Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer

编写机构：

American College of Medical Genetics and Genomics,
American Society of Clinical Oncology,
College of American Pathologist.

2021.02.18

体系变异分类

根据专业调查，文献综述和工作组主题专家共识的结果，提出了一种基于体细胞序列变异的临床意义进行分类的四类系统：

tier I

variants with strong clinical significance;

强临床意义

tier II

variants with potential clinical significance

潜在临床意义

tier III

variants of unknown clinical significance;

临床意义未明

tier IV

variants deemed benign or likely benign.

良性或疑似良性

报告内容调研

通过对44个完成调研的机构情况进行梳理，发现不同实验室在检测组织类型、检测基因数量、是否检测肿瘤组织全外显子组或全基因组、以及其他细节方面都存在较大差别，

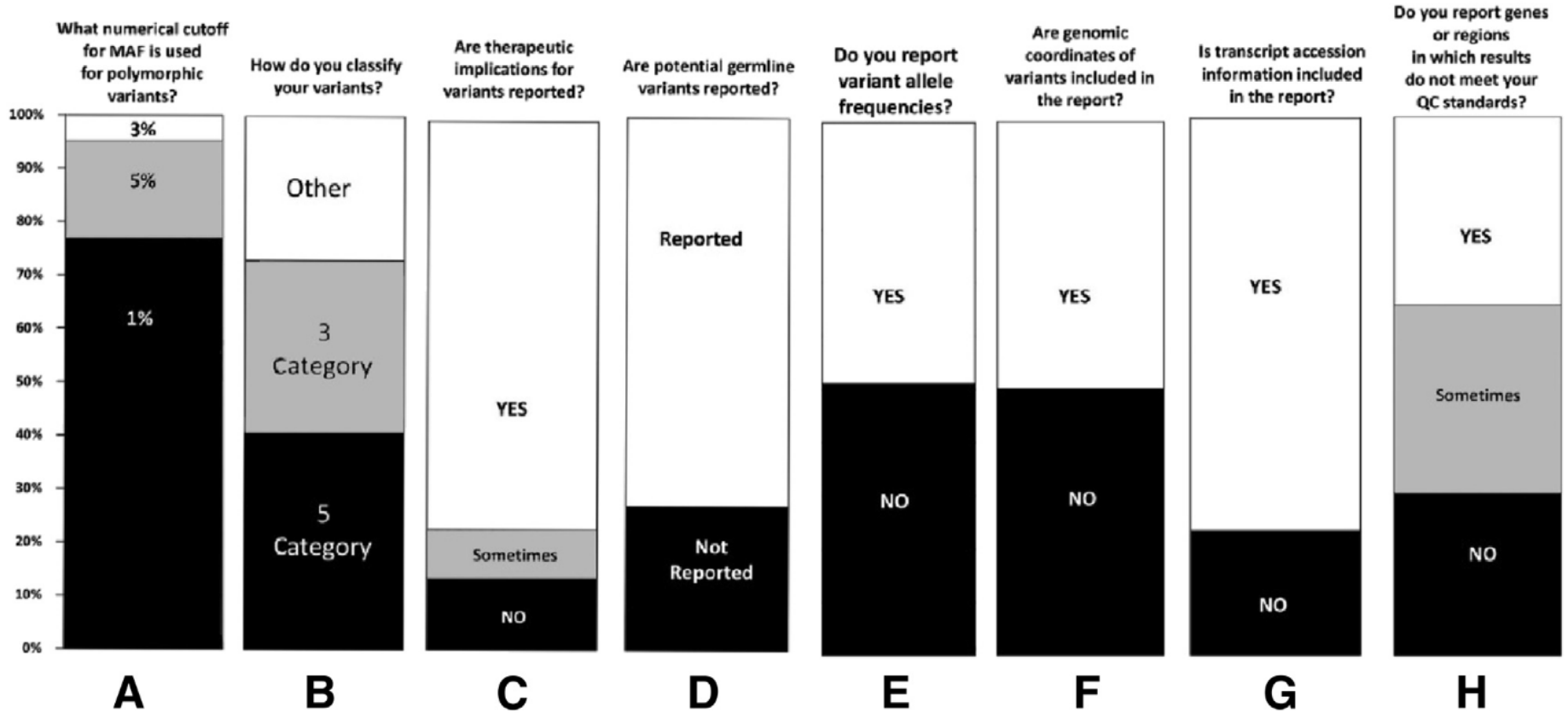


图1 AMP对NGS技术及NGS结果解读的调研

A: MAF阈值.　B: 变异分类数目　C:报告中是否包含治疗性建议 .　D: 报告中是否包含潜在的生殖细胞突变.

E: 报告是否包含变异等位频率Variant allele frequency (VAF)　F: 报告是否包含基因组坐标

G: 报告是否包含转录本ID(Transcript accession)　**H: 报告是否包含不符合质控的基因/区间**

Databases

Databases

1. Genomic Databases
2. Reference Sequence Databases
3. Population Databases
4. Cancer-Specific Databases
5. Constitutional Variant Databases
6. Internal (Laboratory-Generated) Databases

Genomic Databases

随着越来越多的针对各种肿瘤类型的大规模基因组测序项目的发布，全球正在产生大量的基因组信息并将其整合到许多公共数据库中

The Cancer Genome Atlas,
Therapeutically Applicable Research to Generate Effective Therapies,
Cancer Genome Characterization Initiative (https://gdc.cancer.gov).
Catalog of Somatic Mutations in Cancer (http://cancer.sanger.ac.uk/cosmic).

