Performance of ACMG-AMP Variant-Interpretation Guidelines among Nine Laboratories in the Clinical Sequencing Exploratory Research Consortium

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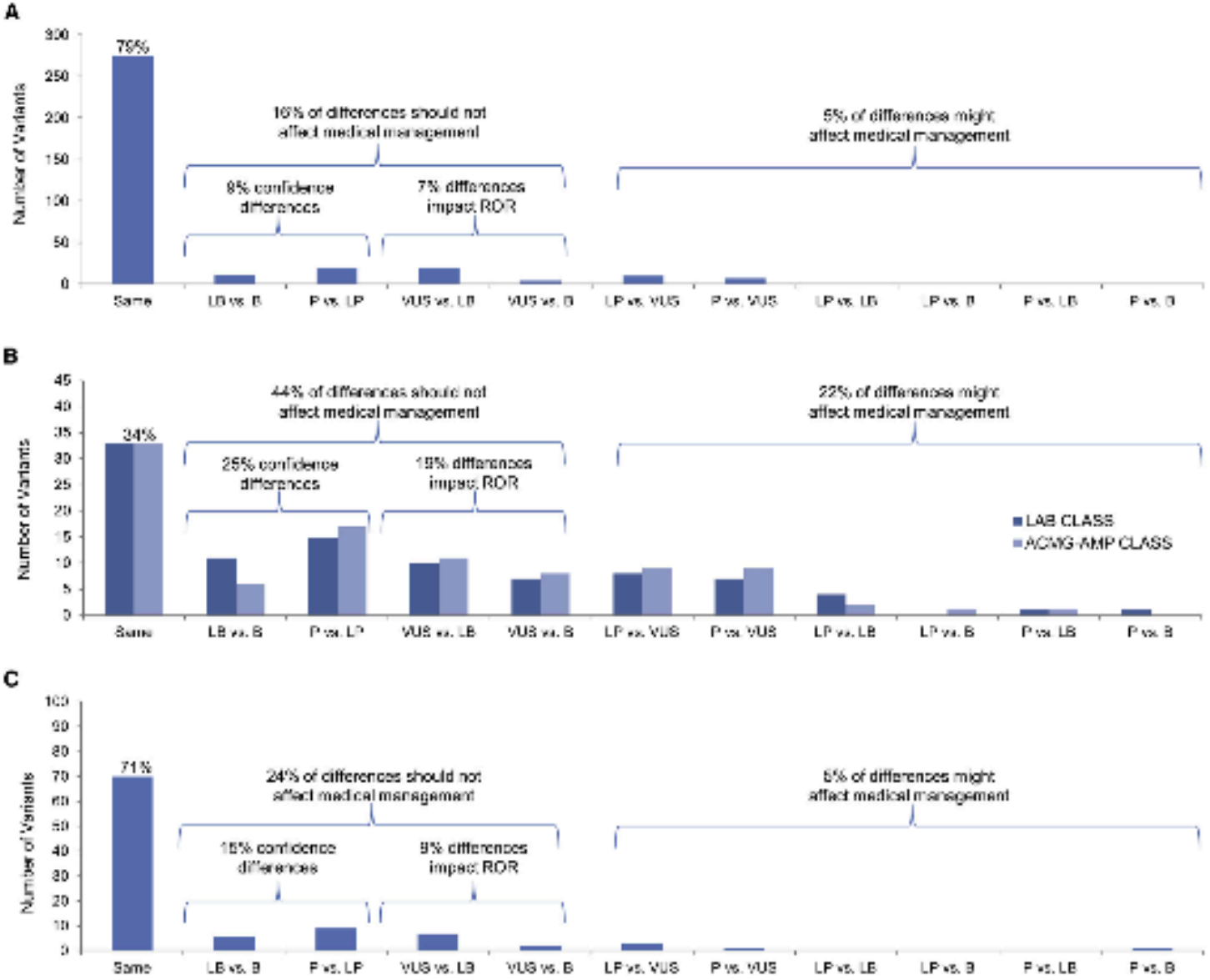

Figure 1. Distribution of Variant-Classification Comparisons according to the Extent of Differences across a Five-Tiered Classification Scheme

(A) Intra-laboratory concordance between laboratory and ACMG-AMP classification systems. This graph compares each site's use of the ACMG-AMP rules to their own laboratory classification methods.

