# Implementation of a Customized Tertiary Analysis Platform for the Reporting of Somatic Variants

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ABSTRACT: Precision medicine for oncology requires the evaluation of variants identified in molecular profiling of solid tumors and hematologic malignancies. This includes evaluation of pre-analytical and postanalytical quality metrics, variant interpretation, classification, and tiering as outlined in established guidelines, association with clinical significance such as FDA approved drugs and clinical trials, and finally comprehensive reporting. This study documents our experience with the customization and implementation of a software platform that facilitates these requirements for effective reporting of somatic variants.

KEYWORDS: Somatic variant interpretation, tertiary analysis, software platforms

Precision medicine for oncology requires the evaluation of variants identified in molecular profiling of solid tumors and hematologic malignancies. This includes evaluation of preanalytical and post-analytical quality metrics, variant interpretation, classification, and tiering as outlined in established guidelines,<sup>1</sup> association with clinical significance such as FDA approved drugs and clinical trials, and finally comprehensive reporting.

Platform customization: To optimize the efficiency and quality of variant evaluation and report generation workflows, our laboratory implemented a third-party solution, the GenomOncology Pathology Workbench as a tertiary analysis platform for the reporting of somatic variants. Once a case is created in the GenomOncology workbench, it reads the VCF files along with applicable ancillary run information output from the Ion Torrent Suite and Ion Reporter Software. Based on the specific requirements of our laboratory, the platform was customized to evaluate sequencing data from QC metrics to report generation (Figure 1A). QC metrics were set based on assay validation characteristics and established thresholds, at the run and sample level (Figure 1B), with the ability to view a list of variants that were filtered out by the platform based on established thresholds including potential technical artifacts. Default settings were used for annotating variants with information from public and privately licensed databases, for automatic classification of variants as having clinical or potential clinical significance, uncertain significance or as benign, and for automatic tiering of variants according to the AMP, CAP, and ASCO guidelines for somatic variant interpretation (Figure 1C and D).<sup>1</sup> The platform was also customized to support multiple tests including disease-specific tests composed of subsets of genes on the backbone of larger panels. To support our offering of a single comprehensive report for each case, the

platform was set up to accept data from a next-generation sequencing (NGS) assay that included both DNA and RNA variants, as well as the results of non-NGS assays, specifically PD-L1 IHC and MSI-PCR, which were then combined into a single analysis for each case (Figure 1E and F). The final report was customized according to our laboratory's requirements and included a detailed summary of FDA approved targeted therapies and potential clinical trials to facilitate treatment and management decisions by oncologists (Figure 1H and I). Once signed out, reports were downloadable in either PDF or Microsoft Word format to enable easy integration into the patient electronic health record.

Testing and implementation: Post customization, the platform was tested using 20 specimens processed using NGS across 2 different validated test systems for solid tumor and hematologic malignancies. The validation included the testing of various parameters including quality metrics output, filters for variant evaluation set per assay thresholds, variant classification as configured per established guidelines, and test specific report generation with review and sign-out capabilities. After validation and setup was completed, the GO Pathology Workbench was implemented for the analysis and reporting of somatic variants for the tertiary analysis of myeloid and pan cancer panels across single nucleotide variants (SNVs), insertion and deletion (InDels), copy number variation (CNVs), RNA fusions, and additional biomarkers.

Performance and impact of the platform: Implementation of the GenomOncology tertiary analysis platform improved the efficiency of somatic variant analysis in 2 main areas-turnaround time (TAT) and comprehensive report generation. Customization of the platform to identify variants based on specific filtration presets, eased the analysis workflow by eliminating false positives, low quality variants, and variants below



the limit of detection. Autoclassification of the variants identified with ancillary evidence and the ability to modify the classification if needed allowed the laboratory team to efficiently assess large amounts of data in a timely fashion. Classification of all clinically significant variants detected in the platform that are reported is verified using established processes including evaluation of functionality and oncogenicity of the variant followed by the AMP/ASCO/CAP criteria for variant classification. Review of literature and usage of databases such as OncoKb, Clinvar, COSMIC, TCGA portals etc. are established variant evaluation workflows. By querying GenomOncology's knowledge base with the identified variants for a given case in the context of patient information such as age, gender, and tumor type, the platform matches patients to specific treatment recommendations based on their molecular and clinical history. These recommendations are automatically pulled into the solution, simplifying case and presentation creation processes, creating a final report to include in an EHR. Ultimately, this process provides relevant, meaningful content to inform a patient's treatment plan (Figure 1). Report customization to include a test result summary, with clinically significant variants tabulated along with

Α	QC Metrics	Potentially	Significant Variants	Uncertain Variants	Benign Variants	Other Assays	Clinical Trials	Patient	Comments	
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	Raw Accuracy (>= 0.99): 99.30 % Total Reads (>= 60000000.00): 61328885.00			Target Base	Target Base Coverage at 1x: 99.98 %					
	% Usable Reads (>= 0.30): 44.40 %			Mean Depth Mapped Res	Mean Depth (>= 800.00): 2498.00 Mapped Reads (>= 3000000.00): 6575236.00					
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	NP ID: NP_004324.2		dbs	SNP: rs121913365 (probable-path	ogenic) QUAL:	390.44				
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(Continued)

