ACGS Best Practice Guidelines
Association for Clinical Genomic Science

Association for Clinical Genomic Science (ACGS) guidelines for the classification of oncogenicity of somatic variants in cancer - recommendations by the UK somatic variant interpretation group (SVIG-UK)

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1. Document version history

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2. Scope

2.1 Abstract

Comprehensive genomic testing in routine cancer care pathways has created the need to interpret the consequences of somatic (acquired) genomic variants beyond the currently well-characterised driver variants in cancer gene hotspots. While several guidelines have been published to determine the oncogenicity of somatic cancer gene variants, they lack a comprehensive and flexible approach that encompasses all available lines of evidence. Individual UK laboratories have developed local approaches to standardise somatic variant interpretation, often based on different sets of published guidelines, but a comprehensive national standardised framework is lacking. The absence of standardisation in approaches to somatic variant interpretation highlights a significant gap in the field of genomic medicine within the UK healthcare system. Key stakeholders from across the UK cancer genomics diagnostic community formed the UK somatic variant interpretation group (SVIG-UK) in September 2018 to develop a consensus approach for interpretation of somatic variants identified through genomic testing in patients with solid tumours and haematological malignancies. SVIG-UK scientists conducted a review of existing somatic variant interpretation classification systems and although they mostly agreed on evidence sources for variant interpretation, differences were identified in how the evidence should be used, weighted and combined. The SVIG-UK team subsequently developed a single, standardised UK-wide approach to somatic variant interpretation which encompassed both solid tumour and haematological cancer genomic testing. This framework was shared with stakeholders across the UK alongside variants for preliminary testing. Outcomes were then reviewed and following engagement sessions across the community, the variant interpretation recommendations were updated and ratified by the UK Association of Clinical Genomics Sciences (ACGS). We present herein the SVIG-UK framework and recommendations, which provide a standardised, comprehensive and flexible approach for classifying the oncogenicity of somatic variants in cancer genes.

2.2 What is already known on this topic

Variant interpretation is key in cancer genomics at both the inherited and somatic level. Germline variant interpretation in cancer and rare disease is well embedded in clinical practice guided by multiple sets of recommendations. Somatic cancer variant interpretation lags behind and is less well embedded in clinical practice. Very few guidelines are available with less flexibility compared to germline.

2.3 What this study adds

We provide the first UK somatic cancer variant interpretation guidelines, a standardised framework combining both solid tumour and haematological malignancies.

2.4 How this study might affect research, practice or policy

We present herein the SVIG-UK framework and recommendations, which provide a standardised, comprehensive and flexible approach for classifying the oncogenicity of somatic variants in cancer genes.

3. Introduction

The complex process of oncogenesis is the result of sequential accumulation of “driver” genomic alterations within tumour cells. The accepted definition of a driver variant is the one

used by Stratton, Campbell & Futreal in their 2009 paper 'The Cancer Genome': "'Driver' mutations confer growth advantage on the cells carrying them and have been positively selected during the evolution of the cancer. They reside, by definition, in the subset of genes known as 'cancer genes'"[1]. It is important to recognise, however, that variants identified in cancer-associated genes are not necessarily drivers and may not have played a role in cancer development. An important additional consideration is that oncogenicity can be acquired in a context dependent fashion, for example resistance variants can be considered oncogenic in certain therapeutic contexts[1]. Distinguishing "driver" from so-called "passenger" variants is therefore a crucial step in discerning clinical relevance; as such, the process of somatic variant interpretation (SVI) should comprise a biological classification, where the oncogenic potential of the variant is evaluated, followed by an actionability assessment, whereby the clinical significance of the variant within the context of a specific tumour type is appraised. Both assessments are necessary to be able to standardise somatic variant assessment in a diagnostic setting.

Since the publication and widespread adoption of the American College of Medical Genetics (ACMG) and Association for Molecular Pathology (AMP) guidelines for interpretation of germline sequence variants in 2015[2], attempts have been made to similarly standardise interpretation of somatic variants detected in tumours. In 2017, the AMP in conjunction with the American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP) published a widely adopted framework for reporting of somatic variants. The classification system focused primarily on clinical actionability, and the guidelines stopped short of providing a systematic oncogenicity framework to address standardised biological classification[3]. In the same year, the Belgian ComPerMed Expert Panel[4] began to address this gap by proposing a two-level workflow, including as a first step a tumour-independent, biological five-tier classification system based on the ACMG/AMP germline guidance[2]. Their workflow describes a process of somatic variant calling and annotation which forms the basis of assigning biological classification, comprising a system designed to limit subjectivity. Point based scores are included but are limited to loss of function variants only, an aspect that has been covered in more detail elsewhere[5]. Helpfully, 'Consensus Pathogenic Variant' (CPV) lists of canonical variants requiring no further classification were included for solid tumours and myeloid tumours[4].

Recognising that existing recommendations for somatic variant interpretation (SVI) were insufficient (being based on limited parameters, lacking in detail, or missing elements required for adequate interpretation), in 2021 a consortium of French experts published a new framework for assessing biological impact of somatic variants[6]. Their system, also based on the ACMG/AMP germline guidance, utilises the same nomenclature (pathogenic>benign) and evidence categories with relative weightings, but adds additional somatic-specific criteria. Subsequently, an international consortium of experts representing Clinical Genome Resource (ClinGen), Cancer Genomics Consortium (CGC) and Variant Interpretation for Cancer Consortium (VICC) developed a standard operating procedure (SOP) for the classification of oncogenicity of somatic variants[7]. Horak *et al*., (2022)[7] who also cite inspiration from the ACMG/AMP germline guidelines, categorise evidence of oncogenicity or benignity as very strong, strong, moderate, or supporting, and introduce a user-friendly, point-based system based on the work by Tavtigian *et al*., (2018)[8]. Tavtigian *et al*., (2018) were able to show that a quantitative Bayesian formulation could be fitted to the qualitative ACMG/AMP variant

classification system, endorsing a simple, additive points-based scoring system for combining evidence[8]. Somatic variants can thus be assigned to one of five categories: oncogenic, likely oncogenic, variant of uncertain significance (VUS), likely benign and benign. Once a biological classification is reached, Horak *et al.*, (2022) advise following the AMP/ASCO/CAP guidelines to assess clinical actionability[3,7].