数据库增长非常快，原则上，使用应该遵循如下规则：

1. 了解数据库的内容以及如何汇总数据。临床实验室应查看与给定数据库有关的文档或公开文献，以确定数据库的来源，类型和意图
2. 特别注意每个数据库的限制，以避免对注释结果的过度解释。
3. 确认人类基因组装配的版本以及mRNA转录本参考，以确保适当的人类基因组变异学会（HGVS）注释。
4. 尽可能使用基因组坐标而不是HGVS命名法来明确查询基因组数据库。
5. 根据出版物或其他数据库的来源，单个或多个特定条目的数量，研究的深度，使用适当的对照，确认变异的体细胞来源，评估提供的基因组数据的质量以及功能和潜在药物反应研究。
6. 验证所提供病理诊断的数据质量（例如，地点，诊断和子类型

Reference Sequence Databases

参考序列数据库提供有关人类基因组装配版本的信息以及相关信息，

例如基因组坐标，以明确表示序列变异。

例如mRNA转录本的登录和版本（例如，BRAF NM_004333.4）和外显子边界定义，对于产生变异的正确HGVS命名法至关重要。

变异位置图谱（编码，非编码，非翻译区和剪接位点）和链信息（正链和负链）可以通过和这些数据库进行比较获得。

一些常用资源包括：

- RefSeq（国家生物技术信息中心参考序列数据库，https：//www.ncbi.nlm.nih.gov/refseq）
- Ensembl（http://www.Ensembl.org）
- Locus Reference Genomic （https://www.lrg-sequence.org）

Population Databases

这些数据库提供了有关大量特定人群中给定基因座上替代（次要）等位基因频率的全面信息。这些数据库通常用于根据次要等位基因频率（MAF）的任意临界值筛选出被认为是多态/良性的变异。

目前尚无用于去除多态或良性变异的MAF的标准临界值。在**没有正常组织配对**的情况下，工作组建议使用**1%（0.01）作为主要阈值**。尽管全球人的MAF最常用，但实验室May考虑特定的患者人群。

在解释体细胞变异时，必须谨慎使用这些数据库，因为在参与研究时，假定参与这些测序研究的个体是**健康的或没有亚临床疾病**。确实，一些众所周知的经典癌症相关的和可靶向的体细胞变异已作为种群数据库的胚系变异包括在内。

例如，变异NM_004972.3（JAK2）：c.1849G> T（c.V617F）通常被看作是叶绿体增生的体细胞变异体肿瘤，可以用FDA批准的Janus激酶（JAK）抑制剂靶向。它也包含在多个人群数据库中。

在评估可能的血液系统恶性肿瘤时应格外小心，因为白血病和骨髓增生异常综合症中的许多常见突变基因也可能在其他健康个体的血液中发生体细胞突变，因此可能被错误地注释为多态性。

Cancer-Specific Databases

这些数据库提供了有关不同癌症和亚型中基因变异的发生率和普遍性的信息，对其他基因组数据库的交叉引用以及对已发表或未进行系统综述的文献的引用，细胞途径，靶向疗法，临床试验 以及结果数据。

从这些数据库中提取的不同癌症中的序列变异体的普遍性和分布，**应谨慎解释**，因为病理诊断标准的代表性较差，缺乏临床级别的文献管理以及提交变异体的来源控制不严（例如，探索性或 发现研究）。

例如，这些**数据库中包括一些常见的胚系良性变异**，例如 the Catalog of Somatic Mutations in Cancer database 中的NM_000222.2（KIT）：c.1621A> C（p.M541L）。

Constitutional Variant Databases

这些数据库也可用于评估在这些数据库中报道了经过充分研究的种系对应物的体细胞变异（例如，TP53和PTEN基因中的某些变异）。

一个常用的数据库是ClinVar（http://www.ncbi.nlm.nih.gov/clinvar）。

ClinVar处理所有种类的稀有种系变异，例如病原体和良性，并在可用时提供相关的临床和实验证据。专家小组对ClinVar中的某些变异进行了有关其致病性的审查。

Internal (Laboratory-Generated) Databases

需要强调的是，临床实验室应该建立一个标注良好的内部数据库，以跟踪实验室中识别出的变异并提供一致的变异注释。

这样的数据库可用于识别可能由测序比对等引起的潜在假阳性检出，以及确定实验室通常遇到的癌症类型的突变频率。我们强烈鼓励体细胞变异数据共享，并敦促临床实验室将精心挑选的变异体贡献到公共变异数据库中，以促进对体细胞变异体的准确解释。

但是，此类提交过程应标准化并符合联邦隐私法规，满足相关法规（the Health Insurance Portability and Accountability Act and the Health Information Technology for Economic and Clinical Health Act.） 。

In Silico (Computational) Prediction Algorithms

预测软件整体可以分为两大类

Table 2 Algorithms for Computational Prediction of Functional Impact of Sequence Variant/Splice Site Changes

Utility/function	Algorithm/software	Location (web address)
Missense SNV	PolyPhen2[37]	*http://genetics.bwh.harvard.edu/pph2*
	SIFT[38]	*http://sift.jcvi.org*
	MutationAssessor[39]	*http://mutationassessor.org*
	MutationTaster[41]	*http://www.mutationtaster.org*
	PROVEAN[45]	*http://provean.jcvi.org/index.php*
	Condel[46]	*http://bg.upf.edu/blog/2012/12/condel-for-prioritization-of-variants-involved-in-hereditary-diseases-and-transfic-for-cancer*
	CoVEC[40]	*https://sourceforge.net/projects/covec/files*
	CADD[47]	*http://cadd.gs.washington.edu*
	GERP++[48]	*http://mendel.stanford.edu/sidowlab/downloads/gerp/index.html*
	PhyloP and PhastCons[49]	*http://compgen.bscb.cornell.edu/phast*
Splice site prediction	Human Splicing Finder[42]	*http://www.umd.be/HSF3*
	MaxEntScan[43]	*http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html*
	NetGene2[44]	*http://www.cbs.dtu.dk/services/NetGene2*
	NNSplice[50]	*http://www.fruitfly.org/seq_tools/splice.html*
	GeneSplicer[51]	*http://www.cbcb.umd.edu/software/GeneSplicer/gene_spl.shtml*

These are not comprehensive lists, and inclusion does not represent an organizational endorsement of any individual database or product. All websites last accessed June 7, 2016.

