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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 pathogenicity/oncogenicity 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.

Table 1– Details of the variants provided for the EQAs

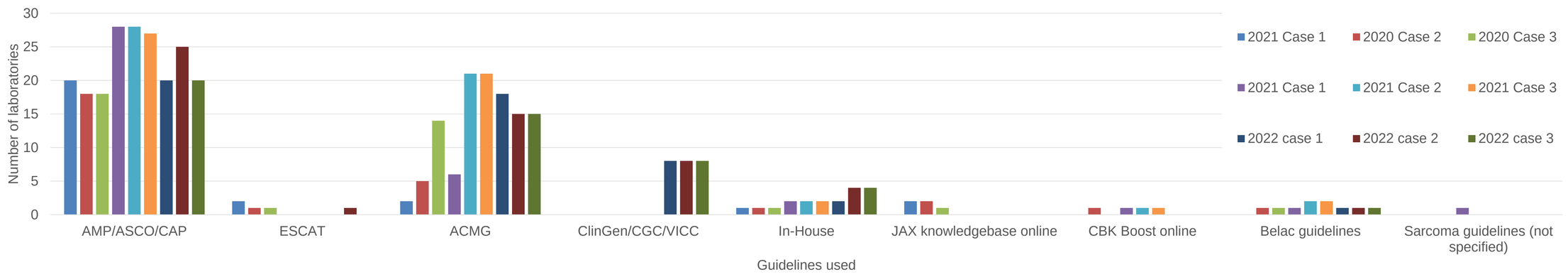
EQA year/ case number	Gene	Referral reason	Variant	Expected classification	Reference sequence
2020/Case 1	*ROS1*	Atypical spitzoid tumour	In-frame ROS1 fusion transcript present: PWWP2A-ROS1 PWWP2A exon 1 ROS1 exon 36	Tier I or Tier IIA (depending on diagnosis or treatment)	*ROS1* LRG_997t1 *PWWP2A* NM_052927.4
2020/Case 2	*IDH1*	Cholangiocarcinoma	c.384C>G p.(Arg132Gly)	Pathogenic Tier I or Tier II	LRG_610t1
2020/Case 3	*BRCA2*	Prostate cancer	c.7007G>T p.(Arg2336Leu)	Likely pathogenic Tier I	NM_000059.4
2021/ Case 1	*NTRK2*	Osteosarcoma	Fusion transcript detected between exon 6 of UFD1 and exon 6 of NTRK2.	Tier III or Tier IV	*UFD1* NM_005659.7 *NTRK* NM_006180.6
2021/Case 2	*BRAF*	Thyroid cancer	c.1797_1811delinsCGA p.(Val600_Trp604delins Glu)	Likely Pathogenic Tier II or Tier III	LRG_299t1
2021/Case 3	*FGFR1*	Small cell lung cancer	c.7007G>T p.(Gly611Trp)	VUS Tier III	NM_001174067.2
2022/Case 1	*BRCA2*	Prostate cancer	c.8021_8023del p.(Lys2674del)	Variant of uncertain significance (VUS)	NM_000059.4
2022/Case 2	*TP53*	Lung cancer	c.811G>A p.(Glu271Lys)	Likely pathogenic /oncogenic Tier IIA	NM_000546.6
2022/Case 3	*PDGFRA*	Gastrointestinal stromal tumour (GIST)	c.1432T>C p.(Ser478Pro)	Benign Tier IV	NM_006206.6

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 guidelines[1] to classify the variants. The use of ACMG[2] or related guidelines such as CanVIG[3] 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/CGC/VICC[4] in 2022 and were starting to be adopted for use by laboratories in the 2022 EQA.

