

Face the Molecular Pathology Decision Support Challenge with Alissa Interpret

Tumor profiling using Next Generation Sequencing (NGS) is rapidly gaining importance in molecular pathology. Adopting this technology requires not only bioinformatics tools to analyze, interpret, and database the large number of variants originating from NGS assays, but also brings challenges in data management and clinical interpretation. Furthermore, the delivery of actionable results from NGS data needs to be clinically robust: informed, traceable and reproducible. Moreover, in a cancer diagnostic setting, fast turnaround times are essential.

Acknowledging these challenges, the Association of Molecular Pathology's (AMP) Europe 2018 meeting hosted a 'Battle of the Bioinformatics Pipelines'. Vendors of NGS analysis and interpretation solutions were provided sequencing data from real samples, which were generated by a routine molecular diagnosis laboratory. The 'battle' objectives were to see how the vendors' solutions can help molecular pathologists and to compare results between the pipelines. Of the original eight vendors who signed up, only Agilent and two other commercial vendors faced the challenge, identifying and annotating variants in samples, and gathering evidence for their classification.

Only Agilent went beyond the pipeline analysis to give pathologists what they really need to face the molecular pathology challenge—a clinical-grade draft report that puts the pipeline in a clinical context and empowers their decision-making.

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Introduction

Both organizers and session participants agreed that, as there is no gold standard for data analysis and interpretation, the expertise of the clinical professional remains of paramount importance to make the right decision on clinical relevance and reporting criteria.*

To do this, molecular pathologists require best-in-class informatics tools to efficiently triage, classify, and database the large number of variants originating from NGS assays while meeting established consensus standards. Moreover, pathology professionals and medical technicians require workflow tracking and annotated reporting, which are essential for satisfying a variety of compliance rules and reimbursement systems.

Here, we showcase how we deployed Alissa Interpret, the variant assessment and reporting module on the Agilent Alissa Clinical Informatics Platform, to:

- Implement an automated pipeline for somatic variant assessment
- Support efficient triage and confident variant classification in context of the tumor's type and supported by public and premium third-party knowledge databases
- Build a curated variant knowledgebase tailored to collect diagnostic evidence for somatic variants
- Deliver comprehensive draft reports based on configurable templates
- Meet professional guidelines

Case

The 'Battle of the Bioinformatics Pipelines' provided vendors with output of a gene panel capture kit performed on a variety of adenocarcinoma biopsies. As an example, in this Application Note we use Alissa Interpret to analyze, interpret, and report on the identified variants for one such tumor biopsy that was provided by the session organizers in VCF format.

* AMP-Led Project Compares Bioinformatics Pipelines for Analyzing Cancer Panel Data, Julia Karow, GenomeWeb, May 03, 2018

In-depth variant review and classification

Variant review in context of up-to-date public and internal knowledge databases

The three variants automatically identified for further review were reviewed in the context of detailed gene and variant information from public and collaboratively built in-house databases and *in silico* variant effect predictions, all collected

in a clear overview on the Variant Review page. For Oncology, the platform integrates with the public ClinVar, COSMIC and CIViC databases to deliver insight in the variant's clinical significance and actionability, including diagnostic evidence. CIViC is an open access, community driven web resource for Clinical Interpretations of Variants in Cancer. Here we show how all the evidence available in CIViC for a variant can be aggregated and consulted in a dedicated tab, ready to be used for variant assessment and curation (Figure 2).

Gene information	Variant information	Managed variant lists	Annotations	Links	Previous occurrences	CIVIC		
G719	Variant G719							
G719A	Variant summary							
MUTATION								
Evidence								
EID	Type	Direction	Significance	Disease	Drug	Citation	Evidence level	Trust rating
4189	👁️	Supports	Sensitivity	Non-small Cell Lung Carcinoma	Gefitinib, Erlotinib	Wu et al., 2011, Clin. Cancer Res.	B - Clinical	★★★
1780	👁️	Supports	Sensitivity	Lung Adenocarcinoma	Erlotinib	Klughammer et al., 2016, J Thorac Oncol	B - Clinical	★★
Evidence description								
EGFR G719 mutations have been associated with increased sensitivity to first generation EGFR tyrosine kinase inhibitors, including erlotinib and gefitinib. In a study of patients treated with erlotinib or gefitinib, a subset of patients with EGFR mutation at codons G719 or L861 (n=30) was associated with improved response rate (47.5% vs 16.5%, P<0.001) median progression free survival (5.0 vs 2.0 months, P<0.001), and overall survival (15.0 vs. 10.4 months, P =0.030), compared to patients with wild-type EGFR (n=272).								

