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Comparing AMP/ASCO/CAP Tier Classifications Across Multiple Variant Annotation Solutions

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Introduction

Variant annotation is a critical step in deciphering the functional impact of genetic variants. With the emergence of numerous next generation sequencing (NGS) technologies, many variant annotator software tools have become available to aid in the interpretation of genomic variants, for both germline and somatic applications. However, the differences in variant classification assignments across these tools remain largely unexplored and these differences can have a significant impact on the interpretation of genetic variants and downstream management. In this study, we analyzed and compared the variant tier classification differences among four commonly-used variant annotator software tools for the interpretation of variants identified from tumor testing: navify® Mutation Profiler (navify®MP; Research Use Only (RUO) in the US - not for diagnostic procedures; Roche)‡, SOPHIA DDM™ (RUO in the US; Sophia Genetics), QIAGEN® Clinical Insight (QCI) Interpret (RUO; QIAGEN), and Franklin (Genoox), a free, publicly-available annotator (see software version #'s in Fig. C). By examining the differences in variant tier assignments and the underlying criteria employed by these software tools, we aim to show the nuances of somatic variant interpretation and offer potential reasons for tier classification differences across the solutions, which, in turn, may help researchers select the most appropriate tertiary analysis software solution for their specific needs.

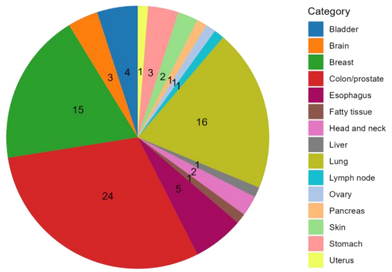
Materials & Methods

Formalin Fixed Paraffin embedded (FFPE) tumor samples from 80 pan-cancer cases were processed by the Illumina TruSight™ Oncology 500 (TSO500; RUO) assay, which contains 523 DNA genes with implications in cancer. Secondary analysis was performed via the Illumina DRAGEN™ pipeline and VCFs were loaded into the four annotation tools mentioned above. In the case of SOPHIA DDM™, secondary analysis calls generated out of the SOPHIA pipeline were compared with Illumina DRAGEN™ calls using a Python script that showed a single nucleotide polymorphism (SNP) match rate of 93.6%. A total of 5,255 unique variants across all 80 cases were successfully classified. The resulting Association for Molecular Pathology (AMP) tier classifications, such as IA, IB, IIC, IID, were compared in several configurations (4-way, 3-way, 2-way); the Positive Percent Agreement (PPA) statistic (95% CI) was used for 2-way comparisons.

COMPARISON ANALYSIS

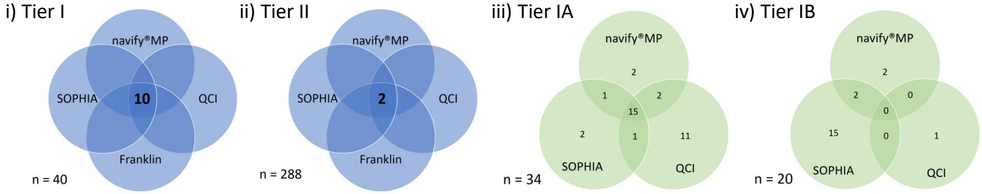
A. Tumor Sample Type

80 real world cases depicted by cancer location



B. Tier I and II concordance profiles

Across all four annotators, concordance was 22.5% (i) for Tier I and 0.69% (ii) for Tier II. Tier IA assignments showed higher concordance (41.2%; iii) across comparable tools than Tier IB assignments (0.00%; iv).



Note: Variant sample numbers represent variants with a classification of Tier I, II, IA, and IB, respectively, in ≥ 1 annotator.

C. Positive Percent Agreement (PPA) for 2-way comparisons

PPA is provided for each Tier I/II comparison as well as the number of variants being compared (in parentheses). Highest PPAs for Tier I comparisons are seen between navify®MP and Sophia DDM™, while the lowest PPAs for Tier I are seen between navify®MP and Franklin.

navify®MP (v 2.3.2.c090e09)	SOPHIA DDM™ (v 5.10.42.1—h275027-1c0c57f)	QIAGEN®Clinical Insights Interpret (v 9.2.1.20231012)	Franklin** (v 2023.7)
Tier IA	84.21% (22)	77.27% (36)	43.48% (29) Tier III: 10 variants Tier IV: 3 variants
Tier IB	99.76% (18)	0.00% (5)	
Tier IIC	26.69% (250)	50.31% (392)	4.66% (324) Tier IV: 90 variants
Tier IID*	N/A	N/A	N/A

*navify®MP had Tier IID calls in this dataset; however, the other annotators did not; thus, a IID comparison was not made in this study. **Tier IA and IB were combined to compare with Franklin's Tier I category; Tier IIC was compared to Franklin's Tier II category.

D. Subjective phrases published in the AMP/ASCO/CAP guidelines (PMID: 27993330): one potential explanation of discordance across tools

Bold, italicized, underlined words are considered "subjective" and require further definition by sites implementing this classification structure.

Tier	Evidence 1	Evidence 2	Evidence 3	# subjective words or phrases
IA	FDA-approved therapy	Included in ***professional*** guidelines		1
IB	***Well-powered*** studies with ***consensus*** from ***experts in the field***			3
IIC	FDA-approved therapies for different tumor types	***Investigational*** therapies	***Multiple***, published, ***small*** studies with ***some consensus***	4
IID	***Preclinical*** trials	A ***few*** case reports ***without consensus***		3

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‡navify® Mutation Profiler (navify®MP) is intended for Research Use Only (RUO) in the US and not for use in diagnostic procedures or decisions. NAVIFY is a trademark of Roche. All other product names and trademarks are the property of their respective owners. Comparisons to other products does not constitute a claim that navify®MP is anything other than an RUO tool.

Results

For Tier I and II variants, comparisons made across all 4 solutions revealed less than 25% concordance (22.5% and 0.69% for Tier I and II, respectively). Tier IA calls for comparable tools were at much higher concordance than Tier IB calls (41.2% vs. 0%, respectively). For 2-way comparisons, although limitations apply to the PPA such as sample size, there are still notable trends. PPA for IA classifications was >77% when comparing navify®MP to QIAGEN®Clinical Insights Interpret and SOPHIA DDM™. However, when comparing navify®MP to Franklin, the PPA was <50% for Tier I calls and <5% for Tier II calls. Of variants navify®MP classified as Tier I or II, 103 of these variants were classified as Tier III or IV by the Franklin annotator. Further investigation of specific variants showing markedly discrepant tier classifications across the annotators suggested that differences may be due to a number of aspects, including differing healthy population thresholds, quality thresholds, primary transcripts, genome builds, variant-naming conventions, and classification contexts (therapeutic, diagnostic, prognostic, and hereditary). Furthermore, we believe that the subjectivity in the evidence definitions published in the AMP/ASCO/CAP guidelines (PMID: 27993330) plays a sizeable role in tier assignments, particularly for Tiers IB and IIC, where there are several words or phrases, leading to multiple, possible interpretations of the definitions.

Conclusions

Overall, the 4 software solutions are user-friendly and far superior to manual curation. These tools generally provide an easy-to-navigate user interface with the characteristics needed to readily interpret variants. However, there were marked disagreements between all annotators, suggesting that it is important to carefully consider the choice of variant annotation software for specific applications. In addition, as reported from the Variant Interpretation Testing Among Laboratories (VITAL) challenge conducted by AMP, variant classification remains challenging and clarifications in the guidelines are warranted (PMID: 35429647).