	Variant		Significance	1	Ion_S5 Inf	o	BAM Inf	fo (click for pileup)
	PPM1D I496V (c.1486 17:58740581:A:G Mutation Type: Subs NP ID: NP_003611.1 NM ID: NM_003620.3 Count: 1/1 (Detected See More Informatio	titution - Missense 3 /Total)	Significance Source: Aut AMP Tier: Ti dbSNP: rs3 pathogenic) 1000 Genor	: Likely Benign ocalculated, 10/14/2022 er 4 5491690 (probable-non- nes: 1.99681E-4 sso of function	Total Read Variant Rea VAF: QUAL: Q Score: Target Edg View all Me	s: 3769 ads: 3329 88.3% 26781.1 (24.4/26.2) es: 55 bp	J.	. G
	TCF3 L120P (c.3597> 19:1627365:A:G Mutation Type: Subs NP ID: NP_003191.1 NM ID: NM_003200.3 Count: 2/3 (Detected See More Informatio	titution - Missense } /Total)	AMP Tier: Ti dbSNP: rs3	ocalculated, 10/14/2022 ier 4 5354874 (non-pathogenic) nes: 0.00838658 one	Total Read Variant Rea VAF: QUAL: Q Score: Target Edg View all Me	ads: 789 50.5% 9142.29 (27.0/26.0) es: 42 bp	Ţ	c a g c
ł	Clinical Trials							
	<b>Relevant Alterations</b>	Trial Details		Title		Conditions		Interventions
	MET Amplification NCT03175224 (ct.gov) Phase 1/Phase 2, Recruiting 2022) Apollomics Inc.		(July 27, APL-101 Study of Subjects With c-Met EXON 14 Skip Mutat c-Met Dysregulation Advanced S Turnors		itions and	ons and Cancer, Gastric Cancer,		APL-101 Oral Capsules
	TP53 C275F Phase 2, Recruiting (August National Cancer Institute (NC		Administration of Autologous T-C Genetically Engineered to Expres Receptors Reactive Against Neos in People With Metastatic Cancer		ess T-Cell oantigens	Endocrine Tumors, Non-Small Cell Cancer, Ovarian Cancer, Breast Cr Gastrointestinal/Genitourinary Can +2 more		Cyclophosphamide, Fludarabine, Aldesleukin Individual Patient TCR- Transduced PBL, Pembrolizumab (KEYTRUDA)
	MET Amplification	NCT01639508 (ct.gov) Phase 2, Recruiting (June 23 Memorial Sloan Kettering Ca	3, 2022) Incer Center	Cabozantinib in Patients With RET Fusion- Positive Advanced Non-Small Cell Lung 2022) Cancer and Those With Other Genotypes: Non-S zer Center ROS1 or NTRK Fusions or Increased MET or AXL Activity		Non-Small Cell Lung Cancer		Cabozantinib
	Report							
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**Figure 1.** GenomOncology Workbench Features. (A) Data analysis tab in GenomOncology that displays all available features for review of case. (B) Quality Control Metrics at run and specimen level. (C) Potentially significant variants—these are tier I/II variants as auto-classified by the platform. (D) Uncertain Variants—variants of uncertain significance (VUS) are displayed on this tab. Other assay tab displays results from ancillary testing such as fusions (E) and PD-L1 status (F). (G) Lists benign variants. Clinical Trials tab (H) displays available clinical trials based on variants identified once approved in B. (I) Report template demonstrating the various sections with relevant information to the case tested, including sections on test details, limitations, and disclaimer as mandated by regulatory guidelines.

potential targeted FDA/NCCN approved therapies and clinical trial recommendations, variant and gene descriptions, a list of pertinent negative genes as applicable to the panel tested, and additional assay information enabled the generation of a high-quality comprehensive report (Figure 1I). Along with the ability to integrate results from different tests performed on a single specimen, the final report accurately illustrates what has been tested for the current patient and what their results indicate as potential treatment in a way that allows oncologists to navigate complex data accurately and effectively.

Salient features of GenomOncology include the ability to edit auto-calculated classification, and real-time manual updates to narrative interpretation of variants as deemed necessary to ensure the most updated relevant information is being provided on a clinical report. Since implementation of the GenomOncology platform, our lab has processed 80 samples across 2 different test types, myeloid and pan-cancer with a substantial increase in efficiency. A 50% decrease in TAT from 7 to 3 days for data analysis and reporting of variants, with positive reviews from oncologists for the content and clarity of the report. Recent improvements to the platform include the ability to evaluate a subset of genes for specific disease allowing the laboratory to expand its test offering menu to include 7 subset panels.

*Future capabilities*: To further enhance the laboratory's clinical decision workflows, the plan is to integrate GenomOncology's Molecular Tumor Board solution which allows a precision medicine laboratory to automate their molecular tumor board processes either in an integrated hospital setting or at the point of care.

# Declarations

### Ethics approval and consent to participate

This study is a validation of variant interpretation and data analysis platform and therefore IRB or ethics approval was not required.

## Consent for publication

No patient relevant data was used in this study. This study is a validation of variant interpretation and data analysis platform and therefore patient consent or approval was not required.

#### Author contributions

Kala F Schilter: Conceptualization, Data curation, Methodology, Validation, Writing—review & editing. Sarah Dubay: Methodology, Software, Writing—review & editing. Matt Stachowiak: Formal analysis, Investigation, Writing—review & editing. Alysia Kaplan: Methodology, Software, Writing review & editing. Abby Stauffenger: Methodology, Software, Writing—review & editing. Honey V Reddi: Conceptualization, Formal analysis, Project administration, Supervision, Validation, Writing—original draft, Writing—review & editing.

#### Acknowledgments

None.

#### Availability of data and materials

Not applicable.

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