary cancer. This technology allowed for characterization of intronic or splice variants, which otherwise might not be reported. Our data illustrates the potential for improved identification of individuals at increased risk, while lowering the number of uncertain variants. This technology will allow for a more precise approach to cancer risk evaluation, and optimize risk management and therapy of cancer.