A SOP for the interpretation of oncogenicity/pathogenicity of somatic variants

(Note: If the somatic variant is in a gene known to cause hereditary cancer, ACMG/AMP ClinGen germline gene specific guidelines might need to be considered in order to take into account gene specific nuances.)

Evidence of oncogenicity/pathogenicity

Category Very strong

OVS1_somatic: null mutation (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a bona fide tumor suppressor gene.

Caveats:

Use caution interpreting LOF mutations at the extreme 3' end of a gene.

Use caution with splice mutations that are predicted to lead to exon skipping but leave the remainder of the protein intact (in frame events). Also use caution if splice mutation leads to expression of well-known alternative isoform which preserves tumor suppressor functionality.

Use caution in the presence of multiple transcripts.

Category Strong

OS1_somatic: Same amino acid change as a previously established oncogenic/pathogenic mutation (by appropriate expert group) regardless of nucleotide change. Example: Val→Leu caused by either G>C or G>T in the same codon
Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

OS2_somatic: Well-established in vitro or in vivo functional studies supportive of an oncogenic/pathogenic effect of the mutation. Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well established.

OS3_somatic: Located in one of the hotspots in cancerhotspots.org with at least 50 samples with mutation at amino acid position and the particular amino acid change count in cancerhotspots.org in at least 10 samples. (Use caution with hotspots driven by truncating mutations.) If mutation is in a tumor type which is not covered well by cancerhotspots.org, resources such as COSMIC or a tumor type specific study could be used. This rule cannot be used if OS1_somatic is applicable.

Category Moderate

OM1_somatic: Located in a well-established mutational hot spot and/or critical and well-established functional domain. (e.g., active site of an enzyme.)

OM2_somatic: Absent from controls (or at extremely low frequency) in 1000 Genomes Project, Genome Aggregation Database (gnomAD) or Exome Aggregation Consortium (ExAC).

Caveat: Population data for insertions/deletions may be poorly called by next-generation sequencing. Population data may contain somatic mutations associated with clonal hematopoiesis.

OM3_somatic: Protein length changes as a result of in-frame deletions/insertions in known oncogene or tumor suppressor gene or stop-loss mutations in known tumor suppressor gene.

OM4_somatic: Novel missense change at an amino acid residue where a different missense change determined to be oncogenic/pathogenic (by appropriate expert group) has been documented Example: Arg156His is oncogenic/pathogenic; now you observe Arg156Cys.

Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

OM5_somatic Located in one of the hotspots in cancerhotspots.org with less than 50 samples with mutation at amino acid position and the particular amino acid change count in cancerhotspots.org is at least 10. (Use caution with hotspots driven by truncating mutations.) If mutation is in a tumor type which is not covered well by cancerhotspots.org resources such as COSMIC or tumor type specific study could be used. This rule cannot be used if OM1_somatic or OM4_somatic is applicable.

Category Supporting

OP1_somatic: Multiple lines of computational evidence support an oncogenic/pathogenic effect of a mutation. (conservation/evolutionary, splicing impact, etc.)

Caveat: Because many in silico algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. Can be used only once in any evaluation of a mutation.

OP2_somatic: Mutation in a gene in malignancy with a single genetic etiology. Example: retinoblastoma is caused by bi-allelic RB1 inactivation.

Caveat: Small fraction of cases may be caused by alternative mechanism; histological similarities may cause misdiagnosis.

OP3_somatic: Located in one of the hotspots in cancerhotspots.org and the particular amino acid change count in cancerhotspots.org is below 10. (Use caution with hotspots driven by truncating mutations.) If mutation is in a tumor type which is not covered well by cancerhotspots.org, resources such as COSMIC or tumor type specific study could be used.

Evidence of benign impact

Category Stand-alone

BA1_somatic: Minor allele frequency is >5% in 1000 Genomes Project, Genome Aggregation Database (gnomAD) or Exome Aggregation Consortium (ExAC). (If the somatic variant is in a gene known to cause hereditary cancer, ACMG/AMP ClinGen germline gene specific guidelines need to be consulted, if such exist, for cut off which take into account disease prevalence.)