SNV, single-nucleotide variant.

错义变异对蛋白质功能的影响的预测

序列变异对剪接的影响

错义和剪接位点预测工具具有中等的特异性（大约60%至80%），并且倾向于过度预测有害影响。

Variant Identification and Annotation

变异检测是一切解读分析的起点。 有许多变异检测软件工具可以满足一种特定的检测，例如SNV，插入缺失，结构变异和CNV 。

Variant caller	Location (URL)
MuTect v1.1.555	https://www.broadinstitute.org/cancer/cga/mutect
Genome Analysis Toolkit (GATK) – MuTect v2	https://www.broadinstitute.org/gatk/guide/tooldocs/org_broadinstitute_gatk_tools_walkers_cancer_m2_MuTect2.php
VarScan 256	http://dkoboldt.github.io/varscan/
VarDict57	https://github.com/AstraZeneca-NGS/VarDict
Sterlka58	https://sites.google.com/site/strelkasomaticvariantcaller/
FreeBayes59	https://github.com/ekg/freebayes
Scalpel60	http://scalpel.sourceforge.net/
Pindel61	http://gmt.genome.wustl.edu/packages/pindel/
SAMtools62	http://samtools.sourceforge.net/
Torrent Suite Variant Caller	https://github.com/iontorrent/TS
SomaticSniper63	http://gmt.genome.wustl.edu/packages/somatic-sniper/

了解变异检测**工具的局限性**很重要。 一些重要指标如 supporting reads (depth of coverage) and variant allele frequency (VAF)，应纳入变异体评估中。 变异检测结果需要输出到标准的格式：vcf、gvcf、gff。

