The Challenges in Classifying Fusion Transcripts

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Introduction

The detection of variants through somatic testing requires the classification of the pathogenicity of the variant and interpretation of clinical significance.

This is challenging for fusion genes as the prediction of a functional protein must be determined and with the availability of pan-cancer treatments based on the presence of *NTRK* gene fusions this has become more of a requirement.

Education and standardisation are required. External quality assessments (EQAs) for variant interpretation have been provided by GenQA since 2013 and demonstrate variable approaches. The classification of somatic variant pathogenicity EQA 2021 required the classification of a *NTRK2* fusion and variable approaches were taken by the participating laboratories.

Methods

Objectives

The aim of the somatic variant classification EQAs is to:

- Review the process employed by laboratories to assign the pathogenicity of variants,
- Assess the classification of the variants,
- Determine the use of published guidelines,
- Educate laboratories on best practice.

EQA case

The EQA was provided in 2021 and one case required the classification of a *NTRK2* gene fusion. The clinical case scenario and details of the fusion were provided to the participating laboratories (see Table 1).

Participants were required to:

- Classify the variant,
- Interpret in the context of the referral provided,
- Provide evidence for the classification and interpretation,
- Indicate if the fusion would be reported by the laboratory.

Referral reason	Result	Expected results	
		Classification[1]	Reporting decision
Presented with a tumour in the right femur which was diagnosed as an osteosarcoma. Lung metastases have been identified on CT scan. Gene fusion testing was requested on the original tumour to determine possibility for targeted therapy options. Tumour content: 50%.	Fusion transcript detected between exon 6 of *UFD1* and exon 6 of *NTRK2*.	Tier III	Dependent on local reporting practice

Gene references: *UFD1 NM_005659.7 and NTRK3 NM_006180.6*

Table 1 – EQA clinical case, details of the fusion and the expected classification

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.

Fusion variant classification and evidence

It should be assumed that the fusion encompasses the whole of exon 6 of *UFD1* and the whole of exon 6 of *NTRK2.* The *UFD1::NTRK2* fusion results in a transcript with a STOP codon present at the start of the *NTRK2* part of the fusion; a productive NTRK fusion is therefore unlikely to be produced.

This fusion is described in osteosarcoma and suggested to be non-fuctional[2] and would be classed as a variant of unknown significance i.e. AMP/ASCO/CAP[1] Tier III.

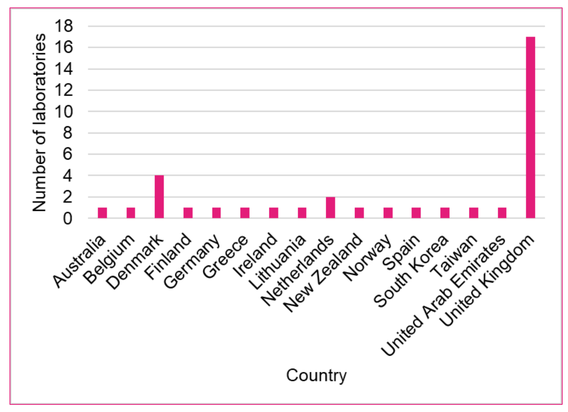
It is expected that participants would:

- ✓ Recognise the fusion as being a non-productive fusion and classify it as being of uncertain significance.
- ✓ Recognise that the there is no evidence that NTRK inhibitor therapy would be appropriate.
- ✓ Report the variant according to local practice.

Results

Participation

Thirty-six laboratories from 16 different countries submitted returns for the EQA case (Graph 1).



Graph 1 – Locations of participating laboratories in the 2021 EQA

Classifications and Guidelines applied

Laboratories received full marks if the variant was classified as either Tier III or Tier IV according to AMP/ASCO/CAP[1] guidelines. Seventeen laboratories using these guidelines (17/28, 61%) classified the variant as either Tier III or Tier IV (Figure 1).

One laboratory did not provide a classification as exact breakpoints for the fusion had not been provided and received full marks. Of the 17 laboratories classifying the variant as Tier III or Tier IV, nine (53%) stated they would not report the variant with eight (46%) stating they would.

Six (13%) laboratories received critical classification errors. Five of these did not correctly determine the production of a non-functional protein. The remaining classed the fusion as Tier I (Figure 1 and Graph 2).

Most laboratories (81%) applied the AMP/ASCO/CAP guidelines[1] to classify the fusion, either stand alone or in combination with other guidance (Figure 2 and Graph 2).

Some laboratories assessed the pathogenicity of an individual variant using ACMG[3] or modified ACMG guidelines and then applied AMP/ASCO/CAP guidelines[1] to independently classify the actionability of the gene. This is incorrect as it is individual variants which should be classified according to AMP/ASCO/CAP guidelines[1].

