Technical Note

Definition of Tiers and Levels of Evidence for QCI® Precision Insights

Introduction

QCI Precision Insights is an expert-based, on-demand curation and clinical interpretation service offered by QIAGEN® to help molecular diagnostic labs dramatically shorten test turnaround time and improve therapeutic decision-making for somatic cancer applications. Powered by a world-class team of molecular biologists and oncologist, QCI Precision Insights delivers concise clinical evidence for each biomarker in the context of the cancer sub-type, listing information on the mutation's molecular characteristics, roles in disease, and therapeutic, prognostic, and diagnostic implications.

QIAGEN utilizes the four-tiered, evidence-based system as outlined by Li et al. (2017) to categorize somatic sequence variations based on their levels of evidence. To provide granular stratification, QIAGEN includes additional subcategories and intermediate categories.

The subcategories enable additional stratification for Level C, which encompasses variants with a wide range of clinical evidence, including variants for which therapies are specifically approved, but in different cancer types, as well as variants that are molecular criteria for a clinical trial. The intermediate categories help to cover edge cases that meet some criteria from one level and some criteria from another level.

In this technical note, the tiers and levels of evidence utilized by QCI Precision Insights are defined and examples of each level are provided.

Tiers and Levels of Evidence, adapted from Li et al. (2017) "Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer" *The Journal of Molecular Diagnostics* 19(1): 4-23.

Tier 1: Variants of Strong Clinical Significance

Level	Definition	Example
A	**Predictive of response**: Therapy is FDA-approved in this disease, based on the presence of this biomarker	EGFR exon 19 deletion for erlotinib, afatinib, gefitinib in NSCLC FLT3-ITD for midostaurin in acute myeloid leukemia (AML)
	Predictive of resistance: Biomarker is included in professional guidelines as providing resistance to therapy	KRAS for cetuximab/panitumumab in colorectal cancer EGFR T790M for erlotinib, afatinib, and gefitinib
	Diagnostic: Biomarker is included in professional guidelines as pathognomonic (required for diagnosis; characteristic of a particular disease)	PML-RARA in acute promyelocytic leukemia (APL)
	Prognostic: Biomarker is included in professional guidelines for clinical decision-making; specifically, the molecular criteria is included in an accepted, clinically relevant prognostic scoring system	NPM1 mutations in the absence of FLT3-ITD have been associated with favorable outcomes in AML, especially in cytogenetically normal AML (CN-AML)
B	**Predictive of response**: Strong evidence (well-powered studies, consensus from experts) that biomarker predicts sensitivity to therapy	BRCA1/2 deleterious variants for niraparib in ovarian carcinoma
	Predictive of resistance: Well-powered studies with expert consensus or smaller studies repeatedly confirmed or reproduced by different groups that variant predicts resistance to therapy	EGFR-G465R resistance to cetuximab/panitumumab
	Diagnostic: Well-powered studies with expert consensus or repeatedly reported in smaller studies with consistent results or reproduced by different groups indicating diagnostic relevance. These markers may be mentioned in professional guidelines, but are suggestive of, rather than conclusive for, a specific diagnosis	ATRX variants in glioma: characteristic but not diagnostic of astrocytoma compared with oligodendroglioma (World Health Organization (WHO) classification of central nervous system (CNS) tumors)
	Prognostic: Well-powered studies with expert consensus or smaller studies repeatedly with consistent results or reproduced by different groups indicating prognostic relevance	ASXL1 mutations in myelodysplastic syndromes: associated with lower overall survival
B/C	**Predictive of response**: Consensus from experts, but lacking well-powered studies that biomarker predicts sensitivity to therapy	ERBB2 exon 20 insertions for ado-trastuzumab emtansine and trastuzumab deruxtecan in non-small cell lung carcinoma
	Not applicable for drug resistance, prognostic, or diagnostic levels of evidence.	NA