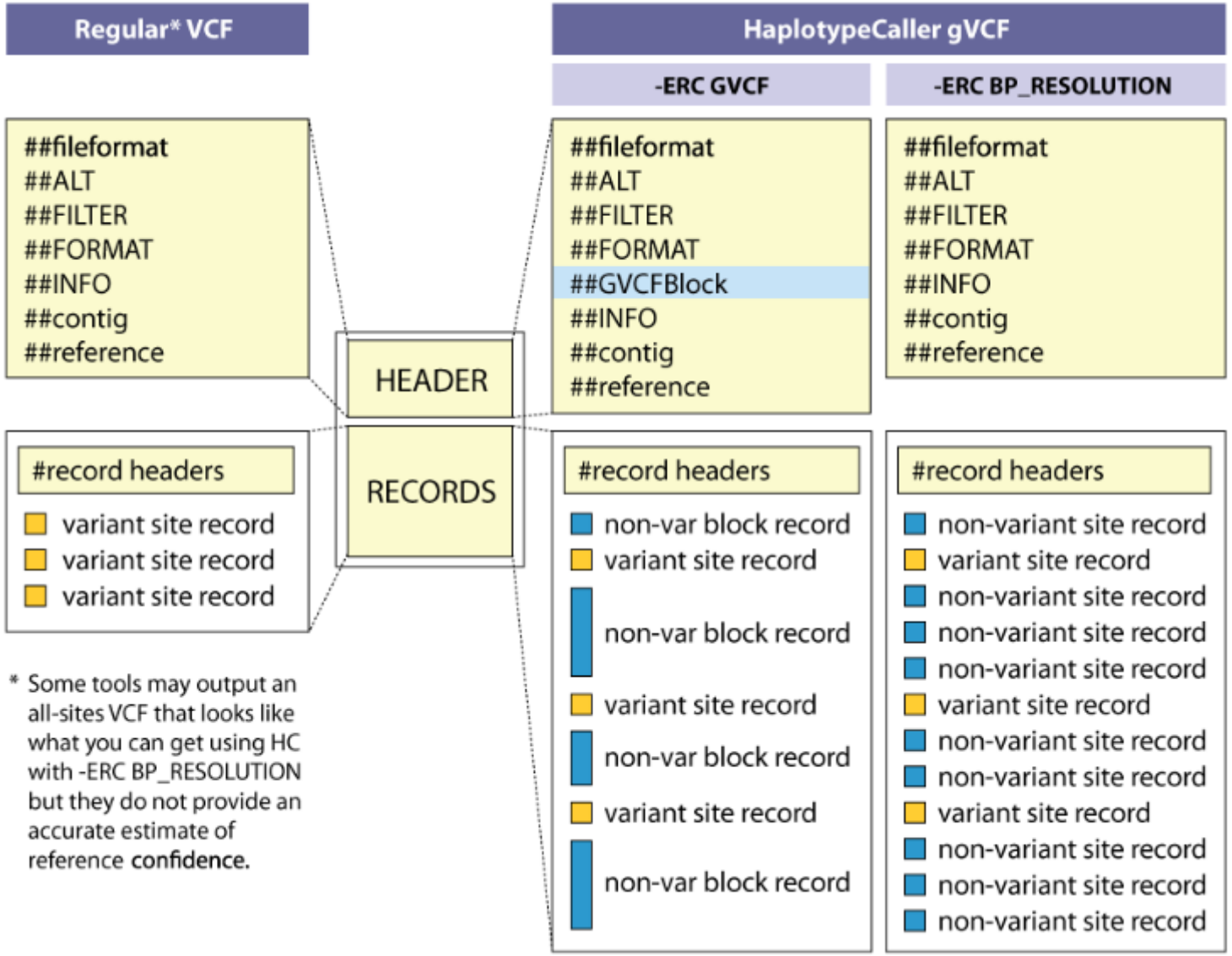
Variant Identification and Annotation

VCF VS gVCF

GVCF和VCF的最大区别是在于GVCF文件会记录所有的点，包括哪些没有突变的点。

在GVCF模式下，那些没有变异的点会形成一个未变异块，non-var block record。

我们需要确定vcf的Info列所包含的具体信息内容：
DP，AD，DP4等等。



Variant Identification and Annotation

为了进一步的临床解释，需要对变异进行进一步的基因注释，为变异添加有意义且易于识别的注释信息，(**gene symbol, variant location, variant type, HGVS nomenclature for cDNA sequence changes, and predicted protein sequence alterations**)。这些注释内容可以参考如下软件进行：

Software	Location (URL)
Annovar64	http://annovar.openbioinformatics.org/en/latest/
snpEff65	http://snpeff.sourceforge.net/
SeattleSeq	http://snp.gs.washington.edu/SeattleSeqAnnotation138/
AnnTools66	http://anntools.sourceforge.net/
NGS-SNP67	https://www.ualberta.ca/~stothard/downloads/NGS-SNP/
VEP (Variant Effect Predictor)15	http://useast.ensembl.org/info/docs/tools/vep/index.html

一个重要挑战就是将基因组坐标（即染色体和位置）转换为相应的cDNA /氨基酸坐标系统（分别为c.和p.syntax）以进行解读。

HGVS系统建议使用右对齐表示法，但VCF规范要求使用左对齐表示法。

存在多个转录本时，必须使用正确的mRNA转录本编号和版本信息，以确保变异描述的准确和一致

Variant Identification and Annotation

肿瘤变异注释阶段常用的一些数据库信息

Table 1 Databases Relevant to Interpretation of Somatic Sequence Variants

Utility/function	Database	Location (web address)
Population databases to exclude polymorphisms	1000 Genomes Project[16]	*http://browser.1000genomes.org*
	Exome Variant Server	*http://evs.gs.washington.edu/EVS*
	dbSNP[17]	*http://www.ncbi.nlm.nih.gov/snp*
	dbVar[18]	*http://www.ncbi.nlm.nih.gov/dbvar*
	ExAC	*http://exac.broadinstitute.org*
Cancer-specific variant databases	Catalog of Somatic Mutations in Cancer[19]	*http://cancer.sanger.ac.uk/cosmic*
	My Cancer Genome	*http://www.mycancergenome.org*
	Personalized cancer therapy, MD Anderson Cancer Center	*https://pct.mdanderson.org*
	cBioPortal, Memorial Sloan Kettering Cancer Center[20]	*http://www.cbioportal.org*
	Intogen[21]	*https://www.intogen.org/search*
	ClinicalTrials.gov	*https://clinicaltrials.gov*
	IARC (WHO) TP53 mutation database[22]	*http://p53.iarc.fr*
	Pediatric Cancer Genome Project (St. Jude Children's Research Hospital—Washington University)	*http://explorepcgp.org*
	International Cancer Genome Consortium[23]	*https://dcc.icgc.org*
Sequence repositories and data hosts	NCBI Genome	*http://www.ncbi.nlm.nih.gov/genome*
	RefSeqGene[24]	*http://www.ncbi.nlm.nih.gov/refseq/rsg*
	Locus Reference Genomic[25]	*http://www.lrg-sequence.org*
	UCSC table browser[26]	*https://genome.ucsc.edu/cgi-bin/hgTables*
	Ensemble BioMart[27]	*http://useast.ensembl.org/biomart/martview*
Other disease/mutation databases useful in the context of variant interpretation for cancer genomics	ClinVar[28]	*http://www.ncbi.nlm.nih.gov/clinvar*
	Human Gene Mutation Database[29]	*http://www.hgmd.org*
	Leiden Open Variation Database[30]	*http://www.lovd.nl*
	dbNSFP (compiled database of precomputed *in silico* prediction scores for nonsynonymous SNVs)[31]	*https://sites.google.com/site/jpopgen/dbNSFP*
	Ensemble Variant Effect Predictor[15]	*http://www.ensembl.org/info/docs/tools/vep/index.html*

These are not comprehensive lists, and inclusion does not represent an organizational endorsement of any individual database or product. All websites last accessed June 7, 2016.

dbSNP, The Database of Short Genetic Variation; ExAC, Exome Aggregation Consortium; IARC, International Agency for Research on Cancer; NCBI, National Center for Biotechnology Information; SNV, single-nucleotide variant; UCSC, University of California, Santa Cruz; WHO, World Health Organization.

Proposed Guideline for Evidence-Based Categorization of Somatic Variants

临床影响应包括治疗，预后，诊断和预防措施。给定变异的临床影响应根据当前可获得的证据确定。

基于变异分类的证据在临床决策中的重要性，可以对其进行不同的权衡。

在文献综述和工作组共识的基础上，我们建议将临床和实验证据分为四个级别：

Table 3 Categories of Clinical and/or Experimental Evidence

Category	Therapeutic	Diagnosis	Prognosis
Level A	1. Biomarkers that predict response or resistance to FDA-approved therapies for a specific type of tumor 2. Biomarkers included in professional guidelines that predict response or resistance to therapies for a specific type of tumor	Biomarkers included in professional guidelines as diagnostic for a specific type of tumor	Biomarkers included in professional guidelines as prognostic for a specific type of tumor
Level B	Biomarkers that predict response or resistance to therapies for a specific type of tumor based on well-powered studies with consensus from experts in the field	Biomarkers of diagnostic significance for a specific type of tumor based on well-powered studies with consensus from experts in the field	Biomarkers of prognostic significance for a specific type of tumor based on well-powered studies with consensus from experts in the field
Level C	1. Biomarkers that predict response or resistance to therapies approved by the FDA or professional societies for a different type of tumor 2. Biomarkers that serve as inclusion criteria for clinical trials	Biomarkers of diagnostic significance based on the results of multiple small studies	Biomarkers of prognostic significance based on the results of multiple small studies
Level D	Biomarkers that show plausible therapeutic significance based on preclinical studies	Biomarkers that may assist disease diagnosis themselves or along with other biomarkers based on small studies or a few case reports	Biomarkers that may assist disease prognosis themselves or along with other biomarkers based on small studies or a few case reports

FDA, Food and Drug Administration.