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



AMP/ASCO/CAP Classification

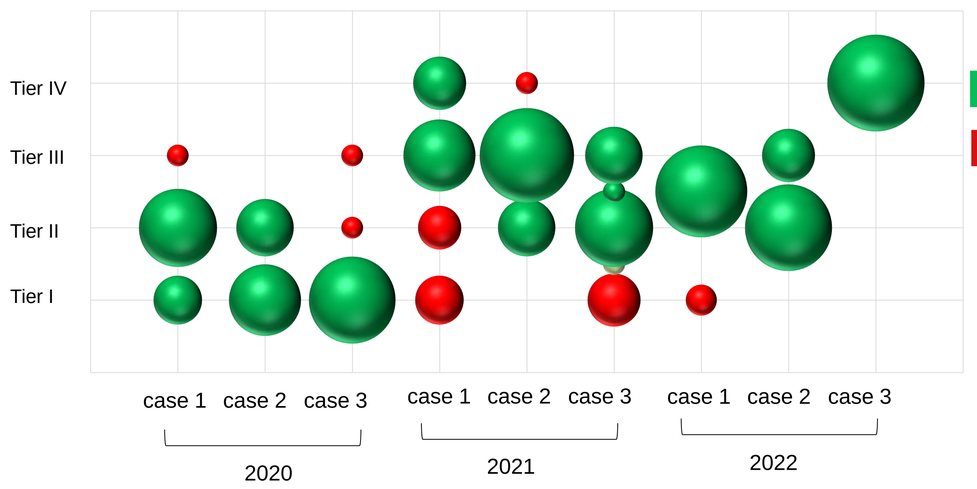


Figure 2 – Summary of the AMP/ASCO/CAP[1] classifications assigned to each variant
The larger the bubble, the increased number of participants using that classification.

ACMG (or ClinGen/CGC/VICC) Classification

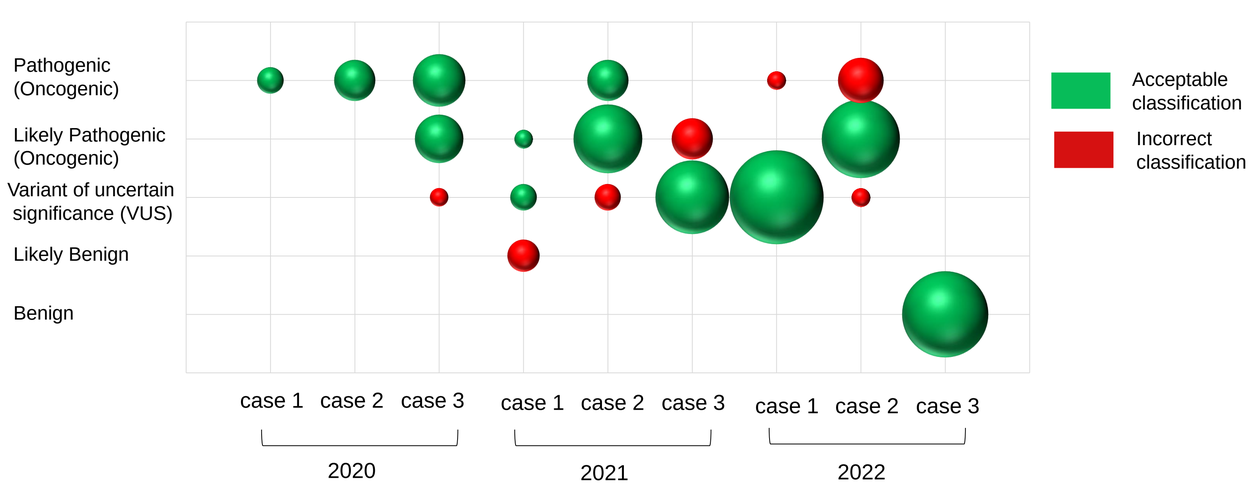


Figure 3 – Summary of the ACMG[2] or ClinGen/CGC/VICC[4] classifications assigned to each variant
The larger the bubble, the increased number of participants using that classification.

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Conflicts of interest: The authors have no conflicts of interest to declare.

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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.

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