Category Strong

BS1_somatic: Well-established in vitro or in vivo functional studies show no oncogenic/pathogenic effects.

BS2_somatic: Minor allele frequency is >1% in 1000 Genomes Project, Genome Aggregation Database (gnomAD) or Exome Aggregation Consortium (ExAC). (If the somatic variant is in a gene known to cause hereditary cancer, ACMG/AMP ClinGen germline gene specific guidelines need to be consulted, if such exist, for cut off which take into account disease prevalence.)

Category Supporting

BP1_somatic: Multiple lines of computational evidence suggest no impact of a mutation. (conservation/ evolutionary, splicing impact, etc.)

Caveat: Because many in silico algorithms use the same or very similar input for their predictions, each algorithm cannot be counted as an independent criterion. Can be used only once in any evaluation of a mutation.

BP2_somatic: A synonymous (silent) mutation for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.

Rules for combining criteria to classify oncogenicity/pathogenicity of somatic variants

Oncogenic/pathogenic

(i) 1 Very strong (OVS1_somatic) AND
  (a) ≥1 Strong (OS1_somatic – OS3_somatic) OR
  (b) ≥2 Moderate (OM1_somatic – OM5_somatic) OR
  (c) 1 Moderate (OM1_somatic – OM5_somatic) and 1 supporting (OP1_somatic – OP3_somatic) OR
  (d) ≥2 Supporting (OP1_somatic – OP3_somatic)

(ii) ≥2 Strong (OS1_somatic – OS3_somatic) OR

(iii) 1 Strong (OS1_somatic – OS3_somatic) AND
  (a) ≥3 Moderate (OM1_somatic – OM5_somatic) OR
  (b) 2 Moderate (OM1_somatic – OM5_somatic) AND ≥2 Supporting (OP1_somatic – OP3_somatic) OR
  (c) 1 Moderate (OM1_somatic – OM5_somatic) AND ≥3 supporting (OP1_somatic – OP3_somatic)

Likely oncogenic/pathogenic

(i) 1 Very strong (OVS1_somatic) AND 1 moderate (OM1_somatic – OM5_somatic) OR

(ii) 1 Strong (OS1_somatic – OS3_somatic) AND 1–2 moderate (OM1_somatic – OM5_somatic) OR

(iii) 1 Strong (OS1_somatic – OS3_somatic) AND ≥2 supporting (OP1_somatic – OP3_somatic) OR

(iv) ≥3 Moderate (OM1_somatic – OM5_somatic) OR

(v) 2 Moderate (OM1_somatic – OM5_somatic) AND ≥2 supporting (OP1_somatic – OP3_somatic) OR

(vi) 1 Moderate (OM1_somatic – OM5_somatic) AND ≥3 supporting (OP1_somatic – OP3_somatic)

Benign

(i) 1 Stand-alone (BA1_somatic) OR

(ii) 2 Strong (BS1_somatic – BS2_somatic)

Likely benign

(i) 1 Strong (BS1_somatic – BS2_somatic) and 1 supporting (BP1_somatic – BP2_somatic) OR

(ii) ≥2 Supporting (BP1_somatic – BP2_somatic)

Uncertain significance

(i) Other criteria shown above are not met OR

(ii) the criteria for benign and oncogenic/pathogenic are contradictory

Intended scope of SOP

This SOP is focused on interpretation of oncogenicity/pathogenicity of small somatic genetic alterations specifically on tumor cells.

Interpretation of oncogenicity/pathogenicity of mutations using this SOP should be done in context of relevant tumor type(s).

This SOP is not intended for interpretation of pathogenicity of fusions and other chromosomal rearrangements.

This SOP should not be used for interpretation of pathogenicity of germline cancer predisposition variants.

This SOP is not intended for determining diagnostic, prognostic or therapeutic value of mutations.