This report describes the development of the UK Somatic Variant Interpretation Group (SVIG-UK) guidelines for the interpretation of somatic variants detected during analysis of DNA originating from tumour cells. These guidelines are focused on single nucleotide variants (SNVs) and small indels and are not intended for assessment of structural and multigenic copy number variants. The oncogenicity framework incorporates elements from existing guidelines and introduces several key/novel aspects which address important gaps in existing guidelines. They are thus expected to encourage harmonisation and improve standardisation of SVI across laboratories.

4. Methods

4.1 Somatic Variant Interpretation Group (SVIG-UK) and Guideline Development

In September 2018, stakeholders from across the UK diagnostic cancer genetics community convened to address issues surrounding SVI. A key outcome of this meeting was a commitment to adopt a consensus approach to SVI, ensuring standardised interpretation and reporting of variants to improve consistency across UK NHS laboratories. Subsequently, the UK Somatic Variant Interpretation Group (SVIG-UK) was formed, comprising Clinical Scientists from multiple NHS laboratories and representatives from key organisations including UK NEQAS for Leucocyte Immunophenotyping (UK NEQAS LI), Genomics Quality Assessment (GenQA), UK Cancer Variant Interpretation Group (CanVIG-UK), the Royal College of Pathologists and the Association for Clinical Genomic Science (ACGS), with the remit of producing a set of guidelines for SVI intended to be adopted by all NHS cancer genomic laboratories.

SVIG-UK scientists began by reviewing published SVI guidelines[3,4,6,7,9–12], placing particular emphasis on the Belgian ComPerMed Expert Panel's recommendations concerning the biological impact of somatic variants[4]. This review aimed to identify key elements for integration into the new guidelines. Due to success and widespread adoption of the ACMG/AMP germline guidance for interpretation of germline variants, the SVIG-UK group decided to adapt the framework set out in these guidelines for SVI, utilising evidence codes supporting either “pathogenicity” (later adapted to “oncogenicity” based upon the ClinGen/CGC/VICC guidance[7]) or “benignity” with relevant weightings. Each weighting is assigned a score and when all scores for codes applicable to the variant under investigation are summed, a final classification can be reached (see Figure 1). The relevance and weighting of each ACMG/AMP code was considered in context of SVI and all applicable codes were retained. The retained codes, and guidance pertaining to application of these codes, was then reviewed and adapted to ensure specificity for investigation of somatic rather than germline variants. To maintain consistency the application of each evidence code aligns, where appropriate, with existing variant interpretation guidelines from ACMG/AMP, ACGS and CanVIG-UK. Additional decisions were made to reorganise the codes according to a logical SVI workflow to enhance usability, to rename the evidence criteria to reflect oncogenicity or

benignity and to remove the weighting from the code titles, instead presenting it as a suffix. Further to this, taking inspiration from the Consensus Pathogenic Variants (CPV) lists in the Belgian ComPerMed Expert panel guidelines, a canonical variants list (see Supplementary Table 3) was developed to enable rapid classification of commonly occurring somatic variants.

In 2022 the ClinGen/CGC/VICC guidelines[7] were released and subsequently compared to the draft SVIG-UK guidance focusing particularly on differences in lines of evidence, evidence weightings, databases, and computational tools, with HCPC-registered Clinical Scientists from the SVIG-UK team classifying three variants (*BRAF* c.1406G>C p.(Gly469Ala), *EZH2* c.1876G>A p.(Val626Met) and *PIK3CA* c.3141T>G p.(His1047Gln) using both the ClinGen/CGC/VICC guidelines and the draft SVIG-UK framework. This comparison highlighted benefits of the draft SVIG-UK framework, such as the inclusion of a canonical variants list (see Supplementary Table 3) and additional lines of evidence not present in the ClinGen/CGC/VICC guidelines[7], such as recurrence in cancer databases, tumour phenotype and gene mode-of-action. Furthermore, the SVIG-UK framework introduces novel somatic permissible and restricted code combinations, aligning with CanVIG-UK[13]. Differences in the layout and evidence weightings were also noted. As a result of this analysis, the SVIG-UK oncogenicity framework was revised to adopt the terminology of Horak et al. (2022)[7], replacing "pathogenic" with "oncogenic", and prefixing oncogenic codes with "O", to better distinguish between somatic and germline classifications. The final set of guidelines, rooted in the original ACMG/AMP germline framework, incorporates elements from consensus specifications developed by ACGS and CanVIG-UK, whilst using the terminology from Horak et al. (2022).

4.2 Community Testing and Consultation

SVIG-UK members performed preliminary testing of the oncogenicity framework on 11 cancer gene variants previously included in various UK NEQAS EQA schemes, allowing a comparison with prior variant assessments by participating laboratories (Table 1). Outcomes were reviewed and the framework adjusted to improve consistency.

In 2023, a wider consultation was performed with the draft SVIG-UK guidelines being made available to all testing laboratories across the UK and the ACGS scientific membership, alongside a list of 20 test variants across 13 cancer genes (Table 1).