Figure 2. The Variant Review tab collects the information needed for an in-depth review and classification of variants on a single page. Evidence present in the CIViC database is directly available in the CIViC tab.

Expert curated evidence on variant actionability

For additional detailed information on actionability, Alissa Interpret facilitated submission to N-of-One, an expert molecular decision support partner, enabling oncologists to provide targeted therapeutic treatment strategies, including clinical trial matching (Figure 3).

For those variants where therapeutic evidence is limited or outdated in public data sources and in the lab's internal knowledge sources, submissions to N-of-One for supplemental insight can be done automatically based on filtering criteria or triggered manually during variant review. In this example, all three variants were submitted to N-of-One.

Submit to N-of-One PrecisionInsight		Show full report	
Third party status	Not submitted (N-of-One PrecisionInsight)	Third party status	Information received (N-of-One PrecisionInsight)
Submitted on		Submitted on	Sep 17, 2018 4:23 PM CEST
Submitted by		Submitted by	nvanderaa
Information received on		Information received on	Sep 18, 2018 4:14 PM CEST

Figure 3. Variants were submitted to N-of-One for in depth curation.

All information present in the N-of-One report is automatically imported and presented in Alissa Interpret, where it is available for variant review and reporting, and tiered according to the AMP guidelines.

Content provided by N-of-One is manually curated by PhD-level scientists, approved by oncologists, and updated on a regular and rolling basis using a quality management system. N-of-One also includes professional guidelines where appropriate (Figure 4).

The screenshot displays the 'Variant information' tab for the EGFR G719A variant. It includes a table with gene information (Gene: EGFR, Transcript: NM_005228.3, cDNA: c.2156G>C, Protein: p.Gly719Ala), variant details (Type: snp, Location: exonic, Exon: 18, Effect: nonsynonymous), and read quality metrics (Read Depth: 4870, Call Quality: 7873.66, Genotype Quality: 7873, Filter status: PASS). A 'Labels' section indicates 'Good Somatic Significant'. To the right, the 'N-of-One PrecisionInsight' section provides a biomarker results summary, biological relevance, and molecular function. Below this, a 'PrecisionInsights' box contains a detailed summary of clinical significance, including a table of predictive variants and a list of trials prioritized by region.

Gene information | **Variant information** | Managed variant lists | Annotations | Links | Previous occurrences | CIVIC

Gene EGFR **Type** snp **db** SNP **Location** exonic **Exon** 18 **Effect** nonsynonymous

Transcript NM_005228.3 **Position** 7:55,241,708 **Allele** G C **AO** . 2076 **AF** . 42.6

cDNA c.2156G>C **Read Depth** 4870 **Call Quality** 7873.66 **Genotype Quality** 7873 **Filter status** PASS

Protein p.Gly719Ala **Labels** Good Somatic Significant

N-of-One PrecisionInsight **EGFR MUTN (seq): G719A** **Biomarker results summary** EGFR-G719A is an activating mutation. EGFR activating mutations or amplification may predict sensitivity to Egrf-targeted therapies, including inhibitors of multiple ErbB family members, and several have received FDA approval in some tumor types. **Biological relevance** **Molecular function** EGFR G719A is a missense mutation within the protein kinase domain of the Egrf protein (UniProt). This exon 18 mutation has been reported to result in ligand-independent activation of the Egrf protein. Mutations at G719 have been frequently reported in combination with other EGFR

PrecisionInsights

2.1. EGFR-G719A
TIER 1: Variant of Strong Clinical Significance
2.1.1 BIOMARKER RESULTS SUMMARY

Marker	Result	Summary
EGFR	- MUTN (seq): G719A	EGFR-G719A is an activating mutation.
	Clinical relevance	EGFR activating mutations or amplification may predict sensitivity to Egrf-targeted therapies, including inhibitors of multiple ErbB family members, and several have received FDA approval in some tumor types (Mok et al., 2009; 19692680, Rosell et al., 2009; 19692684, Tsao et al., 2005; 16014883).