Proposed Guideline for Evidence-Based Categorization of Somatic Variants

解读 **分类** 和 临床和实验证据 **分级** 之间的对应关系：

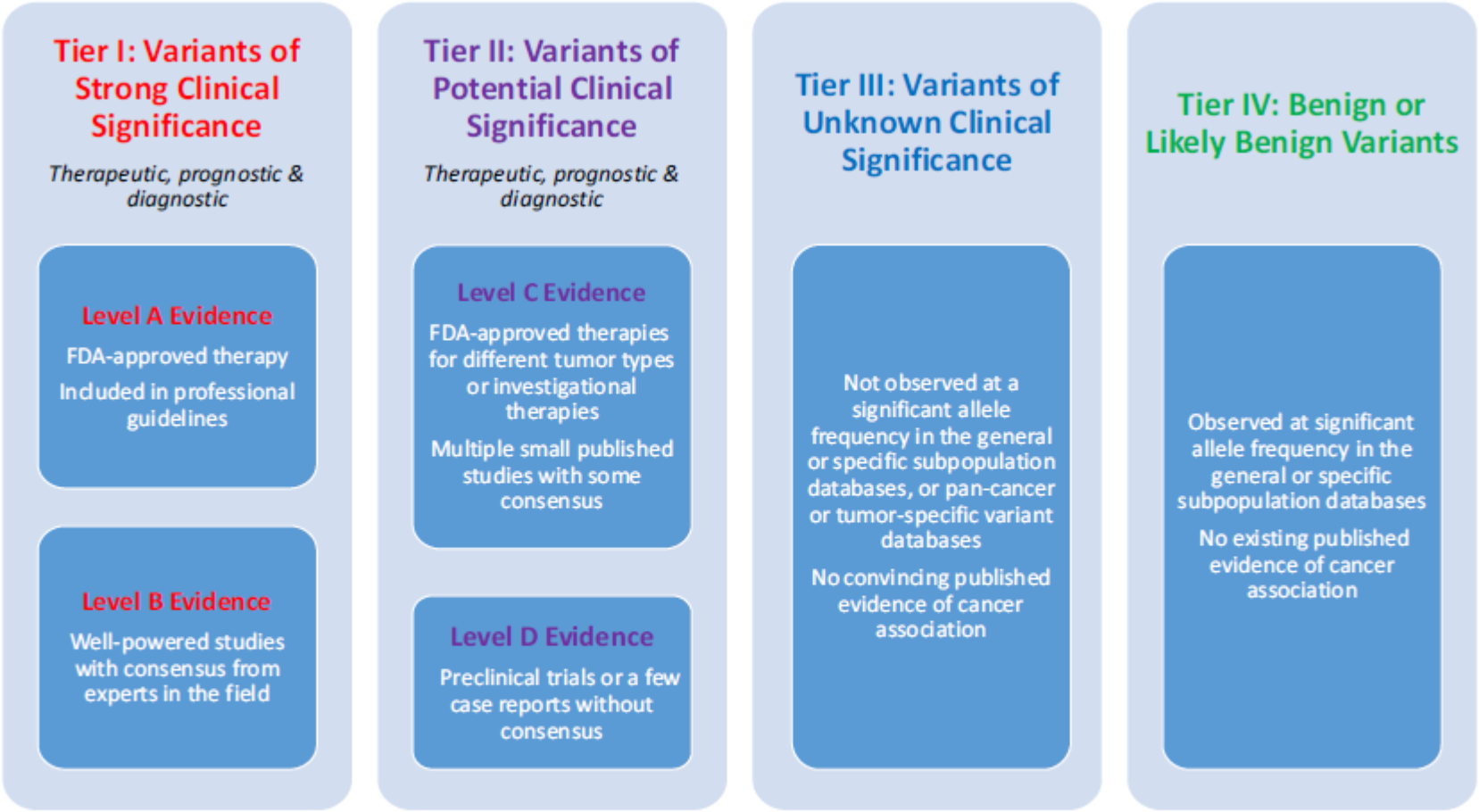


Figure 2 Evidence-based variant categorization. Somatic variants are classified into four tiers based on their level of clinical significance in cancer diagnosis, prognosis, and/or therapeutics. Variants in tier I are of strongest clinical significance, and variants in tier IV are benign or likely benign variants. FDA, Food and Drug Administration.