(B) Inter-laboratory concordance of 97 variants. This graph compares the same calls, based on either the ACMG-AMP rules or the site's rules, between laboratories.

(C) Inter-laboratory concordance after consensus efforts. This graph shows a final comparison of calls between sites after consensus-building efforts.

Box 1. Recommendations and Additional Resources for Increasing Consistency in the Usage of ACMG-AMP Rules

- Develop disease-specific allele-frequency thresholds to enable lowering of the stand-alone benign criteria from a MAF of $\geq 5\%$ to values specific to each disorder.
- Establish a resource of all genes to define whether LOF is a known mechanism of disease.
- Make recommendations for which computational algorithms are best in practice.
- Better define "well-established" functional data and/or distribute a resource that lists functional assays that meet the well-established threshold. Also define when to use reduced strength of the rule.
- Develop quantitative thresholds of evidence for and against segregation of different strengths.
- Promote the development of software tools that automate computable aspects of the ACMG-AMP guidelines to improve accurate use.

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REVIEW

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Current practices and guidelines for clinical next-generation sequencing oncology testing

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Table 1 Summary of valuable references and guidelines relevant to clinical NGS oncology testing

Source	Title	Content summary	Reference
New York State Board of Health	"Next Generation" Sequencing (NGS) guidelines for somatic genetic variant detection	Detailed standards for technical validity	11
ACMG	Standards and guidelines for the interpretation of sequence Variants	Guidelines for clinical validity assessment, particularly for germline/constitutional variants	12
ACMG	ACMG clinical laboratory standards for next-generation sequencing	Broad summaries of major areas of consideration for clinical validation of all NGS assay	13
CDC	Assuring the quality of next-generation sequencing in clinical laboratory practice	Detailed recommendations for technical validity assessment/validation of all NGS assays	14
CDC	Good laboratory practice for clinical next-generation sequencing informatics pipelines	Detailed recommendations for clinical validity assessment for all NGS assays	15
Quest Diagnostics (reference laboratory)	Annotation of sequence variants in cancer samples processes and pitfalls for routine assays in the clinical laboratory	The framework for a repeatable workflow for clinical validity assessment in use at a high volume testing facility is described	16

ACMG: American College of Medical Genetics and Genomics; CDC: United States Centers for Disease Control and Prevention

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A classification system for clinical relevance of somatic variants identified in molecular profiling of cancer

