Title: Utilizing data from fusion transcript analysis by massively parallel sequencing beyond validated gene fusion reporting

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Introduction: Fusion transcript analysis by massively parallel sequencing (MPS) detects gene fusions and novel isoforms with single nucleotide resolution. While the reporting of these specific findings encompasses the validated usage of this methodology in our clinical laboratory, further interrogation of fusion transcript sequencing data may provide additional diagnostic benefit. Here we present a series of cases from our institution that highlight our experience in using data from a fusion transcript panel beyond detection of gene fusions, including to rule out contamination, exclude low level variants as likely artifacts, and suggest additional testing to investigate nonspecific fusion variant calls.

Methods: Select cases from our clinical oncology MPS laboratory were chosen based on the use of data from a fusion transcript panel beyond the reporting of gene fusions. Each case was reviewed to evaluate the impact on variant reporting or the recommendation for additional testing. Immunohistochemical studies were performed in one case to further investigate atypical variant calls in the fusion panel.

Results: Four cases were selected to highlight scenarios in which analysis of fusion transcript data provided benefit beyond detection of fusion transcripts. In one case, concern that an EGFR deletion detected on a gene sequencing panel could be caused by sample contamination was mitigated by detecting the same EGFR deletion in the concurrent fusion transcript panel, which allowed for confident variant reporting. In two cases, an oncogenic isoform was detected on the fusion panel but at levels significantly below our cutoff for reporting. Closer examination of the data demonstrated numerous nonspecific splicing and fusion variants, a phenomenon we repeatedly see when a gene is overexpressed at high levels due to copy number gain, and thus the variant was not reported. Lastly, a lung cancer case demonstrated numerous nonspecific CALCA fusion transcript calls, suggesting the unexpected overexpression of calcitonin in the patient’s tumor. Comparison of CALCA RNA levels across samples on the sequencing run suggested a marked overexpression in the specimen. Immunohistochemical stains for calcitonin were performed to investigate this finding.

Conclusions: Data generated from fusion transcript panels contain a wealth of information that can provide insights beyond detecting gene fusions. These cases highlight scenarios in which additional analysis provided clinical benefit by increasing confidence in variant reporting or uncovering unusual variant patterns with suggested follow-up testing. Thus, investigation of orthogonal data proves useful for a variety of clinical benefits and is applicable across many different laboratory assays.