Proposed Guideline for Evidence-Based Categorization of Somatic Variants

分类标准

华大基因 BGI

Table 4 Tier I: Variants with Strong Clinical Significance

Evidence source/type	Available evidence
FDA-approved therapies, PG, investigational therapies	Therapeutic: FDA approved or investigational with strong evidence*
	Diagnostic: In PG or reported evidence with consensus
	Prognostic: In PG or reported evidence with consensus
Mutation type	Activating, LOF (missense, nonsense, indel, splicing), CNVs, fusions
Variant frequencies	Mostly mosaic
Potential germline†	Mostly nonmosaic (VAF approximately 50% or 100%)
Population database: *ESP, dbSNP, 1000Genome, ExAC*	Absent or extremely low MAF
Germline database: *HGMD, ClinVar*	May or may not be present
Somatic database: *COSMIC, My Cancer Genome, TCGA*	Most likely present
Predictive software: *SIFT, PolyPhen2, MutTaster, CADD*	Mostly damaging; information to be used for reference only
Pathway involvement	Disease-associated pathways
Publications: *functional study, population study, other*	Therapeutic: reported evidence with consensus
	Diagnostic: reported evidence with consensus
	Prognostic: reported evidence with consensus

Italicized text indicates examples provided within each category; these are not comprehensive lists, and inclusion does not represent an organizational endorsement of any individual database or product.

*Strong evidence based on well-powered clinical studies with consensus from experts in the field.

†Confirmation on normal tissue if tested tumor only and genetic counseling should be recommended.

CNV, copy number variation; COSMIC, Catalog of Somatic Mutations in Cancer; dbSNP, The Database of Short Genetic Variation; ExAC, Exome Aggregation Consortium; FDA, Food and Drug Administration; HGMD, Human Gene Mutation Database; indel, insertion and deletion; LOF, loss of function; MAF, minor allele frequency; PG, professional guideline; TCGA, The Cancer Genome Atlas; VAF, variant allele frequency.

Table 5 Tier II: Variants with Potential Clinical Significance

Evidence source/type	Available evidence
FDA-approved therapies, PG, investigational therapies	Therapeutic: FDA approved for different tumor type; investigational therapies with some evidence
	Diagnostic: not in PG but with convincing published data
	Prognostic: not in PG but with convincing published data
Mutation type	Activating, LOF (missense, nonsense, indel, splicing), CNVs, fusions
Variant frequencies	Mostly mosaic
Potential germline*	Mostly nonmosaic (VAF approximately 50% or 100%)
Population database: *ESP, dbSNP, 1000Genome, ExAC*	Absent or extremely low MAF
Germline database: *HGMD, ClinVar*	May or may not be present
Somatic database: *COSMIC, My Cancer Genome, TCGA*	Likely present
Predictive software: *SIFT, PolyPhen2, MutTaster, CADD*	Mostly damaging; information to be used for reference only
Pathway involvement	Involve disease-associated pathways or pathogenic pathways
Publications: *functional study, population study, other*	Therapeutic: evidence of using FDA-approved therapies for different tumor types; phase 2 or 3 clinical trials for investigational therapies
	Diagnostic: multiple lines of reported evidence without consensus
	Prognostic: multiple lines of reported evidence without consensus

Italicized text indicates examples provided within each category; these are not comprehensive lists, and inclusion does not represent an organizational endorsement of any individual database or product.

*Confirmation on normal tissue if tested tumor only and genetic counseling should be recommended.

CNV, copy number variation; COSMIC, Catalog of Somatic Mutations in Cancer; dbSNP, The Database of Short Genetic Variation; ExAC, Exome Aggregation Consortium; FDA, Food and Drug Administration; HGMD, Human Gene Mutation Database; indel, insertion and deletion; LOF, loss of function; MAF, minor allele frequency; PG, professional guideline; TCGA, The Cancer Genome Atlas; VAF, variant allele frequency.

Table 6 Tier III: Variants of Unknown Clinical Significance

Evidence source/type	Available evidence
FDA-approved therapies, PG, investigational therapies	Cancer genes: none
	Noncancer genes (apply to cancer exome/whole genome sequencing): none
Mutation type	Functionally unknown; mostly missense, in-frame indels; less commonly, other types
Variant frequencies	Mosaic or nonmosaic
Potential germline*	Mostly nonmosaic (VAF approximately 50% or 100%)
Population database: *ESP, dbSNP, 1000Genome, ExAC*	Absent or extremely low MAF
Germline database: *HGMD, ClinVar*	Absent or downgraded from pathogenic to VUS
Somatic database: *COSMIC, My Cancer Genome, TCGA*	Absent or present without association to specific tumors (potential germline VUS); present but in very few cases
Predictive software: *SIFT, PolyPhen2, MutTaster, CADD*	Variable; information to be used for reference only
Pathway involvement	May or may not involve disease-associated pathways
Publications: *functional study, population study, other*	None or no convincing evidence to determine clinical/biological significance

Italicized text indicates examples provided within each category; these are not comprehensive lists, and inclusion does not represent an organizational endorsement of any individual database or product.

*Confirmation on normal tissue if tested tumor only and genetic counseling should be recommended.

COSMIC, Catalog of Somatic Mutations in Cancer; dbSNP, The Database of Short Genetic Variation; ExAC, Exome Aggregation Consortium; FDA, Food and Drug Administration; HGMD, Human Gene Mutation Database; indel, insertion and deletion; MAF, minor allele frequency; PG, professional guideline; TCGA, The Cancer Genome Atlas; VAF, variant allele frequency; VUS, variant of unknown clinical significance.

Table 7 Tier IV: Benign/Likely Benign Variants

Evidence source/type	Available evidence
FDA-approved therapies, PG, investigational therapies	None
Mutation type	Functionally benign or unknown; mostly missense; less commonly, other types
Variant frequencies	Mostly nonmosaic (VAF, approximately 50% or 100%)
Potential germline*	Mostly nonmosaic (VAF, approximately 50% or 100%)
Population database: *ESP, dbSNP, 1000Genome, ExAC*	MAF $\geq$ 1% in the general population; or high MAF in some ethnic populations
Germline database: *HGMD, ClinVar*	Absent or present but downgraded to benign/likely benign
Somatic database: *COSMIC, My Cancer Genome, TCGA*	Absent or present without association to specific tumors (potential rare germline polymorphism)
Predictive software: *SIFT, PolyPhen2, MutTaster, CADD*	Mostly benign; information to be used for reference only
Pathway involvement	May or may not involve disease-associated pathways
Publications: *functional study, population study, other*	Reported evidence supportive of benign/likely benign; or none

Italicized text indicates examples provided within each category; these are not comprehensive lists, and inclusion does not represent an organizational endorsement of any individual database or product.