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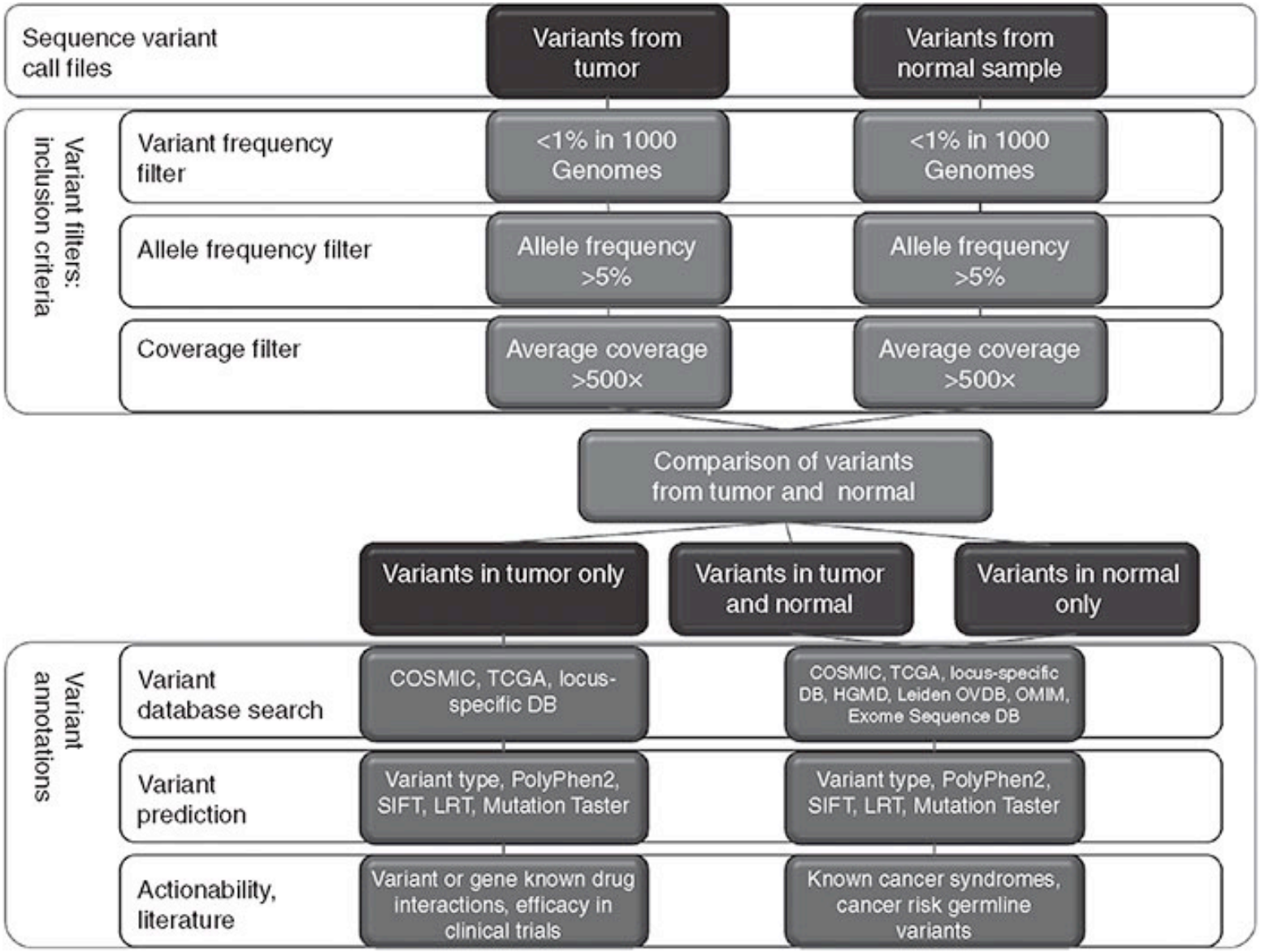
Sequence variant call files
Variants from tumor
Variants from normal sample
Variant filters: inclusion criteria
Variant frequency filter
<1% in 1000 Genomes
<1% in 1000 Genomes
Allele frequency filter
Allele frequency >5%
Allele frequency >5%
Coverage filter
Average coverage >500×
Average coverage >500×
Comparison of variants from tumor and normal
Variants in tumor only
Variants in tumor and normal
Variants in normal only
Variant annotations
Variant database search
COSMIC, TCGA, locus-specific DB
COSMIC, TCGA, locus-specific DB, HGMD, Leiden OVDB, OMIM, Exome Sequence DB
Variant prediction
Variant type, PolyPhen2, SIFT, LRT, Mutation Taster
Variant type, PolyPhen2, SIFT, LRT, Mutation Taster
Actionability, literature
Variant or gene known drug interactions, efficacy in clinical trials
Known cancer syndromes, cancer risk germline variants

