Application Note

Alissa Interpret

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Face the Molecular Pathology Decision Support Challenge with Alissa Interpret

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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.

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**

Efficient variant triage

A flexible Decision Tree was created using Alissa Interpret's intuitive Classification Tree tool (Figure 1), capturing the data analysis and interpretation steps. Decision Trees can be validated and stored as part of a Standard Operating Procedure (SOP) for automated execution on future samples.

In this example classification tree, the filter steps we used included:

- A Target Panel: limiting the variants for investigation to those in a set of genes defined by the Battle Organizers. User defined target panels allow pathologists to focus their analysis on the genes of interest, avoid unwanted incidental findings and to be in control of their assay offering.
- Presence in public databases that represent frequent variants. In particular, this protocol will label variants as "germline" as soon as two out of five databases (including ExAC, gnomAD and 1000 Genomes and dbSNP) have this variant with a minor allele frequency of over 1%.
- A transcription effect other than "synonymous"; only variants with a likely effect on the protein are considered.
- A check against a broad set of relevant databases:
  - The COSMIC cancer variant database: Flagging variants with evidence in COSMIC. This can be done in context of the sample's tumor type.
  - The CIViC community driven Cancer Precision Medicine database: Flagging variants with evidence in CIViC. This can be done in context of the sample's tumor type.
  - Lab-specific databases built using Alissa Interpret using the Managed Variant List (MVL) functionality, collecting curated variants for this tumor type and capturing diagnostic evidence: Flagging previously curated variants.

It should be noted that the filters used are only an example. Many more filters, databases, algorithms, and strategies are available in Alissa Interpret tailored to support somatic cancer data interpretation in molecular pathology labs, as well as inherited disease data interpretation in clinical genetics labs.

In this molecular pathology case, the automated filtration strategy flagged three variants out of the 168 variants present in the VCF file for in-depth review (Figure 1), in the EGFR, TP53, and RNF43 genes.

A.

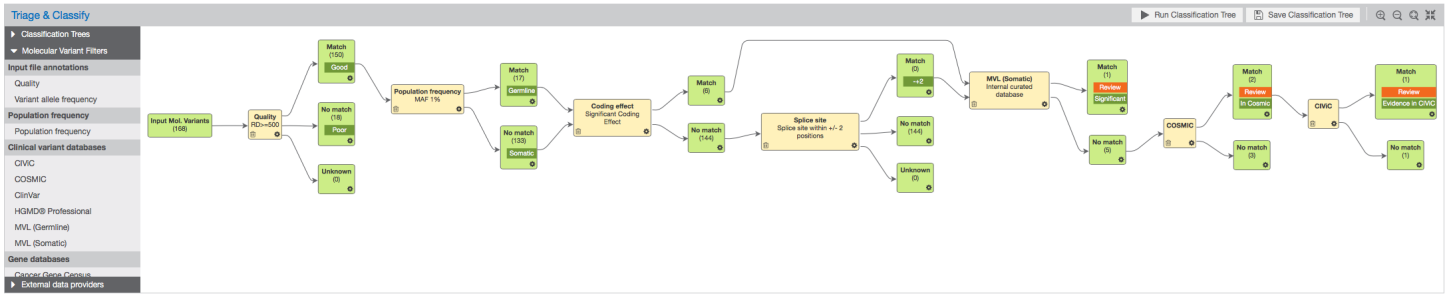
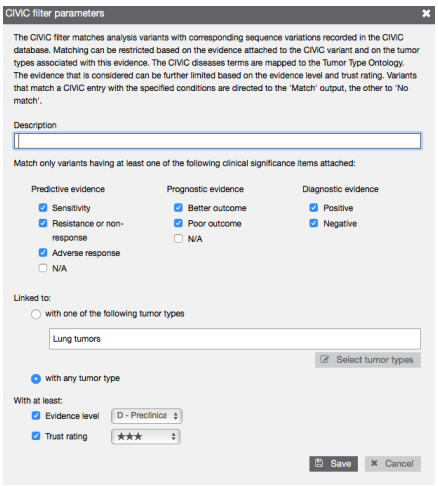
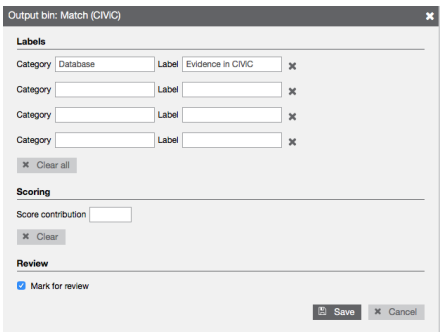


Figure 1A. The filtration strategy is captured in a Decision Tree. Variants flowing through the Decision Tree are classified according to the protocol.