*Confirmation on normal tissue if tested tumor only and genetic counseling should be recommended.

COSMIC, Catalog of Somatic Mutations in Cancer; dbSNP, The Database of Short Genetic Variation; ExAC, Exome Aggregation Consortium; FDA, Food and Drug Administration; HGMD, Human Gene Mutation Database; MAF, minor allele frequency; PG, professional guideline; TCGA, The Cancer Genome Atlas; VAF, variant allele frequency.

体细胞变异分类	等级	解释
I类变异（强临床意义）	A	FDA / NMPA 批准用于患者肿瘤治疗有响应或耐药的生物标志物
	A	专业指南（NCCN）明确对患者肿瘤治疗有响应或耐药的生物标志物
	A	专业指南（NCCN）明确对患者肿瘤有诊断或预后意义的生物标志物
	B	专家共识研究明确对患者肿瘤治疗有响应或耐药的生物标志物
	B	专家共识研究明确对患者肿瘤有诊断或预后意义的生物标志物
II类变异（潜在临床意义）	C	FDA/NMPA批准用于其他肿瘤治疗有响应或耐药的生物标志物
	C	专业指南（NCCN）推荐对其他肿瘤治疗有响应或耐药的生物标志物
	C	已经作为临床试验筛选入组标准的生物标志物
	C	多项小型研究结果表明有诊断或预后意义的生物标志物
	D	临床前研究表明具有潜在治疗意义的生物标志物
	D	有小型研究和病例报道或结论未形成共识，评估疾病诊断或预后意义的生物标志物并伴有其他生物标志物
III类变异（临床意义未明变异）	-	在人群数据库、特定亚人群数据库、泛癌种数据库、肿瘤特异性数据库中未检出；
	-	无确凿证据与癌种相关；
IV类变异（良性多态性和疑似良性变异）	-	在人群数据库、特定亚人群数据库检出，不存在与癌症相关证据

截图自前端培训PPT。

Germline Variants Identified during Cancer Testing

大多数遗传变异，是传代下来的，并且通常在100%的细胞中具有该变异体，导致等位基因分数为0.5或1.0。体细胞变异是在出生后获得的，通常是由于DNA复制或修复错误或环境污染引起的。体细胞变异的等位基因分数通常<0.5，因为存在污染的正常组织，即使在表面纯净的肿瘤样品中也是如此。

实验室必须正确识别可能与诊断，预后，治疗干预和/或临床试验选择有关的体细胞突变，并且不得将高频体细胞突变错误识别为种系（高频 ≠ 胚系变异），因为这可能具有重大的临床意义。

当分析中未包括匹配的对照时，实验室应具有可用于推断变异为体细胞生殖细胞的标准。指定胚系的主要标准是VAF，对于杂合变种应为大约50%，对于纯合变种应为100%。由于变异等位基因的序列同源性缺失，某些胚芽变体（例如大插入缺失）可能导致正常等位基因的偏好扩增（在基于扩增子的测试中）或捕获（在基于捕获的测试中），导致胚系变异的VAF最终小于50%。

当在已知的癌症易感综合征基因（例如，TP53或BRCA1）中检测到表观生殖细胞变异时，有关疾病发作年龄的临床信息（年轻人与致癌基因中遗传性生殖系突变的风险较高相关），肿瘤的侧面性（更可能遗传双侧肿瘤）以及癌症的家族史或个人病史可以帮助确定癌症易感性的可能性。还可以参考相关数据库：人类在线孟德尔遗传， Human Gene Mutation Database，ClinVar和locus-specific databases。

生殖系变种的解释应遵循ACMG / AMP标准和解释生殖系序列变体的指南。当仅在肿瘤试验中怀疑有致病性生殖系变体时，应建议使用正常组织样本确认该变体，并进行适当的遗传咨询。

Nomenclature

所有检测到的遗传变异均应按照HUGO基因命名委员会的指定进行注释和报告:

基因名参考 ：HGNC :http://www.genenames.org

变异写法参考：HGVS，http：//www.hgvs.org

SNV和插入缺失应使用p.和c. 符号进行报告（例如，BRAFp.V600E，c.1799T> A）。

SV，列出两个融合的基因伴侣之间**用斜杠分隔**（例如，EML4 / ALK fusion）。

CNV应以表格形式报告为拷贝数GAIN或LOSS。

如果适用，可以包括基因/基因组基因座的基因组坐标。
可以在适当的时候报告数字拷贝数的变化[例如，EGFR拷贝数GAIN（拷贝数比25）； CDKN2A拷贝数LOSS]。

使用标准术语不会超过与临床团队进行清晰明确沟通的需要。 根据需要，除了标准术语外，还应包括口语命名法，以便医生阅读报告并使用它们来确定治疗时向医生清晰地传达含义。例如，TERT启动子变体的报告可以为1-124C> T（ HGVS命名法，然后是括号中的口语命名法（TERTC228T）。使用HGVS命名法报告基因组变体以明确将变体重新映射到参考基因组，而口语命名法则向临床团队传达了明确的信息。

Other Reporting Elements

除了检测到的变体之外，报告还应包含其他一些元素，这些元素可能与更深入的结果分析或与随时间推移从该患者获得的其他结果进行比较有关，例如基因组座标，基因组构建和转录参考序列（例如NM_004333.4），前提是此信息不会损害患者和临床提供者解释该报告中与之直接相关的基本要素的能力。