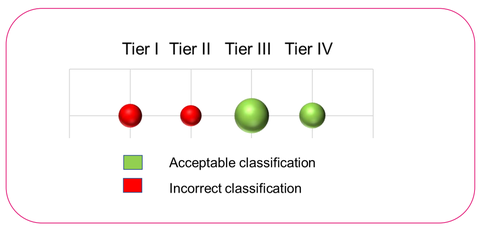


Figure 1 – Participant variant classifications according to AMP/ASCO/CAP guidelines[1]

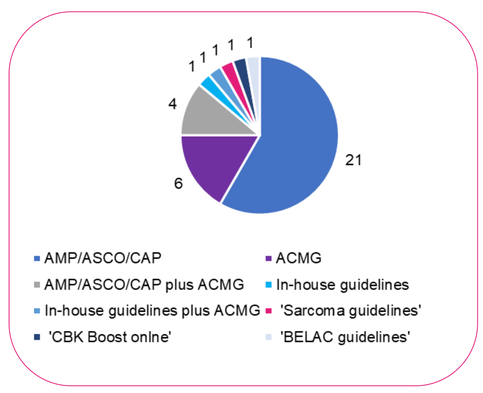
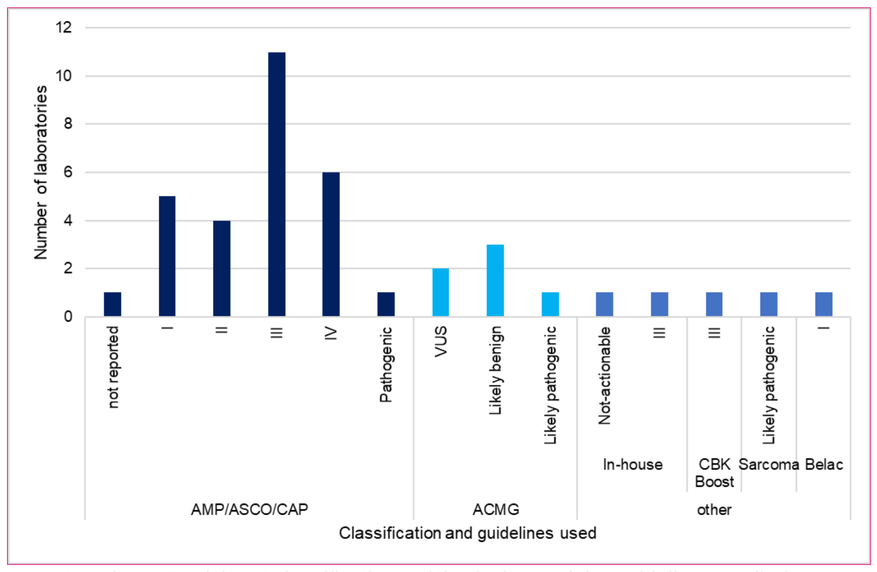


Figure 2 – Breakdown of guidelines used



Graph 2 – Participant classifications of the fusion and the guidelines applied

Results

Critical errors

Six laboratories (13%) received critical classification errors:

- Two laboratories classified the variant as Tier I according to AMP/ASCO/CAP guidelines[1] with no mention of whether or not the fusion was in-frame.
- One laboratory classified the variant as likely pathogenic according to ACMG guidelines[3] and assumed the fusion was in-frame despite quoting a paper which described the fusion as not in-frame.
- One laboratory classified the variant as pathogenic quoting unspecified 'guidelines'; the laboratory did not state whether the fusion was in-frame.
- One laboratory classified the variant as of uncertain significance using ACMG guidelines[3] but Tier I according to AMP/ASCO/CAP guidelines[1]; this was considered contradictory and therefore the laboratory received a critical error. This laboratory, however, did provide the correct interpretation for the variant.

Six other laboratories received deductions from their classification scores as follows:

- Three laboratories classified the variant as Tier I according to AMP/ASCO/CAP guidelines[1], however stated that it should be determined whether the fusion was in-frame; this was therefore not considered a critical error.
- Two laboratories classified the variant as Tier II according to AMP/ASCO/CAP guidelines[1], however, stated that it should be determined whether the fusion was in-frame; this was therefore not considered a critical error.
- One laboratory classified the variant as Tier II and suggested further functional studies therefore this was not considered a critical error.

Conclusions

Tumours with NTRK fusions have an approved therapy[4] which can be used regardless of the tumour type. However, as NTRK inhibitors target the kinase domain of NTRK proteins, it is important to determine whether a functional kinase domain will be expressed in the fusion to aid treatment options.
The EQA has demonstrated variability in the interpretation of fusions and the need to understand the biological impact of the creation of the fusion with regards to protein function and subsequent predicted effect of the variant. Guidelines are used in different ways and standardisation is required to ensure optimal clinical management is available.

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References: 1. Li. M. *et al.* (2017) Journal of Molecular Diagnostic 19(1): 4-23; 2. Ameline B. *et al.* (2020) J Pathol Clin Res.Apr;6(2):107-112; 3. Richards S. *et al*. (2015) Genetics in Medicine;17(5):405-23
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