How to integrate somatic and germline NGS into routine clinical oncology practice

The importance of identifying germline mutations in cancer cases and how bioinformatics tools can help your lab discern between somatic and germline variant origins

Introduction

The advent of massively parallel sequencing technologies has initiated the integration of large-scale tumor molecular profiling programs across institutions worldwide. The evolution of the technology, the growth in the field of molecular oncology, the development of new targeted treatments, and the decreasing costs of sequencing have fuelled the expansion of large tumor sequencing panels reaching up to hundreds of genes for one patient. Molecular pathology has become fundamental to drive therapeutic decisions by revealing a large number of druggable, tumor-detected molecular aberrations.

Even though testing for somatic mutations in tumors is an evolving standard of care across many cancer types, many challenges for its wide implementation still exist. These challenges include tumor heterogeneity, implementing drug combinations, clinical interpretation of co-existing mutations, clinical trial design, validation, and managing incidental findings (1).

Since a substantial proportion of genes included in large tumor panels confer a heritable predisposition to cancer, tumor genomic profiling is associated with the possibility of identification of incidental germline aberrations. When such a variant is detected in a tumor, it is unclear whether the variant is germline in origin (it is present in all or most tissues, including the germline), or it has been somatically acquired and represents the characteristic of that particular tumor. Such variants are sometimes referred to as tumor-detected pathogenic variants of unknown origin, or variants of unknown origin (2). Mutations identified during tumor sequencing may be acquired somatically, caused by postzygotic mosaicism, or inherited through the germline.

Because of the importance of determination of the mutation origin, there must be a systematic approach to guide clinicians on how to recognize genetic variants that may be germline. However, guidelines on how to determine the potential germline origin of variants detected through somatic tumor profiling are not widely available, and there is no established standard of care for those who require further germline genetic tests (3).

Why identifying incidental germline mutations is important

The desire to identify somatic mutations as actionable mutations with therapeutic relevance often overshadows the importance of recognizing hereditary germline variants that have important clinical implications for patients and their family members (4). Recent studies report that tumor genomic profiling will detect a germline pathogenic variant in 4% to 12% of patients (5-7).

Incidental germline alterations as prognostic and predictive biomarkers

Likely pathogenic (LP) and pathogenic (P) germline mutations in a patient can potentially be used as prognostic biomarkers for assessing the disease stage, the disease prognosis, and disease remission (8). For example, pathogenic germline variants in CDKN2A, CDK4, ATM, POLH, MRE11A, RECQL4 and XPC genes are associated with poor survival in stage III/IV melanoma patients (9). BRCA1/2 serve as biomarkers of poor prognosis in breast cancer (10); but, interestingly, women with a BRCA1/2 related ovarian cancer have better survival than non-carriers, particularly if they receive platinum-based therapy (11).

Germline mutations in cancer susceptibility genes confer higher risks for second primary cancer, which implicate the necessity of personalized clinical management of these patients. Carriers of germline MLH1 and MSH2 mutations have high lifetime risks of developing colorectal, endometrial/uterine, stomach, ovarian, small bowel, and other types of cancer (12). However, BRCA1 carriers are at high risk for breast cancer, ovarian cancer, second breast cancer, prostate cancer, and male breast cancer (13).

Germline mutations in DNA damage repair pathways, such as homologous recombination, have emerged as predictive biomarkers for response to novel targeted therapies in many human cancers. For example, defects in homologous recombination repair genes suggest potential susceptibility to poly ADP ribose polymerase inhibitors (PARPi) and platinum-based chemotherapy in metastatic prostate cancer (14), and breast and ovarian cancers (15). Germline mutations in mismatch repair (MMR) genes have been shown to result in a clinically significant response to immune checkpoint inhibitors (16).

Implication of incidental germline alterations for family members

Once a LP/P germline mutation in a cancer-associated gene is detected, this information carries important implications not only for the patient, but for their family members as well. The suspicion of the existence of such mutations in the family implicates the necessity of confirmatory germline genetic testing, expert genetic counselling, and predictive genetic testing for blood relatives. In cases where healthy family members inherit high-risk cancer-associated mutations, clinical surveillance and risk-reduction interventions should be offered in specialized clinics.

How to recognize incidental germline mutations during somatic mutational profiling

'On-tumor' and 'off- tumor' associations

In cases where a LP/P mutation is found in the cancer for which this particular gene confers high risk, the mutation is considered to be 'on-tumor'. For example, if a BRCA1 mutation is detected in ovarian cancer tissue, it is considered to be 'on-tumor' since BRCA1 confers elevated risk for ovarian cancer.