Participating laboratories were invited to return a classification for either the solid tumour, haemato-oncology or both sets of test variants, depending upon their specialism, and up to five additional variants of their choice. Participants, mainly UK-based HCPC registered clinical scientists, were asked to classify the variants using both the draft SVIG-UK framework and their existing in-house SVI assessment procedure for comparison. Regular participant workshops were held during the consultation to provide opportunity to discuss the guidelines and to gather participant feedback.

Participants were also asked to complete an exit questionnaire to allow detailed feedback on individual codes, weightings and general suggestions for improvement.

Gene	Transcript	Variant	Tumour type
APC	NM_000038.6	c.7422del*	Colorectal
ASXL1	NM_015338.6	c.2495_2498del*†	Query AML
ATRX	NM_000489.6	c.6514G>T*	Dedifferentiated liposarcoma
BRAF	NM_004333.6	c.1797_1811delinsCGA* c.1457_1471del15† c.1333G>C†	Thyroid Melanoma Lung
CALR	NM_004343.4	c.1152C>G†	MPN
DDX41	NM_016222.4	c.138+1G>C†	Query AML
EZH2	NM_004456.5	c.1696C>A* c.2045C>T† c.2050C>T†	AML cell line MDS AML
FGFR1	NM_001174067.2	c.1831G>T*	Small cell lung cancer
FLT3	NM_004119.3	c.1007C>T* c.2505T>A†	AML cell line Query AML
GATA2	NM_032638.5	c.1085G>A*	Query AML
IDH1	NM_005896.4	c.94T>G†	Glioblastoma multiforme
KIT	NM_00222.3	c.1708T>A*	Melanoma
KRAS	NM_004985.5	c.194G>T†	Colorectal
PIK3CA	NM_006218.4	c.3203dup† c.3193C>T†	Breast Breast
PTEN	NM_000314.8	c.389G>A†	Glioblastoma multiforme
RB1	NM_000321.3	c.1981C>T†	Small cell lung cancer
TET2	NM_001127208.3	c.2599T>C*†	Query AML
TP53	NM_000546.6	c.139C>T† c.217G>A† c.403T>G† c.517G>A† c.892G>A†	Non-small cell lung cancer AML MPN/MDS Non-small cell lung cancer MDS
UBA1	NM_003334.4	c.122T>C*‡	Query VEXAS syndrome/MDS

Table 1. Genetic variants assessed during internal and external testing of SVIG-UK framework. In total, 29 core variants across 19 cancer genes underwent internal (11 variants) and external (20 variants) review by SVIG-UK members and participating laboratories, two of which were assessed both internally and externally**.** *Indicates variants tested during the internal first round testing among SVIG-UK members. †Indicates variants tested during external second round testing with participating laboratories, whereby local existing in-house SVI and SVIG-UK classifications were recorded. ‡At the time of selecting variants for testing, the *UBA1* hotspot variant associated with VEXAS/MDS was newly described and assumed to have an oncogenic function. Subsequently it has become clear that the prevalence of MDS in VEXAS is much lower than originally reported and the association between *UBA1* variants and cancer is uncertain[14]. *UBA1* is not a typical tumour suppressor gene and therefore*, based on current evidence,* the use of SVIG-UK is not recommended for classification of variants in *UBA1.* AML: acute myeloid leukaemia, MPN: myeloproliferative neoplasm, MDS: myelodysplastic syndrome, VEXAS: vacuoles, E1-ubiquitin-activating enzyme, X-linked, autoinflammatory, somatic.

5. Results

5.1 Consultation outcomes

Following wider consultation, 26 responses were received for the ten solid tumour variants and 36 for ten haematological malignancy variants. A further 30 variants across 18 genes were submitted by seven participating laboratories as part of the consultation: 22 from haemato-oncology cases and eight from solid tumour cases. SVIG-UK performed a detailed analysis of the codes applied and final classification for each variant interpretation returned, reviewed feedback, and made adjustments to the oncogenicity framework based on the collective input from participating laboratories. For example, in-frame deletions in oncogenes were included within the test set of variants to challenge this aspect of the guidelines. Analysis of in-frame insertions and deletions was specifically targeted in the exit questionnaire and consensus was subsequently reached to implement an evidence code enabling case-counting within the guidelines for these variant types.

Following the initial analysis and adjustments to the guidelines based on laboratory feedback, the consultation group was provided with an opportunity to review and comment on the consultation outcomes and key developments to the guidelines in early 2024. The revised draft guidelines were then presented at the ACGS annual conference and CanVIG-UK meetings in summer 2024 to update laboratories on current progress. During the consultation period, the Royal College of Pathologists Genomics Special Advisory Committee ratified the SVIG-UK Guidelines, and a second round of consultation and feedback was conducted with the ACGS membership as part of their formal ratification process. This multi-stage consultation and ratification process ensured broad input and consensus across the UK genomics community.

6. Proposed criteria for classification of somatic sequence variants in cancer

6.1 Scope of guidance

The proposed criteria have been developed for the biological classification (determination of oncogenicity) of somatic variants detected during analysis of DNA originating from tumour cells. It is focused on SNVs and small indels and is not intended for structural and multigenic copy number variants. SVIG-UK guidance is not intended for classification of variants of germline origin.

Consistent with Horak *et al*., (2022)[7], the SVIG-UK guidelines use the terminology oncogenic, likely oncogenic, VUS, likely benign and benign for variant classification. It is however essential to have an awareness of alternative terminology, such as pathogenic or driver, describing the biological significance of variants when searching evidence sources.