2.1.2 BIO

Markers	Trial ID	Title	Phase	Targets	Locations/contact
1 EGFR	NCT03239340	A Molecular Profiling Study of Patients With EGFR Mutation-positive Locally Advanced or Metastatic NSCLC Treated With Osimertinib	Phase 2	EGFR	•Overall contact: AstraZeneca Clinical Study Information Center, information.center@astrazeneca.com, 1-877-240-9479 •CA (1), CT (1), GA (2), IL (1), MA (1), Italy (7), Korea, Republic of (8), Malaysia (5), Spain (6)
2 EGFR	NCT02917993	An Open-Label Phase 1/2 Study of INCB039110 in Combination With Osimertinib in Subjects With Non-Small Cell Lung Cancer	Phase 1/Phase 2	EGFR, JAK1	•Overall contact: Incyte Corporation Call Center, 1-855-463-3463 •AZ (1), CA (3), CO (1), DC (1), FL (1), MA (1), MI (2), NJ (1), NY (2), OH (1), OR (1), PA (1), TX (4), UT (1), VA (1), WV (1), Barcelona (1), Madrid (1), Seoul (3), Taipei (1), Taipei City (1), Valencia (1)

*Note: the trials listed above were matched to the biomarkers identified in this tumor; the list may not be comprehensive.

Figure 4. All information present in the AMP-tiered report delivered by N-of-One can be easily viewed in the Variant information tab and included in the Alissa Interpret lab reports.

Internal Database Building

Alissa Interpret's internal database building feature enables labs to leverage their curation efforts to increase efficiency in variant review and classification in future analyses. In this case, the EGFR variant was curated and stored in a Managed Variant List (MVL) for further reference (Figure 5).

Figure 5. Curated variant assessments, report abstracts, and evidence can be saved in the Managed Variant List. In this example, curated variant information obtained from by N-of-One, Inc. and evidence gathered from CIVIC was stored in the MVL, to be leveraged for future variant assessments.

The screenshot shows the internal database building interface for the variant NM_005228.3 c.2156G>C. It includes a table with gene information (Gene: EGFR, Transcript: NM_005228.3, cDNA: c.2156G>C, Protein: p.Gly719Ala), variant details (Type: snp, Location: exonic, Exon: 18, Effect: nonsynonymous), and a 'Curated version' section. Below this, there is a 'Clinical relevance' section with a 'Very high' rating. The 'Variant information' section includes a text area with a report abstract and a table of evidence. The evidence table lists the variant type, significance, tumor type, drugs, reference, evidence level, trust rating, and actions.

Somatic variants

NM_005228.3 c.2156G>C

Gene EGFR **Type** snp **Location** exonic **Exon** 18 **Effect** nonsynonymous

Transcript NM_005228.3 **Curated version** Version awaiting curation

cDNA c.2156G>C **Clinical relevance** Very high

Protein p.Gly719Ala **Last updated on**

Variant information

EGFR G719A is a missense mutation within the protein kinase domain of the Egrf protein (UniProt). This exon 18 mutation has been reported to result in ligand-independent activation of the Egrf protein (Bivona et al., 2013; ASCO 2013, Abstract 11067, Kobayashi et al., 2015; 26206867, Furuyama et al., 2013; 23387505). Mutations at G719 have been frequently reported in combination with other EGFR alterations and shown to confer sensitivity to the Egrf tyrosine kinase inhibitors erlotinib and gefitinib, both as single and complex mutations (Kobayashi et al., 2013; 23242437, Karaha et al., 2011; 21252719, Chen et al., 2006; 16205628, Bivona et al., 2013; ASCO 2013, Abstract 11067, van Noessel et al., 2015; 23358982, Wu et al., 2011; 21531810, Lynch et al., 2004; 15118073, Han et al., 2005).