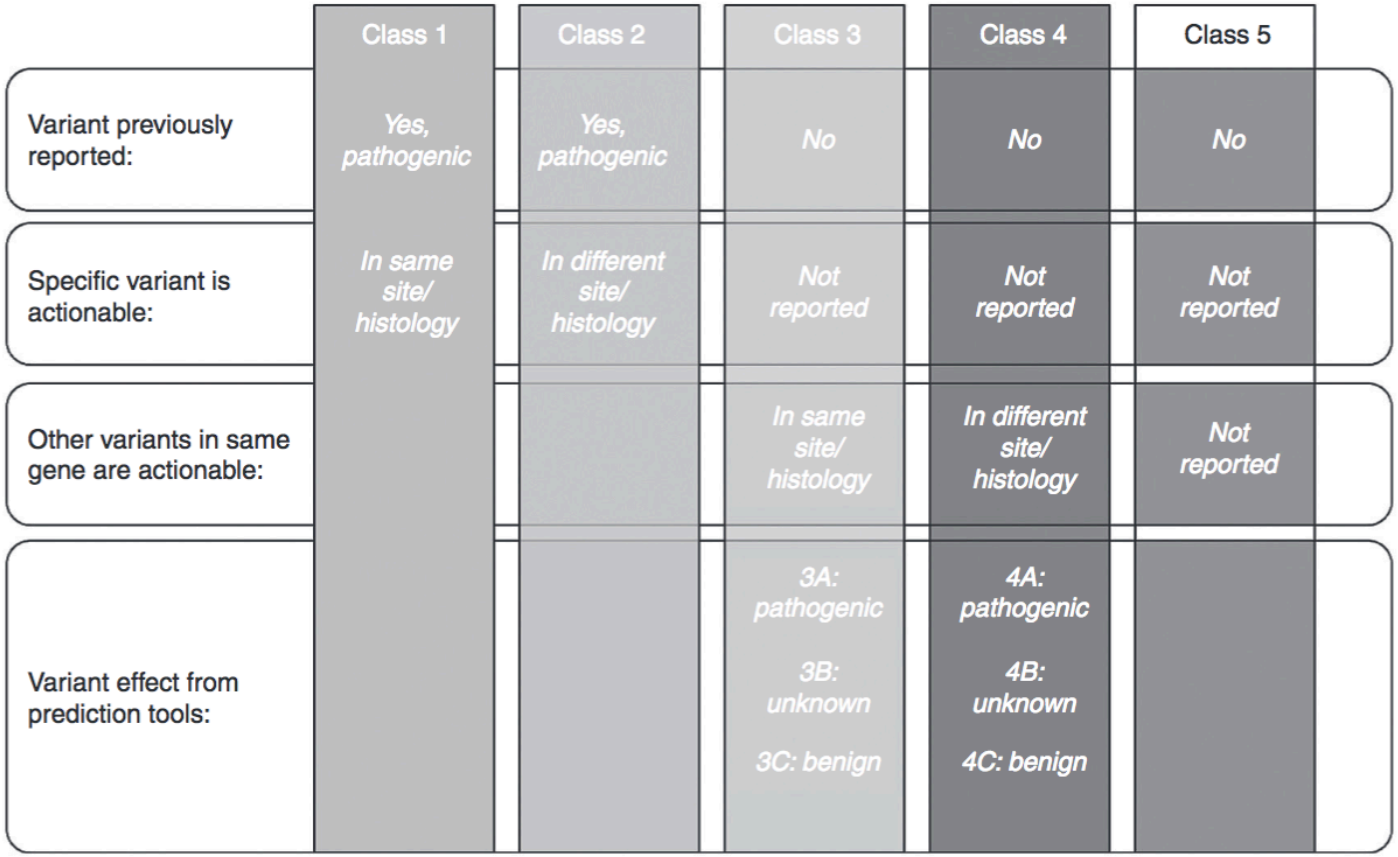

Figure 2 Summary of the proposed somatic variant classification. Variants are classified as classes 1 through 5, based on information around actionability (same variant: classes 1 and 2; other variants in the same gene: classes 3 and 4; no data: class 5), tumor site/histology, recurrence in the literature, and variant effect from prediction tools.

Table 1 Detailed description of the somatic variant classification scheme

Category	Description
1	Variants in this class can be used to DIRECT PATIENT CARE This variant is established as clinically actionable (druggable/predictive/prognostic and/or with diagnostic/classification implications) in the disease primary site & histology in which it has been identified
2	Variants in this class can be used for direct patient care AT THE DISCRETION OF THE TREATING ONCOLOGIST This variant is established as actionable in a DIFFERENT disease site and/or histology; however, in this site/histology, actionability (or non-actionability) has not been established
3	Variants in this class can be used for direct patient care AT THE DISCRETION OF THE TREATING ONCOLOGIST. Variants of this gene in this primary site/histology are established as actionable; however, this specific sequence variant is not one of the recurrently reported variants (nor is it an established benign single-nucleotide polymorphism) in this gene. Functional prediction algorithms have been used to determine the PREDICTED effect of the mutation on protein function: A. Functional prediction algorithms indicate that the identified variant LIKELY DOES modify protein function B. Functional prediction algorithms indicate that the identified variant MAY OR MAY NOT modify protein function C. Functional prediction algorithms indicate that the identified variant LIKELY DOES NOT modify protein function
4	Variants in this class may or may not be used for direct patient care AT THE DISCRETION OF THE TREATING ONCOLOGIST Variants of this gene in a different primary site and/or histology are established as actionable; however, in this site/histology, actionability (or non-actionability) has not been established, and this specific sequence variant is NOT one of the recurrently reported variants (nor is it an established benign SNP) in this gene. Functional prediction algorithms have been used to determine the PREDICTED effect of the mutation on protein function: A. Functional prediction algorithms indicate that the identified variant LIKELY DOES modify protein function B. Functional prediction algorithms indicate that the identified variant MAY OR MAY NOT modify protein function C .Functional prediction algorithms indicate that the identified variant LIKELY DOES NOT modify protein function
5	Variants in this class are of UNKNOWN SIGNIFICANCE No actionability has been established for any variant in this gene in any disease site/histology. This category is further subdivided into: A. Adequate studies have not been done to establish actionability for variants in this gene, or for this specific variant, at any primary site & histology B. This variant or variants of this gene in general have been established as NOT CLINICALLY ACTIONABLE at this primary site & histology

