EQA for classifying somatic solid tumour sequence variants: helping to harmonise and improve patient results.

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Introduction
The introduction of large next generation sequencing (NGS) panels for the analysis of solid tumours has led to more variants being identified which have potential clinical impact. Classification systems are required to attempt to stratify these variants into those with clinical potential and those without. A number of different systems have been proposed which address pathogenic/oncogenic and actionability to assist with choice of targeted therapies, prognosis and diagnosis.

External quality assessments (EQAs) for variant interpretation have been provided by GenQA since 2013 with somatic variants introduced in 2020. These EQAs have demonstrated that there is variability in the guidelines used and evidence applied to classify variants.

Methods
In each EQA year, participants were provided with three variants along with a clinical case scenario for each variant. They were required to classify the variant, interpret in the context of the referral provided, provide evidence for the classification and interpretation and indicate if the variant would be reported by their laboratory. Details of the variants supplied are shown in Table 1. The classification and supporting evidence was supplied independently by two expert advisors.

The laboratory submissions were assessed by an expert panel, anonymously against peer reviewed marking criteria. Post assessment, participants received an individual laboratory score report detailing the outcome of the assessment and any feedback comments as deemed appropriate by the expert panel along with a Summary EQA report summarising the expected results and EQA findings.

Results
The majority of laboratories reported acceptable classifications with 83% in 2020, 72% in 2021 and 71% in 2022 receiving maximum marks. The number of critical classification errors i.e. those errors which could potentially result in incorrect patient management has been variable, however the results for 2022 (6% critical error rate) showed an improvement from those for 2020 (12.5%) and 2021 (28%).

Across the three rounds of EQA offered to date the majority of laboratories have applied AMP/ASCO/CAP guidelines1 to classify the variants. The use of ACMG2 or related guidelines such as CanVIG3 was more variant dependent with them being used mainly for single nucleotide variants (SNVs). Criteria for classifying the oncogenicity of variants were published by ClinGen/GCG/VICC4 in 2022 and were starting to be adopted for use by laboratories in the 2022 EQA.

Table 1– Details of the variants provided for the EQAs

<table>
<thead>
<tr>
<th>EQA year</th>
<th>Case number</th>
<th>Gene</th>
<th>Clinical scenario</th>
<th>Variant</th>
<th>Expected classification</th>
<th>Reference sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>2020</td>
<td>Case 1</td>
<td>ROS1</td>
<td>Apical epidermoid tumour</td>
<td>c.[898_901del5insG]</td>
<td>Likely pathogenic</td>
<td>NM_002648.2 (exon 16)</td>
</tr>
<tr>
<td>2020</td>
<td>Case 2</td>
<td>BOLA2</td>
<td>Breast cancer</td>
<td>c.700G&gt;T (p.Arg233Leu)</td>
<td>Likely pathogenic</td>
<td>NM_000059.4</td>
</tr>
<tr>
<td>2020</td>
<td>Case 3</td>
<td>BRCA2</td>
<td>Endometrial tumour</td>
<td>c.829C&gt;T (p.Leu279Met)</td>
<td>Likely pathogenic</td>
<td>NM_000059.4</td>
</tr>
<tr>
<td>2020</td>
<td>Case 4</td>
<td>TP53</td>
<td>Lung cancer</td>
<td>c.810G&gt;T (p.Ala271Val)</td>
<td>Likely pathogenic</td>
<td>NM_000054.6</td>
</tr>
<tr>
<td>2020</td>
<td>Case 5</td>
<td>PTPN11</td>
<td>Neuroblastoma</td>
<td>c.1432T&gt;C (p.Glu478Pro)</td>
<td>Benign</td>
<td>NM_006200.6</td>
</tr>
</tbody>
</table>

Figure 1 – Graph showing different guidelines used by laboratories in the EQAs

Figure 2 – Summary of the AMP/ASCO/CAP classifications assigned to each variant

Figure 3 – Summary of the ACMG or ClinGen/GCG/VICC classifications assigned to each variant

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References

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Conclusion
It is important to provide accurate variant classification is to ensure the patient receives the correct result and clinical management particularly with respect to targeted therapies in solid tumours. This EQA has demonstrated variability in the use and application of the guidelines and has highlighted the continued need for EQAs to educate and promote standardisation.