Tier 2: Variants of Potential Clinical Significance

Level	Definition	Example
C	**Predictive of response**: Therapy is FDA-approved for a different disease, based on the presence of this biomarker; or, therapy is FDA-approved for similar alterations in this gene (C.1);	BRCA1/2 deleterious variants for rucaparib in a disease other than ovarian; PIK3CA mutations not included in companion diagnostic for alpelisib in any disease
	or, criteria for a clinical trial or investigative therapies with some clinical evidence that the biomarker predicts sensitivity (C.2).	PALB2 deleterious variants for niraparib in any disease
	Predictive of resistance: Preclinical data strongly suggests resistance; reported in clinical cases	ALK-D1203N resistance to crizotinib
	Diagnostic: Small studies, diagnostic for a group of related cancers or variants that are supportive of a diagnosis along with other genomic variants	TET2, SRSF2, ASXL1 or SETBP1 in CMML
	Prognostic: Multiple small studies providing prognostic relevance	SF3B1 alterations in MDS
C/D	**Predictive of response:** Case reports or small case series including exceptional responders that indicate sensitivity to therapy	EGFR-A289V for afatinib and erlotinib in non-small cell lung cancer
	Not applicable for drug resistance, prognostic, or diagnostic levels of evidence.	NA
D*	**Predictive of response**: Plausible sensitivity to therapy based on preclinical studies, which do not need to be disease specific	TP53-R175H for ganetespib
	Predictive of resistance: NA	NA
	Diagnostic: Small studies or a few case reports support this variant alone or in combination with other biomarkers as assisting diagnosis of this disease	DNMT3A or TET2 in MDS
	Prognostic: Small studies or a few case reports support this variant alone or in combination with other biomarkers as assisting with prognostic assessment in this disease	No examples yet curated
E**	**Predictive of response**: Poor evidence that biomarker predicts sensitivity to an approved therapy	KRAS mutations for trametinib
	Not applicable for drug resistance, prognostic, or diagnostic levels of evidence.	**NA**
None found	**Diagnostic:** There are no studies with diagnostic information, or there is evidence that is not sufficient to call this a diagnostic marker	No examples provided

Level	Definition	Example
None found	**Prognostic:** There are no studies with prognostic information, or there is evidence that is not sufficient to call this a prognostic marker Not applicable for drug response or resistance	No examples provided NA
Not determined	**Diagnostic:** Level still needs to be determined **Prognostic:** Level still needs to be determined Not applicable for drug response or resistance	No examples provided No examples provided NA

* QCI Precision Insights will not display variants with a Predictive of resistance Level of Evidence D in PDF or XML reports.
** QCI Precision Insights will not display variants with Level of Evidence E in the summary table in PDF reports.

Tier 2.5: Variants with Clear Biochemical Effect but No Therapies

This category includes variants that have been reported in cancer-related genes and have been reported in cancers. For these variants, the biochemical effect has been determined either experimentally or predicted based on type and location of the mutation, such as uncharacterized variants in hotspot locations or truncation mutations that result in the loss of a key functional domains.

For oncogenes, the variant results in (or is predicted to result in) increased activity. For tumor suppressor genes, the variant results in (or is predicted to result in) reduced activity. However, no approved or experimental therapies are available clinically that target this variant, and it does not meet the criteria for prognostic or diagnostic significance in the disease.

Examples:

- RB1 loss in many solid diseases; well understood effect in cell cycle, but no therapies
- ASXL1 in chronic myeloid leukemia

Note: QCI Precision Insights will display Tier 2.5 variants with Tier 2 variants in the PDF report.

Tier 3: Variants of Uncertain Clinical Significance

This category includes variants where there is insufficient information to confidently determine the variant effect, and may include variants reported in cancers where the effect has not been determined or passenger variants—variants that occur in cancer-related genes, but have not been reported in cancers. Some variants may be classified as Variants of Unknown Significance (VUS) based on the type and location of the alteration, such as a missense mutation outside of a functional domain.

Examples:

- NRAS-E132K
- ASXL1-R1353K

Tier 4: Benign or Likely Benign Variants

Variants reported as germline variants are placed into Tier 4. Support for this classification may include: evidence in literature, population frequency, and status in dbSNP or ClinVar.

Examples:

- VHL-P25L
- CEBPA-H195_P196dup

References

Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, et al. (2017). 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. 19(1):4-23. doi: 10.1016/j.jmoldx.2016.10.002.

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