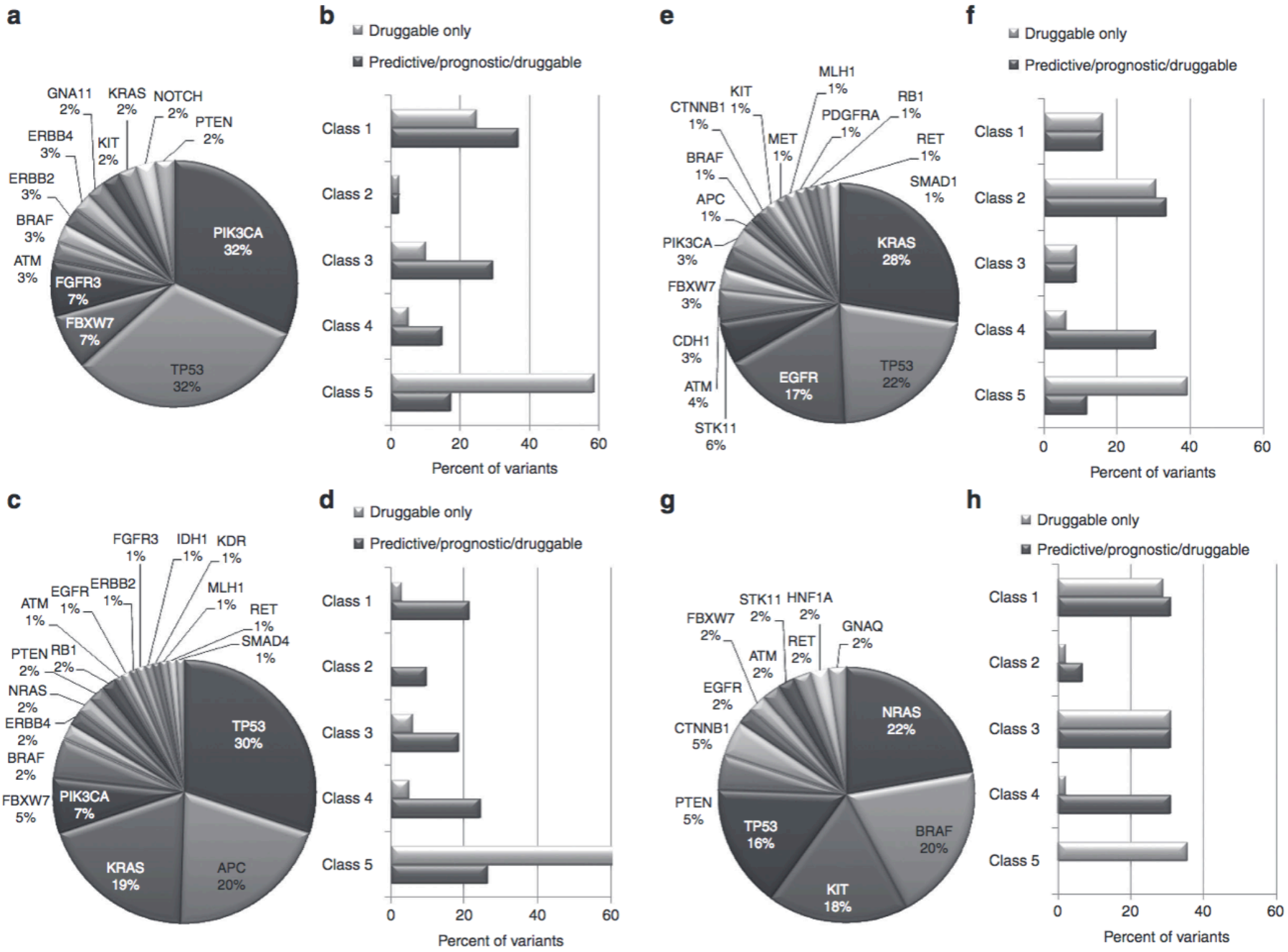
a
GNA11 2%
KRAS 2%
NOTCH 2%
PTEN 2%
ERBB4 3%
KIT 2%
ERBB2 3%
BRAF 3%
ATM 3%
FGFR3 7%
FBXW7 7%
PIK3CA 32%
TP53 32%
b
Druggable only
Predictive/prognostic/druggable
Class 1
Class 2
Class 3
Class 4
Class 5
0
20
40
60
Percent of variants
c
FGFR3 1%
IDH1 1%
KDR 1%
EGFR 1%
ERBB2 1%
MLH1 1%
ATM 1%
RET 1%
SMAD4 1%
PTEN 2%
RB1 2%
NRAS 2%
ERBB4 2%
BRAF 2%
FBXW7 5%
PIK3CA 7%
KRAS 19%
APC 20%
TP53 30%
d
Druggable only
Predictive/prognostic/druggable
Class 1
Class 2
Class 3
Class 4
Class 5
0
20
40
60
Percent of variants
e
KIT 1%
MLH1 1%
CTNNB1 1%
RB1 1%
PDGFRA 1%
MET 1%
RET 1%
BRAF 1%
SMAD1 1%
APC 1%
PIK3CA 3%
FBXW7 3%
CDH1 3%
ATM 4%
STK11 6%
KRAS 28%
EGFR 17%
TP53 22%
f
Druggable only
Predictive/prognostic/druggable
Class 1
Class 2
Class 3
Class 4
Class 5
0
20
40
60
Percent of variants
g
STK11 2%
HNF1A 2%
FBXW7 2%
GNAQ 2%
RET 2%
ATM 2%
EGFR 2%
CTNNB1 5%
PTEN 5%
NRAS 22%
TP53 16%
BRAF 20%
KIT 18%
h
Druggable only
Predictive/prognostic/druggable
Class 1
Class 2
Class 3
Class 4
Class 5
0
20
40
60
Percent of variants