Following assignment of biological classification using these guidelines it is expected that oncogenic/likely oncogenic variants will then be assessed for actionability and classified within one of the four tiers described in the AMP guidelines[3]. It should be noted that whilst oncogenicity is informed by the biology of the variant, actionability is additionally informed by geography, for example regulatory approvals (such as NICE or FDA), local licensing, access or clinical trial arrangements. It is therefore anticipated that a more localised approach for standardising actionability assessment is likely to be required. Further guidance on standardising clinical assessment/actionability assessment is in development and will follow.

It is important to note that whilst oncogenicity and actionability are presented as separate considerations in this document there is clearly interplay between the two. Information on clinical context is essential to accurate interpretation of somatic variants, for example when interpreting oncogenicity of potential resistance variants within a particular treatment context.

To ensure the most biologically relevant classification is obtained, variants should be annotated against the most clinically relevant transcript for the tissue/tumour type under investigation. The Matched Annotation from the NCBI and EMBL-EBI (MANE) project[15] defined a genome-wide set of biologically and clinically relevant transcripts which are useful for clinical reporting and are often the default for display on browsers and key genomic resources. It is therefore recommended that the MANE Select transcript and, where applicable, the MANE Plus Clinical transcripts are used. However, it is important to note that transcripts utilised in the Clinical Genome Resource (ClinGen) Sequence Variant Interpretation (SVI) working group recommendations should be used in preference over MANE Select/MANE Plus Clinical transcripts in genes where these are discrepant.

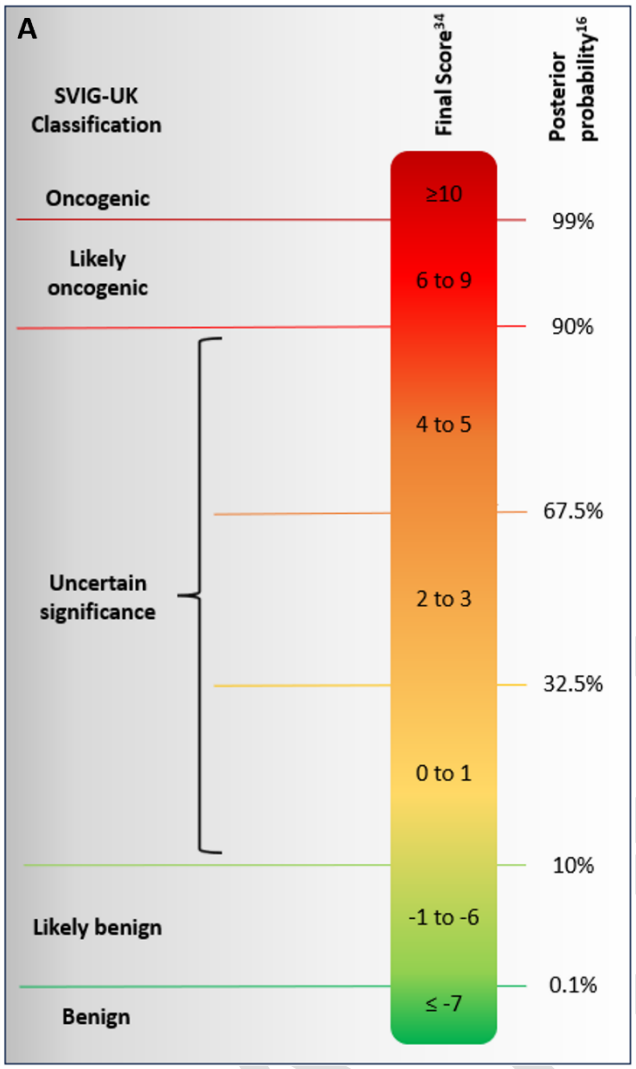
It is the intention of the SVIG-UK group that these guidelines are comprehensive; however, due to the complex nature of variant interpretation, these guidelines are not exhaustive, and it is therefore essential that professional judgement is always applied.

6.2 Points-Based System for Classification

This framework uses a points-based system based upon the study by Tavtigian *et al*., (2018)[8] and the CanVIG-UK recommendations for combining evidence[16]. Codes have been assigned to each line of evidence (summarised in Tables 2 and 3) and weighted scores for each code have been determined by the strength of support for oncogenicity or benignity (Figure 1). The sum of evidence scores allows assignment to an oncogenicity category from oncogenic to benign.

Each code may only be applied once and complementary evidence within each code should not be ‘stacked’ to enable application of a code at a higher strength. For example, entries in different databases cannot be combined to apply O4 at a higher strength than permitted by the use of one database alone. Note also that there are codes which are mutually exclusive and cannot be applied together or can only be applied with some restrictions (see Supplementary Figures 1 and 2).

It is recognised that SVI is a time-consuming process and therefore SVIG-UK acknowledge that where a (likely) oncogenic or (likely) benign classification can be reached, and additional evidence would not upgrade or downgrade the final classification, it may not be necessary to consider all lines of evidence. The classification criteria have been designed with this in mind, placing the most relevant and easiest codes to apply earliest in the workflow. It is essential that scientific judgement is applied to prevent misclassification of variants.



B

Evidence strength	Oncogenicity exponent score	Benignity exponent score
Very strong	+8	-8
Strong	+4	-4
Moderate	+2	-2
Supporting	+1	-1

C

Classification category	Exponent sum
Oncogenic	≥10
Likely oncogenic	6 to 9
Variant of uncertain significance	0 to 5
Likely Benign	-1 to -6
Benign	≤-7

Figure 1 (also Supplementary Figure 4): Points-Based System for Oncogenicity Classification. A) Classification point ranges B) Evidence elements weighting C) Classification categories and exponent sums. Figure adapted from Garrett *et al.* (2021)[16] and UK Best Practice Guidelines for Variant Classification v1.2[34].