B.



C.



Figures 1B and 1C. Filter setting can be configured to match the lab's protocol for variant triage. All filtered variants can be automatically assigned custom labels to facilitate variant assessment and marked for in depth review.

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).

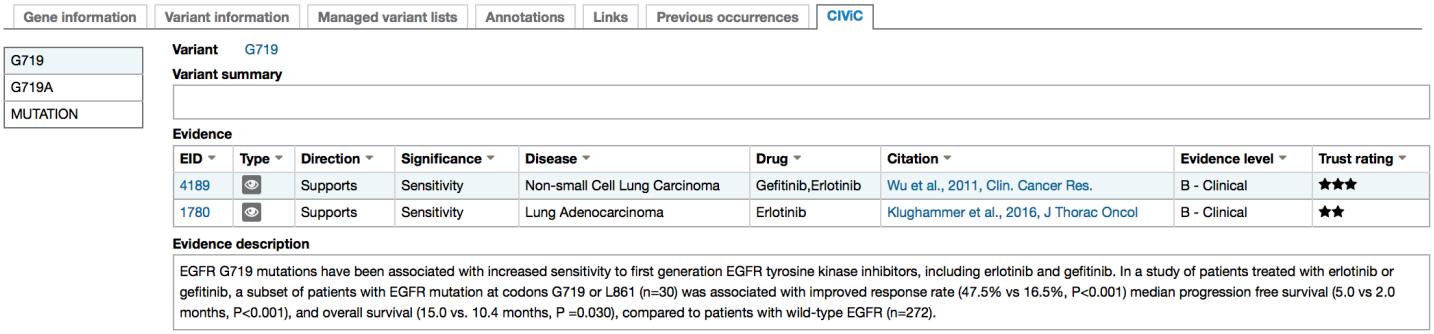


Figure 2. The Variant Review tab collects the information needed for an in-depth review and classification of variants on a single page. Evidence present in the CIViC database is directly available in the CIViC tab.

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.

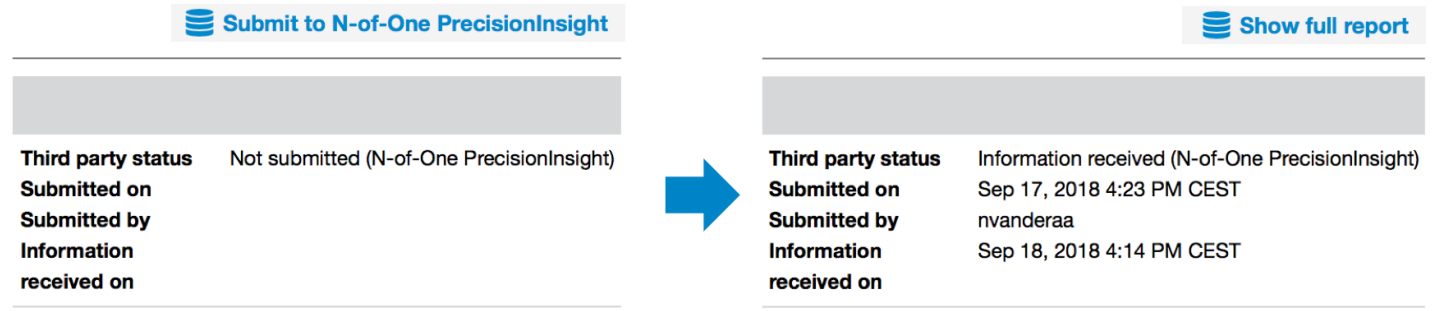


Figure 3. Variants were submitted to N-of-One for in depth curation.

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).