本节或对结果进行扩展说明的另一节中，远离顶部结果。应评估等位基因分数（VAF）和覆盖率，并在适当时包括在报告中。该报告应包括所用NGS测定的测序覆盖率临界值。在报告中应声明所有未满足最低要求的测序覆盖率标准的基因和/或热点均已失败。

报告不应仅限于肯定的发现。 I类药物/癌症组合应包括相关的阴性结果（例如，肺癌患者中明确缺乏EGFR突变或黑色素瘤患者中明确缺乏BRAF突变）。如果存在不确定性， 必须在报告中进行沟通；这包括序列质量，样品充分性，肿瘤含量和生物医学知识的问题。

目前质控都是整体芯片层面的，在指南中指出，应该声明为满足测序要求的基因/热点信息。这部分可以添加上。来提高结果的可靠性。

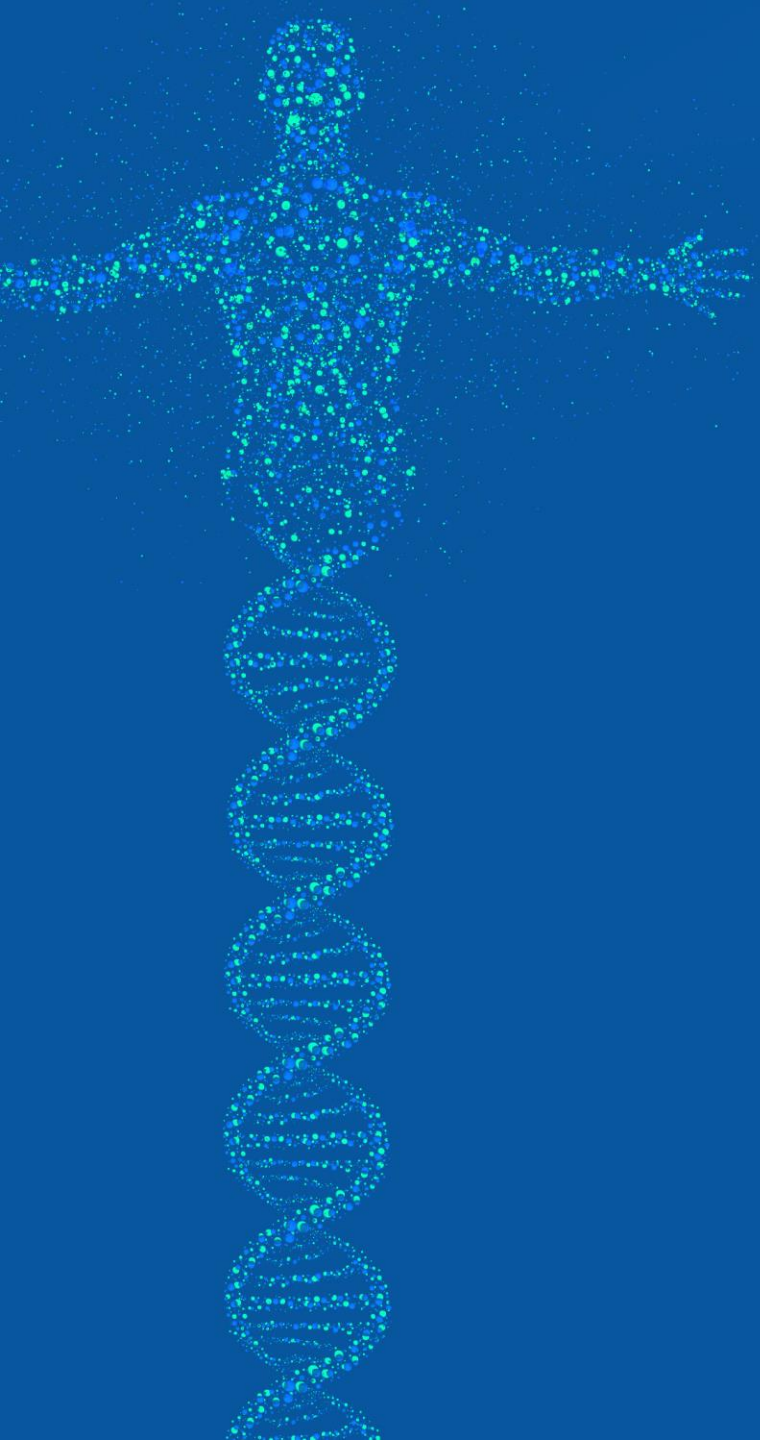
Reporting of Germline Variants

当不使用成对的种系样品时，NGS分析不能区分种系和体细胞变体，测序结果可能包含这两个发现。在这种情况下，可以用免责声明报告使用的NGS测试不能明确区分胚系和体细胞变异。在某些情况下，可能会怀疑胚系变异（例如MAF 40%至60%）。

然而，**这种解释应谨慎进行**，并与肿瘤细胞数量有关。如果怀疑胚系变异，如果在适当的患者同意后临床上有指示，可以建议对患者胚系样本（例如，实体瘤患者的血液）进行测试。报告中应包含一份声明，说明在体细胞和胚系变化之间进行区分的方式，并在适当情况下指示仍存在不确定性。

先单样本后补胚系的建议报告方案：

- 将包含胚系样品发现的结果发布到癌症报告的附录中，
- 在胚系报告中单独发表一份关于胚系变体的报告，并附上唯一的解释性声明，并在初始癌症报告中添加一份附录。
- 作为单独的胚系报告和另外的单独报告发布，该报告整合了癌症和胚系样品的结果。



THANK YOU

感谢观看