6.3 Combining Evidence

To mitigate the risk of a false-positive classification, with the exception of stand-alone evidence criteria, a minimum of two items of evidence is required to reach a (likely) oncogenic or (likely) benign classification. As such, a single line of 'very strong' evidence achieving +8 points will still be classified as a VUS unless a second evidence code supporting oncogenicity can also be applied. Similarly, a single evidence code supporting benignity (-1 point to -4 points) should still be classified as a VUS unless corroborating evidence from a second criteria is applicable. Standalone oncogenicity (code O1) or benignity (specific application of code B1) codes are the sole exception to this requirement.

Where conflicting evidence exists, with applicable codes supporting oncogenicity and benignity, scientific judgement should be applied. Where the strength of evidence for and against oncogenicity is considered equal, it is reasonable to assume that such variants should be classified as a VUS. However, it is not appropriate to apply O3 (absent or rare in population

databases) to classify a variant as a VUS if all other evidence (minimum two criteria) indicates benign status.

To avoid double counting evidence, combinations tables describing comprehensive rules for combining evidence codes towards oncogenicity and benignity have been created (Supplementary Figures 1 and 2).

7. Evidence Criteria

7.1 SVIG-UK Canonical Variants List (O1)

Well characterised, canonical cancer somatic variants supported by robust functional data in the cancer type in question provide the highest level of evidence of biological consequence. Supplementary Table 3 describes a list of such canonical variants in haematological and solid tumours for UK NHS practice, inspired by Froyen *et al*., 2019. Variants in these lists have been reviewed by at least two Clinical Scientists from the SVIG-UK team, are determined to be oncogenic (minimum 10 points) with no apparent discrepant criteria and are also enriched in tumour databases (meeting at least requirement for O4_strong). By exception, a number of very well-established variants that have historically been reported as oncogenic, but only achieve a 'likely oncogenic' rating using the SVIG-UK framework, have also been included in the approved list. The list is not exhaustive and further development of this resource, and its governance, is anticipated.

7.2 Null variant in a tumour suppressor gene (O2)/Mode of action (B2)

Key to establishing the biological significance of a variant is determining whether the variant type aligns with the known mode of action of the gene in the given clinical context. For example, variants predicted to be disruptive are typically associated with tumour suppressor genes, whereas activating variants are found in oncogenes. Additionally, it is essential to consider the downstream implications in the specific tumour type being analysed.

The Cancer Gene Census[17] and the Cancer Genome Interpreter[18] (which sources mode of action data from the Cancer Gene Census) are recommended resources for determining the mode of action of cancer-related genes. If the mode of action for a gene in the cancer under investigation is not available from the Cancer Gene Census, robust evidence must be identified and documented before applying the relevant codes.

Where the mode of action of a gene is ambiguous, it is critical to determine its mode of action in the specific cancer type under investigation. For example, *EZH2* acts as a tumour suppressor gene in myeloid disorders (e.g., MDS/AML), whereas in lymphoid disorders (e.g., B-cell lymphoma), *EZH2* variants are activating.

Driver variants in oncogenes will typically be activating alterations such as missense variants, in-frame insertions and deletions, amplifications, in-frame fusions or translocation of functionally intact regulatory elements. Protein disrupting variants can, however, sometimes result in oncogene activation; for example, 4 bp duplication or insertion in the final exon of the *NPM1* gene results in aberrant localisation of mutant NPM1, which is critical to its role in leukemogenesis[19]. Occasionally, splicing mutations can also be activating; for example, exon 14 splice variants in the oncogene *MET* cause exon skipping[20]. Indels that indirectly affect the essential splice positions are also potential drivers.

Driver variants in tumour suppressor genes are inactivating alterations predicted to impact functional domains important in cancer. This can include missense variants, protein disrupting variants (including premature stop, frameshift and essential splice-site variants), deletions or disruptive structural variants (for example out-of-frame fusions). Canonical splice-site variants affecting the +1, +2 and -1, -2 positions in tumour suppressor genes may be considered as disruptive if *in silico* tools indicate an impact on splicing, although caution should be exercised with splice variants predicted to result in in-frame exon skipping while leaving the remainder of the protein intact[21,22]. If RNA or cDNA-based assays demonstrate that a variant impacts splicing, the O2 (RNA) code may be applied, even if the O2 code would not typically be used based on the variant type or location. The applicable strength of O2 (RNA) is determined by evaluating the impact of the observed RNA change(s) as per general or gene-specific PVS1 recommendations[5,23]. Gene-specific guidance includes CanVIG-UK and ClinGen SVI working group recommendations (available at https://www.cangene-canvaruk.org/gene-specific-recommendations and https://cspec.genome.network/cspec/ui/svi/, respectively).

7.3 Incidence in population database (O3/B1)

A strong indicator that a variant is benign is its recurrence at a high frequency in the general population (B1). The absence of a variant from population databases provides evidence of rarity, supporting its potential biological significance (O3). However, this should not be used as evidence against benignity if two or more benign criteria support a (likely) benign classification.

We recommend using gnomAD; at the time of writing the latest release (v4.1.0) includes data from 807,162 individuals spanning 730,947 exome sequences and 76,215 whole-genome sequences from unrelated individuals of diverse ancestries (https://gnomad.broadinstitute.org/). Be aware that indels are less readily identified by next generation sequencing, and so it is important to ascertain whether other indels are prevalent within the region.

