APPENDIX

REPORTING OF VARIANTS FOR GERMLINE TESTING

For germline testing, variants are typically classified according to standards and guidelines for interpretation of sequence variants published by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP)[1] These guidelines classify variants into pathogenic, likely pathogenic, uncertain significance, likely benign, and benign, as summarized in Table 1.

Variants that are pathogenic or likely pathogenic have evidence for association of that variant with a disease state. When a pathogenic or likely pathogenic variant is detected through germline testing, the patient should be referred for genetic counselling and cascade testing of family members. Variants of unknown significance have either a lack of evidence demonstrating that the alteration is pathogenic, or conflicting evidence about whether it is pathogenic or benign. Most variants of unknown significance are later downgraded to benign or likely benign [2]. Patients with variants of unknown significance may be offered referral to genetic counselling, or they may wait until further information is available reclassifying the variant. Variants that are benign or likely benign are not reported and unless there is a strong personal or family history suggesting hereditary cancer, usually no further action is warranted. If there is a strong personal or family history of cancer, testing can be redone using a larger panel of hereditary cancer genes.

Table 1. Summary of ACMG/AMP variant classification scheme[1]

Reported		**Not reported**
Pathogenic/likely pathogenic	Variant of unknown or uncertain significance (VUS)	Benign/likely benign
Evidence of association of gene alteration with disease state	Lack of, or conflicting evidence regarding whether the alteration is pathogenic or benign Most VUS are later downgraded to benign or likely benign[2]	Evidence to suggest no association of gene alteration with disease state

Next steps: Refer for genetic counselling, cascade testing of family members	Next steps: May be offered referral to genetic counselling, or treat as non-actionable until further information is available	Next steps: Usually no further action

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REPORTING OF VARIANTS FOR TUMOR TESTING

For tumor testing, variants are typically classified according to guidelines for interpretation and reporting of sequence variants in cancer by the Association of Molecular Pathologists, the American Society for Clinical Oncology, and the College of American Pathologists (AMP/ASCO/CAP) [3], although some laboratories may use the ACMG/AMP standards and guidelines described above [1].

The AMP/ASCO/CAP scheme classifies sequence variants into four tiers. This system focuses on the therapeutic, prognostic, and/or diagnostic impact of the sequence variant for clinical management. In the first part of the interpretation process, evidence is used to decide if the variant has a pathogenic or benign impact on the function of the gene, following which the impact of the variant on clinical care is determined.

Tier I and II variants are often grouped together for the purposes of reporting and have evidence of strong clinical significance or potential clinical significance. When a Tier I/II variant is detected, the patient should be referred for germline testing to determine whether the variant is somatic or germline. These variants are generally considered actionable for treatment with the associated targeted therapy, if there is an approved therapy. Tier III variants have unknown clinical significance and are treated as non-actionable with respect to clinical management. The patient can be referred for germline testing if they are eligible. It is important to report Tier 3 variants in case they are present in the germline and are later upgraded to pathogenic/likely pathogenic, as this could trigger cascade testing of family members. Tier IV variants are benign or likely benign, and are not reported. A patient with no actionable variants detected on tumor testing may still be referred for germline testing if they are eligible.

Clinicians should be aware that there may be differences in the interpretation of the same variant in the context of germline testing versus tumor testing since different guidelines are used for interpretation. For example, a variant that is defined as a Tier 3 (variant of uncertain clinical significance) on a tumor testing report because of uncertain therapeutic, diagnostic, or prognostic clinical implications for the patient's cancer may be pathogenic or likely pathogenic in the context of germline testing.

Table 2. Summary of AMP/ASCO/CAP variant classification scheme[3]		
Reported		**Not reported**
Tier I/II	**Tier III**	**Tier IV**
Strong clinical significance (tier I) or potential clinical significance (tier II)	Unknown clinical significance	Benign or likely benign
Next steps: Refer for germline testing, consider treatment options (PARP inhibitors)	Next steps: Treat as 'non-actionable' until further information is available Refer to germline testing if eligible	Next steps: Refer to germline testing if eligible

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REFERENCES

1. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405-24. https://doi.org/10.1038/gim.2015.30
2. Mersch J, Brown N, Pirzadeh-Miller S, et al. Prevalence of variant reclassification following hereditary cancer genetic testing. *JAMA* 2018;320:1266-74. https://doi.org/10.1001/jama.2018.13152
3. Li MM, Datto M, Duncavage EJ, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: A joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn* 2017;19:4-23. https://doi.org/10.1016/j.jmoldx.2016.10.002