Conversely, if a BRCA1 mutation is found in bladder cancer tissue, it is considered to be 'off-tumor', since germline BRCA1 mutations do not confer elevated risk of bladder cancer. In cases of 'on-tumor' detected pathogenic variations, there is a higher probability that the mutation comes from the germline, as opposed to being somatically acquired. In case of 'off-tumor' detected mutations, these mutations are usually somatically acquired and are not related to inherited predisposition (2).

Clinical actionability of germline variants

Clinical actionability in the realm of hereditary cancers encompasses the risk of associated cancer (penetrance) and the availability of clinical management options such as screening, prophylactic measures, and lifestyle adjustments. The American College of Medical Genetics (ACMG) has assembled a set of 25 cancer-associated genes of higher actionability for which they have advised analysis and reporting for LP/P variants (17). The list of hereditary cancer genes that have sufficient clinical validity to be considered cancer susceptibility genes is still evolving. While analyzing variants detected through somatic tumor profiling, special attention should be paid to the list of genes associated with inherited conditions.

Frequency of somatically mutated cancer susceptibility genes (TP53 example)

Germline TP53 pathogenic mutations are usually found in a hereditary cancer syndrome known as Li-Fraumeni syndrome (LFS). TP53 mutation carriers have an increased chance of developing soft tissue sarcoma, osteosarcoma, female breast cancer, brain tumors, adrenocortical carcinoma (ACC), leukemia, and other types of cancer. TP53 mutations are frequently detected in different human carcinomas when somatic tumor profiling is performed. However, the general germline conversion rate is only 1% — with a slightly higher percentage in the 'on-tumor' setting (2%) (2). This means that a somatically detected TP53 mutation is usually somatic in origin, unless it is detected in a patient with a personal and/or family history of Li-Fraumeni syndrome. In these cases, germline testing should be considered.

Founder mutations

The recognition of a somatically detected variant as a founder mutation suggests that its origin is probably in the germline (18). Genes that have a higher mutation rate in the germline and have known founder mutations are more likely to be germline in origin. For example, BRCA1/2 founder mutations detected during somatic testing have been confirmed germline with additional testing (19).

Discriminating between somatic and germline variants

Bioinformatic tools and machine learning protocols are vital to discerning whether a certain variant is germline or somatic (20). The proposed models predict the somatic vs. germline status of each alteration by modelling the alteration's allele frequency (AF), and taking into account the tumor content, tumor ploidy, and the local copy number. Additional factors that are considered are variant population frequency, variant allele frequency (VAF), VAF of the adjacent polymorphisms, nucleotide composition, damaging effects, and presentation of a variant in databases. Since these methods still have their limitations (some of them are intended only for the discrimination of SNVs but not indels), further manual curation and inspection are still recommended.

Bioinformatic tools should be used as an aid in distinguishing the somatic vs. germline origins of variants by assessing VAF, presence of a variant in databases, nucleotide composition, CNV analysis, and more. Due to the insights provided by software tools, users can further manually inspect the criteria for potential additional testing and/or genetic counselling. It is important that the prediction of the variant origin is completed speedily, so that users can manually inspect the data and direct further patient management.