Table 2 Level of evidence assessment for clinical actionability data

Level of evidence	Descriptor
HIGH	☐ US Food and Drug Administration approval
	☐ Regulatory guidelines
	☐ Results of molecular targeted therapy trials (large multicenter), phase II/III
	☐ Results of large phase III trials with prospective/retrospective biomarker analysis
	☐ Meta-analyses (multiple large studies, very large population size)
	☐ Results of large retrospective biomarker studies (multi-center)
	☐ Results of large multicenter biomarker studies
	☐ Combination of results from multiple smaller trials and/or retrospective studies
MODERATE	☐ Small phase III trial with prospective/retrospective biomarker analysis
	☐ Large phase II trial (not targeted, single-center, or multi-center) with prospective/retrospective biomarker analysis
	☐ Small phase II/III biomarker trials
LOW	☐ Small phase II trial (single-center or multi-center), with or without biomarker analysis
	☐ Phase I trial (any size/type)
	☐ Small patient cohort studies (any size-type)
INSUFFICIENT	☐ In vivo models
	☐ Cell culture models
	☐ In silico predictions
	☐ Studies in progress
	☐ Abstracts published at academic conferences

OXFORD

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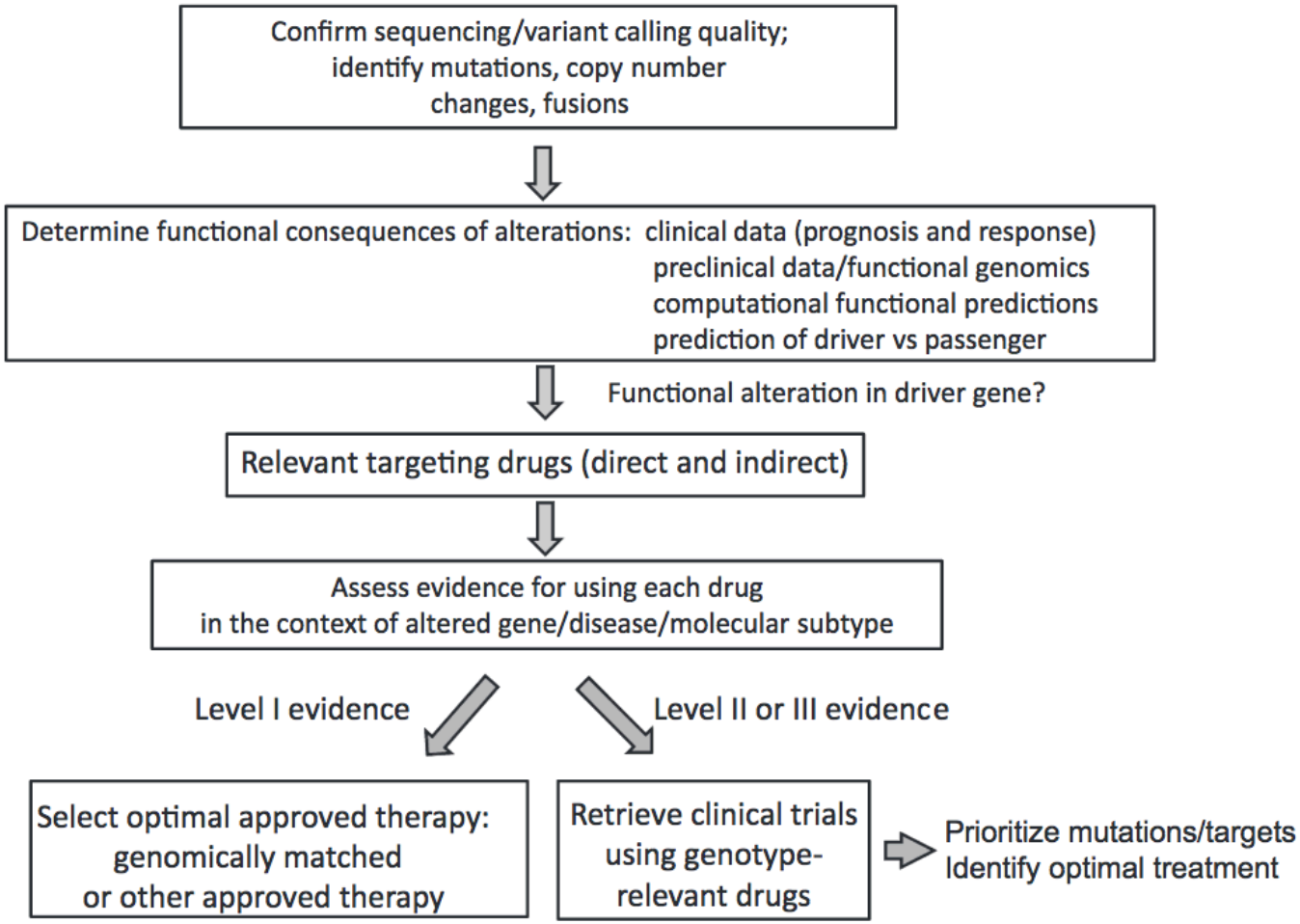
Commentary

COMMENTARY

A Decision Support Framework for Genomically Informed Investigational Cancer Therapy

Funda Meric-Bernstam, Amber Johnson, Vijaykumar Holla, Ann Marie Bailey, Lauren Brusco, Ken Chen, Mark Routbort, Keyur P. Patel, Jia Zeng, Scott Kopetz, Michael A. Davies, Sarina A. Piha-Paul, David S. Hong, Agda Karina Eterovic, Apostolia M. Tsimberidou, Russell Broaddus, Elmer V. Bernstam, Kenna R. Shaw, John Mendelsohn, Gordon B. Mills


Confirm sequencing/variant calling quality;
identify mutations, copy number
changes, fusions
Determine functional consequences of alterations: clinical data (prognosis and response)
preclinical data/functional genomics
computational functional predictions
prediction of driver vs passenger
Functional alteration in driver gene?
Relevant targeting drugs (direct and indirect)
Assess evidence for using each drug
in the context of altered gene/disease/molecular subtype
Level I evidence
Level II or III evidence
Select optimal approved therapy:
genomically matched
or other approved therapy
Retrieve clinical trials
using genotype-
relevant drugs
Prioritize mutations/targets
Identify optimal treatment