7.4 Enriched in a somatic variant database (O4)

Drivers are defined as variants under positive selection, meaning they confer a selective advantage to cells, such as promoting growth or survival in cancer. This characteristic can be objectively measured by analysing protein-level alteration frequencies across large datasets. Variants under positive selection are observed more frequently than would be expected by random chance, reflecting their role in driving disease processes. This definition underscores the importance of large-scale genomic datasets in distinguishing driver mutations from neutral "passenger" mutations.

Recurrence in appropriately curated somatic databases therefore provides evidence of oncogenicity. It is recommended that the GENIE dataset[24] or any national (multicentre) curated database is used to assess enrichment of a variant in a large genomic dataset (O4) (see Supplementary Table 5). Other databases such as the Catalogue Of Somatic Mutations In Cancer (COSMIC)[25] are available may not permit open access.

In the absence of robust statistical measures to determine the significance of somatic variant recurrence, thresholds for publicly available datasets (e.g. GENIE dataset) have been set based upon the expert scientific judgement and experience of SVIG-UK. When utilising other genomic datasets it is important to consider adjusting thresholds based on the strengths and

weaknesses of the specific dataset to ensure accurate interpretation of recurrence data. For example, in solid tumours, thresholds should be much higher if COSMIC is being used compared to GENIE. Higher thresholds can account for known limitations of the COSMIC database including contamination with constitutional variants, instances where constitutional variants are misclassified as somatic, duplication of the same sample under different identifiers or inclusion of multiple samples from the same case, and inclusion of cell line data rather than primary patient samples. Additionally, within the COSMIC dataset the number of solid tumour samples is much higher compared to haematological samples resulting in a bias towards higher recurrence in solid cancer samples.

Notably, many large genomic datasets, including the GENIE dataset, remain underpowered for recurrence analyses in less well-represented solid tumours, haematological cancers and paediatric malignancies; as such, absence of a variant should not be taken as evidence of benignity. Recurrence in a somatic database may be supplemented by incidence in the literature where it has been confirmed that the cited data is not present in the database utilised, although stacking entries from different databases is not permitted.

Where multiple versions of a dataset are available the most current should be utilised and the version number documented as part of the analysis. Every effort should be taken to exclude duplicate entries when establishing recurrence in somatic databases.

7.5 Variants that affect same location and /or result in a similar impact (O5)

In line with PM5 recommendations[2], the presence of alternative previously established oncogenic or likely oncogenic amino acid changes at the same position as the variant under investigation can provide evidence of oncogenicity. Additionally, alternative previously established oncogenic or likely oncogenic nucleotide changes at a position predicted to affect splicing may also be used as evidence of oncogenicity. The strength at which this code can be applied is determined by comparison of computational predictions of oncogenicity between the two variants.

As the SVIG-UK guidelines are primarily based upon amino acid change rather than nucleotide change (with the exception of splicing variants), an equivalent code to PS1[2] has not been incorporated as it is likely to lead to double counting of evidence, particularly recurrence in somatic databases (O4).

7.6 Computational evidence and missense constraint (O6/B3/O8)

Whilst *in silico* tools should never be used as a sole line of evidence for establishing driver status or clinical decision making[3], when used with caution they can provide useful evidence supporting oncogenicity.

Various *in silico* prediction algorithms are available to aid in the interpretation of missense variants. SIFT[26], PolyPhen[27] and Align GVGD are commonly used examples; however, it is recommended that a meta-predictor tool, such as REVEL, replaces the use of multiple prediction tools that each assess overlapping subsets of the evidence[28,29].

In silico tools also exist to predict the impact a variant may have on splicing, including creation of a cryptic splice site, disruption of splice acceptor and donor sites, and the disruption of other essential splicing motifs, such as branch points and the polypyrimidine tract. The deep

learning-based splice prediction tool, SpliceAI (https://spliceailookup.broadinstitute.org/), has been shown to outperform other single algorithm approaches for predicting splicing impact[30]. This tool is currently recommended for application of O6 and B3 using delta score thresholds of 0.2 or higher (as recommended by the tool developers), and 0.1 or lower[23], to support oncogenicity and benignity respectively.

A low rate of benign missense variation in a gene or region in which missense variants are a common mechanism of disease can be used as evidence of oncogenicity. Missense constraint scores for whole genes or specific gene regions are available from a number of sources, including gnomAD, DECIPHER (https://www.deciphergenomics.org/) and MetaDome (https://stuart.radboudumc.nl/metadome/). When available, regional constraint scores should be used over gene-level constraint scores for the application of O8; however, where there is also enrichment for oncogenic variants within specific regions (e.g. functional domains), the use of O7 may be more appropriate.

Computational evidence permitting the use of O6, B3 and O8 are limited to a supporting level of strength. Evidence has been published defining thresholds for the application of codes at higher levels of strength for specific computational tools; however, within the current framework, this has the potential to result in oncogenic/likely oncogenic classification of a variant based on computational evidence and absence from population databases alone.

See Supplementary Table 5 for links to available computational resources.

7.7 Mutational hotspots and functional domains (O7)

Cancer Hotspots (cancerhotspots.org) is a resource for statistically significant mutations in cancer[31,32]. Chang *et al*., (2016) define a somatic mutational hotspot as a single amino position in a protein-coding gene that is mutated more frequently than would be expected in the absence of selection. To determine the statistical significance of mutational hotspots, the Cancer Hotspots algorithm[31,32], takes into consideration the mutability at a given amino acid and the underlying gene/position-specific mutation rates.

The published recommendations from the ClinGen Germline/Somatic Variant Subcommittee[33], specified that the PM1 criterion (O7 equivalent code) can be applied to somatically detected hotspots with ≥10 occurrences in Cancer Hotspots or downgraded to supporting for fewer occurrences. We have extended upon these recommendations to permit the use of O7 at up to strong for ≥50 occurrences at the same position where at least 10 of these are the same amino acid change (as also proposed in the guidelines by Horak *et al*., 2022). It should be noted that whilst solid tumours have good coverage within this database the coverage is more limited for haematological malignancies, and alternative resources may need to be considered when assessing the application of O7.