Report abstract

The protein encoded by this gene is a transmembrane glycoprotein that is a member of the protein kinase superfamily. This protein is a receptor for members of the epidermal growth factor family. EGFR is a cell surface protein that binds to epidermal growth factor. Binding of the protein to a ligand induces receptor dimerization and tyrosine autophosphorylation and leads to cell proliferation. Mutations in this gene are associated with lung cancer. (provided by RefSeq, Jun 2016)

Evidence

Type	Significance	Tumor type	Drugs	Reference	Evidence level	Trust rating	Actions
Sensitivity		Pulmonary adenocarcinoma - non-small cell lung cancer (NSCLC)	Afatinib	A - Validated	★★★★		
Sensitivity		Pulmonary adenocarcinoma - non-small cell lung cancer (NSCLC)	Erlotinib, Gefitinib	21531810	B - Clinical	★★★	

Generating clinical-grade reports

Customizable to meet SOP and compliance

A comprehensive lab report can be automatically drafted. The report templates available in Alissa Interpret can be fully customized towards lab needs, both in content as well as layout. In this case, the report we generated contained information on the identified variants of clinical significance, including treatment suggestions and information on available clinical trials obtained from CIViC, the lab's internal knowledge base, and N-of-One (Figure 6).

Clinical-grade draft reports can also include information on the wet lab protocols used, as well as information on database and protocol versions to warrant full traceability in a clinical context.



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NGS Report

Accession No: AMPBat_S3

Analysis: A_AMPBat_S3

Gender: Male

Target panel: None selected

Tumor type: Lung adenocarcinoma

Reported variants

Variants with high clinical relevance

Gene	Transcript	Nucleotide change	Amino acid change	RD	VAF
EGFR

Variant interpretation

FULL DESCRIPTION

EGFR - p.G719A

Biomarker Results

EGFR-G719A is an inhibitor of multiple tyrosine kinases (TKs) (Lynch et al., 2009; 1969268).

Biological Relevance

Molecular function

EGFR G719A is a mutation that results in a constitutively active receptor with increased ligand-independent activity.

Biomarker-matched Clinical Trials

Trials prioritized by clinical specificity (*)

Trial ID	Title	Phase	Target
NCT02448251	Safety, Pharmacokinetic and Preliminary Efficacy Study of AC0010MA in Advanced Non Small Cell Lung Cancer	Phase 1	EGFR
NCT03239340	A Molecular Profiling Study of Patients With EGFR Mutation-positive Locally Advanced or Metastatic NSCLC Treated With Osimertinib	Phase 2	EGFR

Analysis details

Domain: Solid tumor Manual analysis

Creation date: 2018-09-17 14:08:01.0 Genome build: GRCh37.p13

Annotation sources

COSMIC (version 11) COSMIC release v85

ClinVar (version 12) NCBI ClinVar 20180401

OMIM (version 7) OMIM 2018-05-23

CIViC (version 7) CIViC release 01-May-2018 -

(*) Note The trials listed above are not intended to be a comprehensive list of all available clinical trials.

Figure 6. An example of an automatically drafted report based on a report template.

Conclusion

Agilent Alissa Interpret is tailored to tackle the NGS challenge in molecular pathology.

With features that support standardization and automation of the variant assessment workflow, the fully-hosted, web-based platform enables very significant speedup in variant triage and classification in the context of clinical oncology, in adherence with recognized international standards.

By partnering with Agilent as a gateway to public, collaborative, and premium knowledgebases, the clinical specialist has all the knowledge and tools at hand to efficiently identify clinically relevant variants and draft comprehensive reports with confidence.

www.agilent.com/lifesciences/alissa

Alissa Interpret is a USA Class I Exempt Medical Device, Europe CE IVD, Canada and Australia Class I IVD Device.

N-of-One is a molecular decision support company. N-of-One does not provide medical services, nor is any N-of-One employee engaged in the practice of medicine for or on behalf of N-of-One.

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PR7000-1979
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Printed in the USA, October 17, 2018
5994-0349EN