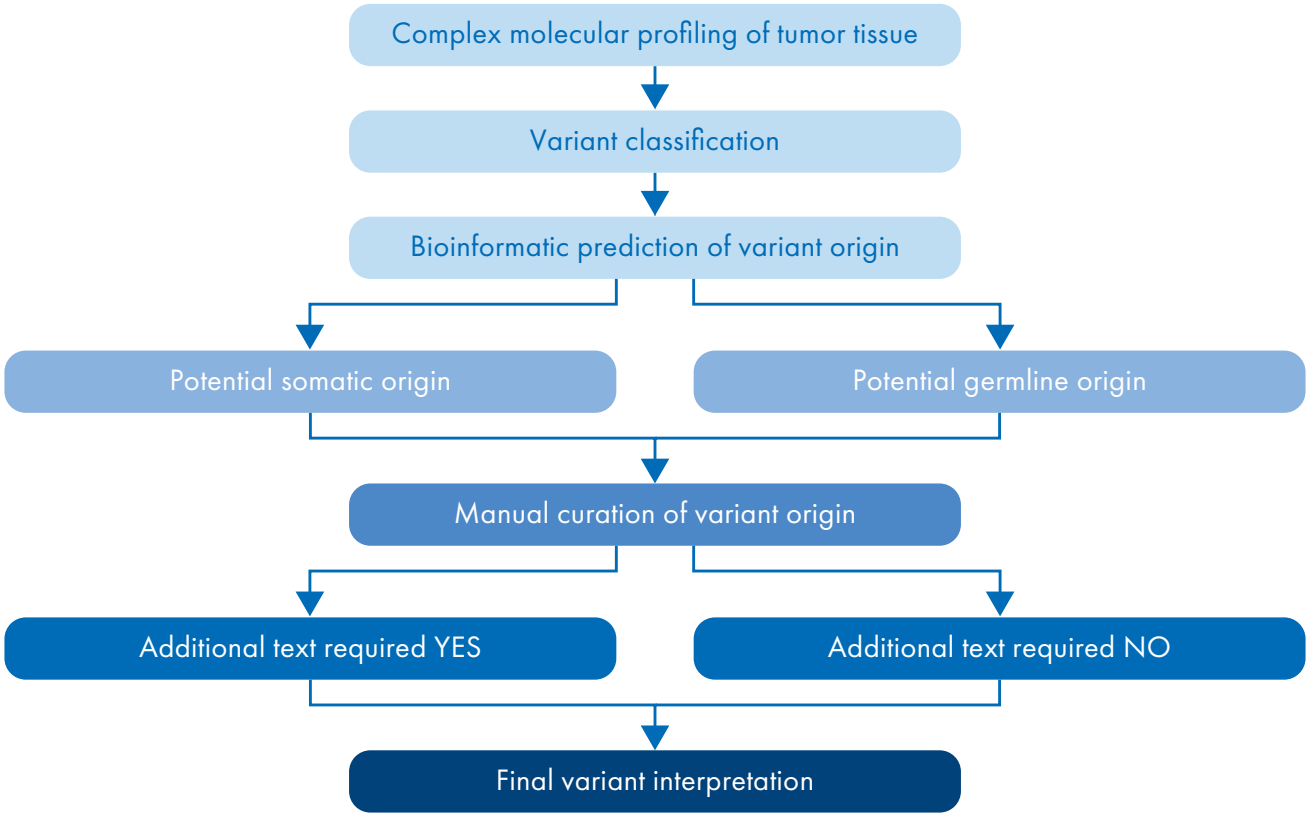


Figure 1. Schematic representation of a pipeline for discerning between germline and somatic origins of detected variants.
Once tumor tissue has undergone complex molecular profiling, a list of annotated variants is available. While performing variant analysis, software computationally predicts whether a certain variant is germline in origin. The prediction is based on several factors such as VAF, population frequency, VAF of the adjacent polymorphisms, nucleotide composition, potential damaging effects, and the presentation of a variant in databases.
For example, pathogenic variants are assumed to be of germline origin when the VAF is approximately 50%; but, a range between 30% and 70% is commonly accepted as representative of a heterozygous pathogenic variant. Or, if a variant is frequently found in somatic databases and is located within a gene that is frequently somatically mutated, the probability of it being of somatic origin is higher.
Bioinformatic analysis should be used as an indicator and aid for further manual inspection. Manual curation considers the assessment of personal and family history of cancer, evaluation of the ACMG list of cancer susceptibility genes, evaluation of the existence of founder mutations, on- and off- tumor associations, variant pathogenicity, and more. Based on the computational and manual analysis, further germline testing may be indicated for a final confirmation of a mutation's origin.

Using QCI® Interpret to discern between somatic and germline mutations

Identify potential germline mutations while analyzing a cancer case

QCI Interpret is a clinical decision support software powered by Augmented Molecular Intelligence. Connected to the exclusive QIAGEN Knowledge Base — the industry's most comprehensive, manually curated resource that is updated weekly — QCI Interpret contains content on over 4.9 million characterized germline variants. PMID references are available for every published germline variant in over 300 cancer risk associated genes, including variant and disease specific associations.

To determine if a variant is indeed pathogenic and associated with a hereditary phenotype, QCI Interpret supports the computation of all 28 ACMG criteria. And users can further manually inspect the evidence. QCI Interpret's curated literature describes the origin of a mutation (somatic or germline), association with a phenotype of interest, genetic inheritance patterns, and if the variant has been detected in other affected unrelated individuals. In addition, the Human Gene Mutation Database (HGMD®) and ClinVar database are included as additional evidence to determine disease-causing variants.

Real-world use-cases for QCI Interpret

Case 1

The patient is a 63-year-old white woman diagnosed with advanced stage, high-grade serous ovarian cancer. Tumor genomic profiling identified a variant in BRCA1 p.K748fs*2. The patient has no family history of cancer other than a maternal grandfather who developed lung cancer and was a smoker. Her mother and father were unaffected by cancer, and she has two children, a son and a daughter.