As per ACGS guidelines 2024[34], *in silico* protein modelling data and/or protein-protein paralogs of pathogenic variants can be included as evidence when assessing functional domain status (supporting the use of O7) as this may provide evidence that the variant is located within an important functional domain. Useful plots of functional domains, gnomAD variants and reported disease-causing variants are available on the DECIPHER website. MSK

and the TCGA should be excluded from this count to enable the use of Cancer Hotspots (O7) without double counting evidence.

7.8 Protein length change due to in-frame deletions/insertions in a non-repeat region and stop-loss variants or truncating variant in the final exon of an oncogene predicted to result in a gain-of-function (O9/B5)

Small insertion or deletion variants of one or more amino acids (less than one exon in size) and variants that result in abolition of the natural termination codon and elongation of a protein (stop-loss variants) that are not subject to non-stop decay (NSD) have a high prior likelihood of oncogenicity due to length changes in the protein. Conversely, where these length changes affect repetitive or poorly conserved regions the likelihood of oncogenicity is reduced and this can be considered as evidence of benignity.

As O2 is only applicable to loss-of-function variants, O9 can also be applied at a moderate level for truncating variants in the final exon of an oncogene predicted to result in a gain-of-function[23] (Supplementary Figure 3).

7.9 Functional studies (O10/B6)

Well established studies demonstrating an impact, or conversely lack of impact, of a variant on protein function is an important criterion in classification of variants. The ClinGen Sequence Variant Interpretation working group (ClinGen SVI WG) published recommendations for the application of functional evidence in variant interpretation (also known as PS3/BS3 in the original ACMG/AMP guidelines)[35]. Although small functional studies do not easily fulfil these strict criteria, they help to avoid discrepancy and inappropriate application of this line of evidence[35,36]. SVIG-UK recommend the use of the Brnich *et al.*, (2019) framework to assess functional evidence in line with the ACGS and CanVIG-UK guidance. Please note that in accordance with the recommendations from the ClinGen SVI Splicing Subgroup, RNA splicing assays have been incorporated into O2_(RNA) where suitable *in vitro* assays are supportive of an effect on splicing and B4_(RNA) when no impact on splicing is demonstrated[23]. SVIG-UK recommend using the CanVIG-UK guidelines[13] for the assessment of splicing studies and the applicable evidence weighting with supplemental guidance from the ClinGen SVI Splicing Subgroup recommendations[23].

Recent large functional studies, commonly known as multiplexed assays of variant effect (MAVEs), have been instrumental in improving variant classification and, according to ClinGen SVI WG recommendations, often achieve a strong weighting for the application of functional evidence[37]. Many such studies have been published for cancer genes including *TP53*, *BRCA1*, *BRCA2*, *EGFR* and mismatch repair (MMR) genes[38–43].

7.10 Tumour phenotype (O11/B7)

This is a novel line of evidence applicable to somatic variant interpretation and is comparable with the ACMG/AMP criteria of patient's phenotype or family history being highly specific for a disease with a single genetic aetiology (PP4)[2].

As part of a comprehensive genomic testing strategy within the context of a multi-disciplinary diagnostic pathway, additional tumour-specific molecular and cellular phenotypic information may be available, for example biochemical analysis or immunohistochemical (IHC) testing of

the relevant protein (e.g. loss of MSH2/MSH6 in a tumour with a variant in *MSH2* gene); absent or supportive germline test findings; alternative supportive genomic testing (such as microsatellite instability [MSI], tumour mutation burden (TMB], homologous recombination deficiency [HRD] or accompanying loss of heterozygosity). SVIG-UK propose a framework for integrating tumour molecular and cellular phenotypic data into the classification of a variant. Further examples and more details can be found in Supplementary Table 4.

7.11 Synonymous variants (B4)

Synonymous variants have traditionally been interpreted as phenotypically silent events (passengers) but increasingly they are being revealed as tumour drivers through alteration of regulatory sites, mRNA stability or effects on translation[44]. For example, synonymous changes at the last base of exon 4, exon 6 and exon 9 of *TP53* should be classed as driver variants as they affect splicing of these exons[45]. It is therefore recommended that synonymous variants are not universally assumed to be passengers and in addition to the investigation of impact upon splicing, the use of resources such as SynMICdb (https://synmicdb.dkfz.de/rsynmicdb/) may enable further investigation.

7.12 Constitutional and Gene Specific Guidance

SVIG-UK recognise that constitutional classifications using expert-curated, gene-specific guidelines (e.g. ClinGen Variant Curation Expert Panels [VCEPs] and CanVIG-UK recommendations) already exist for classification of variants detected within several genes, including cancer susceptibility genes. For *BRCA1, BRCA2* and MMR genes, classification using these guidelines is advisable; the use of other gene-specific expert-curated guidelines is permissible but at the discretion of individual laboratories based on the gene, the variant and the case under review. It is acknowledged that specific lines of evidence within constitutional gene-specific guidelines, such as defined mutational hotspots, well-established functional domains and application of functional evidence, may be helpful to laboratories when classifying somatic variants and therefore local decisions can be made regarding the use of these lines of evidence within the current framework of the SVIG-UK guidelines and at the permitted evidence strengths. It is essential that scientific judgement is applied to ensure constitutional guidelines are used in an appropriate manner for classification of somatic variants.