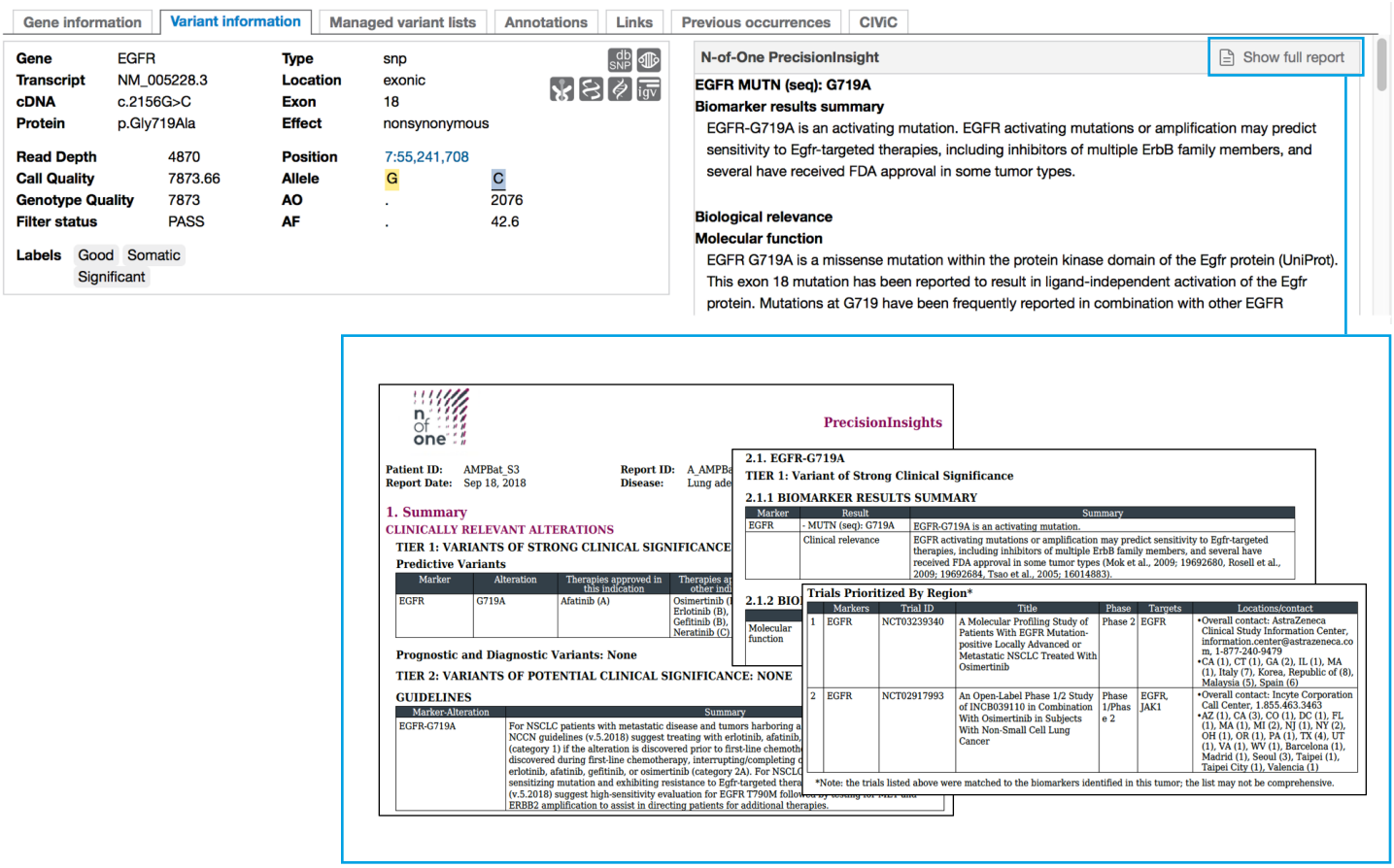


Figure 4. All information present in the AMP-tiered report delivered by N-of-One can be easily viewed in the Variant information tab and included in the Alissa Interpret lab reports.

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).

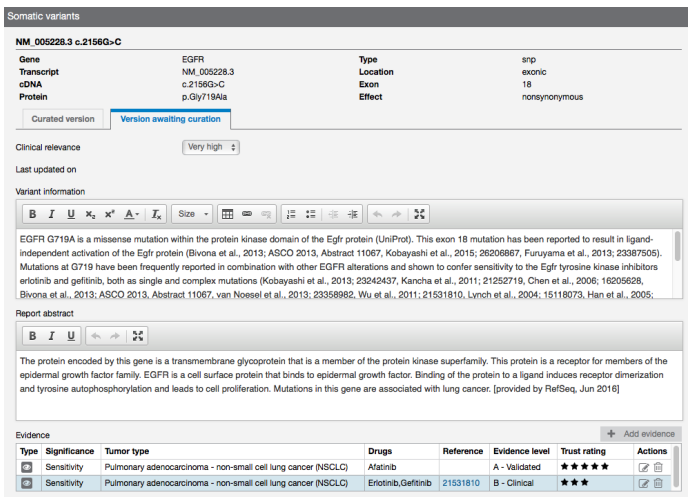


Figure 5. Curated variant assessments, report abstracts, and evidence can be saved in the Managed Variant List. In this example, curated variant information obtained from by N-of-One, Inc. and evidence gathered from CIViC was stored in the MVL, to be leveraged for future variant assessments.

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.

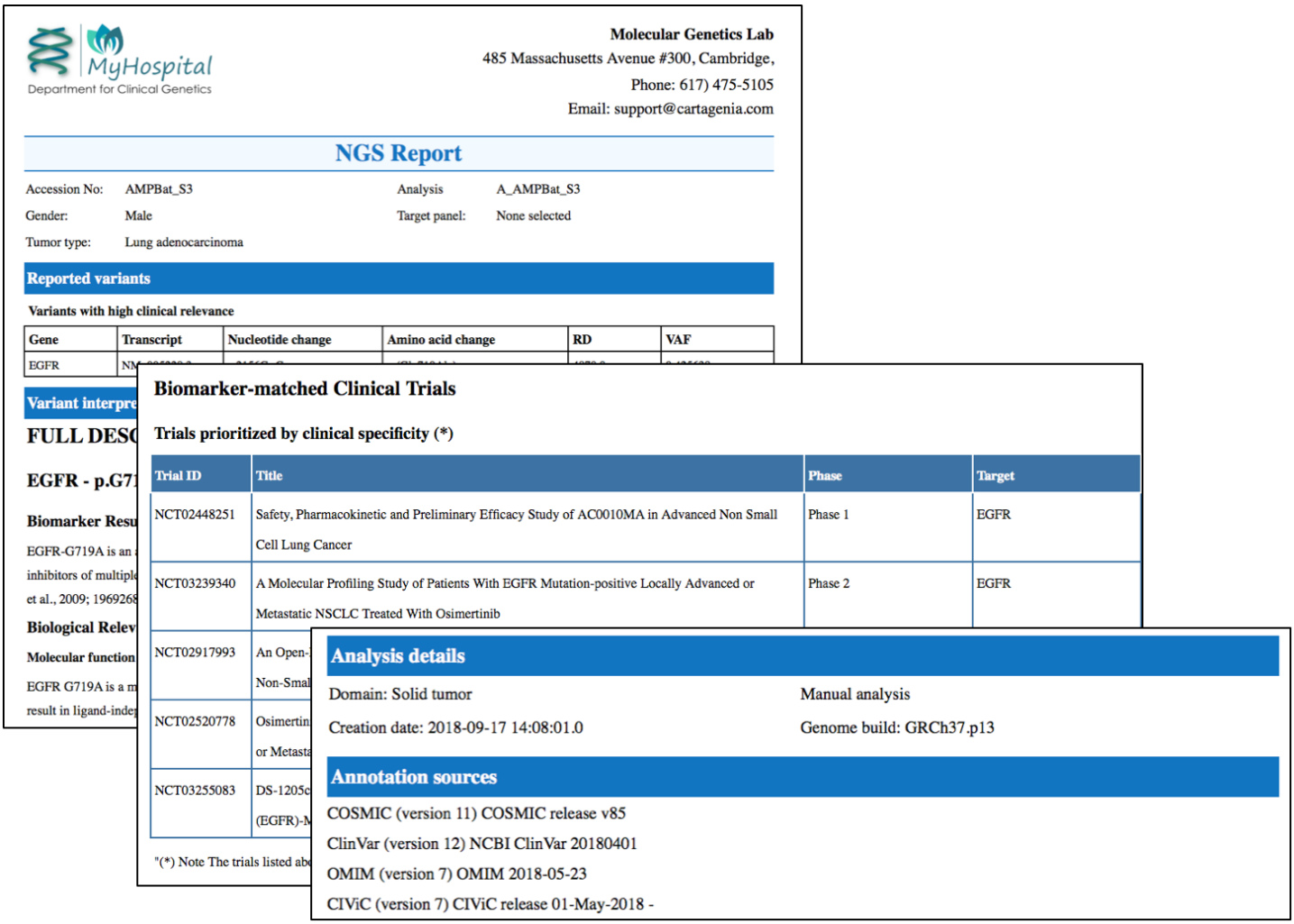


Figure 6. An example of an automatically drafted report based on a report template.

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 knowlegebases, 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.

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