7.13 Variant Reclassification

Variant classifications may be updated over time due to change of guidance and the emergence of new evidence, either supportive or contradictory. When the classification of a variant changes across a significant threshold (e.g. VUS to likely oncogenic), there may be clinical consequences resulting from this change. The implication of reclassification may differ between variants and patients, and is based on many factors including clinical actionability, tumour type, patient history and treatment. A wider multidisciplinary approach including laboratory scientists, pathologists, oncologists and other relevant medical professionals may be required to provide local guidance for reinterpretation and reporting of reclassified variants, considering the impact that this may have on patient care. Reclassification is therefore outside the scope of these guidelines.

Evidence criteria		Evidence strength
O1	SVIG-UK Canonical Variants List (see Supplementary Table 3)	Stand-alone Oncogenic
O2 / O2 (RNA)	Null variant in a tumour suppressor gene (TSG)	Very strong [+8] Strong [+4] Moderate [+2] Supporting [+1]
O3	Absent or very rare in population database (e.g gnomAD)	Moderate [+2] Supporting [+1]
O4	Enriched in a somatic variant database (compared to prevalence in controls)	Strong [+4] Moderate [+2] Supporting [+1]
O5	Variants that affect same location and /or result in a similar impact	Moderate [+2] Supporting [+1]
O6	Computational evidence supports a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact etc).	Supporting [+1]
O7	Located in a mutational hotspot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation	Strong [+4] Moderate [+2] Supporting [+1]
O8	Overall constraint for missense variation, at the level of the gene or domain/region, where missense variants are a common mechanism of disease	Supporting [+1]
O9	Protein length change due to in-frame deletions/insertions in a non-repeat region and stop-loss variants or truncating variant in the final exon of an oncogene predicted to result in a gain-of-function	Moderate [+2] Supporting [+1]
O10	Well-established *in vitro* or *in vivo* functional studies demonstrating a functionally abnormal result consistent with the mechanism of disease	Very strong [+8] Strong [+4] Moderate [+2] Supporting [+1]
O11	Tumour phenotype	Moderate [+2] Supporting [+1]

Table 2: Summary of oncogenicity evidence criteria. For the full criteria and detailed guidance see Supplementary Table 1.

Evidence Criteria		Evidence Strength
B1	Variant is present at a high level in a general population database (e.g. gnomAD)	Stand-alone benign Strong [-4]
B2	Variant does not fit the mode of action of the gene for the cancer under investigation	Stand-alone VUS
B3	Computational evidence DOES NOT support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact).	Supporting [-1]
B4 / B4 (RNA)	Synonymous / intronic variants (outside canonical splice sites; at +7/-21 or beyond)	Strong [-4] Supporting [-1]
B5	In-frame deletion/insertion in a repetitive region with unknown function	Supporting [-1]
B6	Well-established *in vitro* or *in vivo* functional studies demonstrating no damaging effect on protein function or RNA splicing	Strong [-4] Moderate [-2] Supporting [-1]
B7	Tumour phenotype	Moderate [-2] Supporting [-1]

Table 3: Summary of benignity evidence criteria. For the full criteria and detailed guidance see Supplementary Table 2.

7.14 Additional considerations

This is a generic framework which cannot address all gene-specific nuances. Consequently, several very well-established variants historically reported as oncogenic are only able to achieve a 'likely oncogenic' classification when using the SVIG-UK framework (for examples, see Supplementary Table 3). Of note, gain-of-function variants in oncogenes can be particularly difficult to classify for several reasons, including their low frequency in large somatic variant databases and the difficultly in interpretation of their functional consequence (e.g. modest increases in protein activity or poor understanding of the role of the protein in cancer). The stringency of current guidance around the use of functional studies frequently prevents the application of O10 above a supporting level, most notable in the assessment of older studies which often do not meet current standards defining a well-controlled/well-validated assay. As a result, many potential and even 'established' gain-of-function variants will not be scored as (likely) oncogenic using this framework due to a lack of adequately robust evidence. Overall, it is acknowledged that, pending the availability of additional functional data and expanded population and patient cohort data or gene-specific somatic guidance, it may be appropriate to 'uplift' the classification of some variants to (likely) oncogenic based upon scientific/clinical judgement and local policy.

8. Conclusions

Somatic variant interpretation plays a pivotal role in an ever-expanding range of key clinical decisions across nearly all tumour sites. The consequences of misclassification can be severe, and the impact of geographical variation in interpretation of somatic variation also presents a clear challenge to the delivery of equitable care. Furthermore, the lack of a common language to systematically describe the biological classification of variants and the processes taken to reach these classifications hinders data aggregation and the development of shared resources and expertise between institutions and healthcare systems.

To address these challenges, we present a detailed framework to enable a standardised approach to oncogenicity assessment in the UK, designed to be combined with the well-establish AMP four-tier framework to overlay clinical actionability. Building upon existing work in rare disease and cancer genomics, the SVIG-UK oncogenicity framework provides detailed recommendations for applying and integrating both established and novel lines of evidence to classify somatic variants. The resulting structure is applicable across all cancer types. Whilst this framework focuses on the interpretation of somatic small variants, it has also been designed to provide a foundation for the subsequent development of systems to classify copy number and structural variation in cancer. Throughout the development of this framework, we have consulted with a broad range of stakeholders and are optimistic that the SVIG-UK guidelines will be widely adopted as the standard for describing the biological classification of somatic variants within UK diagnostic genomic laboratories and perhaps beyond.

Provision of this framework for biological classification of variants provides a standardised approach that has the potential to improve patient care and the equity of access to harmonised genomic analysis across diagnostic genomic laboratories in the UK and represents a significant step towards a more unified and streamlined approach to somatic variant interpretation.

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