Interpretation by QCI Interpret:

The detected BRCA1 variant is a frameshift variant classified as pathogenic for hereditary breast and ovarian cancer and as actionable (Tier 2C) with a detected VAF of 77%. It is a very rare mutation with low frequency in somatic mutation databases. This mutation is not present in the Catalogue of Somatic Mutations in Cancer (COSMIC). The user can manually inspect the literature to understand if the variant has been detected in other affected unrelated individuals.

QCI Interpret provides in-depth information on a detected variant and suggests potential germline origins based on the type of mutation and VAF. All 28 ACMG criteria are supported and can be transparently reviewed in the assessment section (backed by evidence that can be reviewed). QCI Interpret utilizes the ACMG 73 Genes list to alert users of pathogenic variants causative of associated phenotypes and provides the pathogenicity classification for associated phenotype if VAF >40%.

Thanks to the information provided by the software, users can further manually inspect the criteria for potential additional testing and/or genetic counselling. QCI Interpret delivers the necessary information and recommendations for confirmatory germline testing (Figures 2-7).

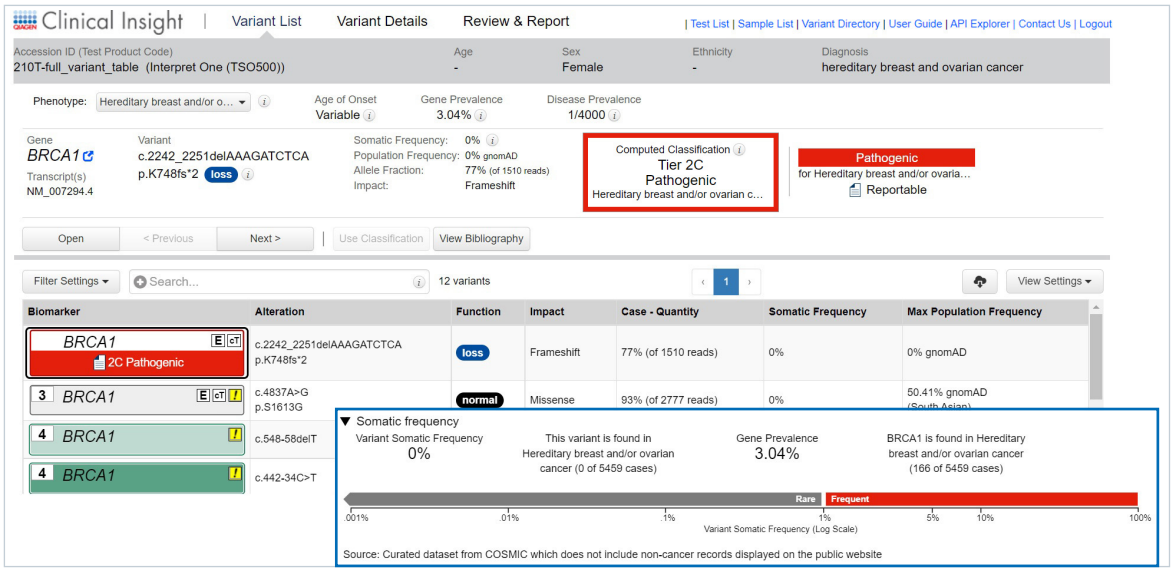


Figure 2.
The list of detected variants and the somatic frequency of *BRCA1* p.K748fs*2

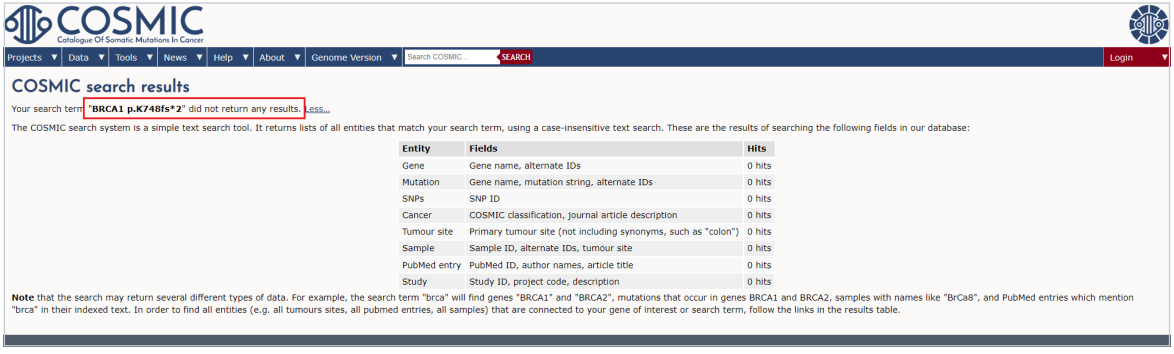


Figure 3.
BRCA1 p.K748fs*2 is not present in COSMIC

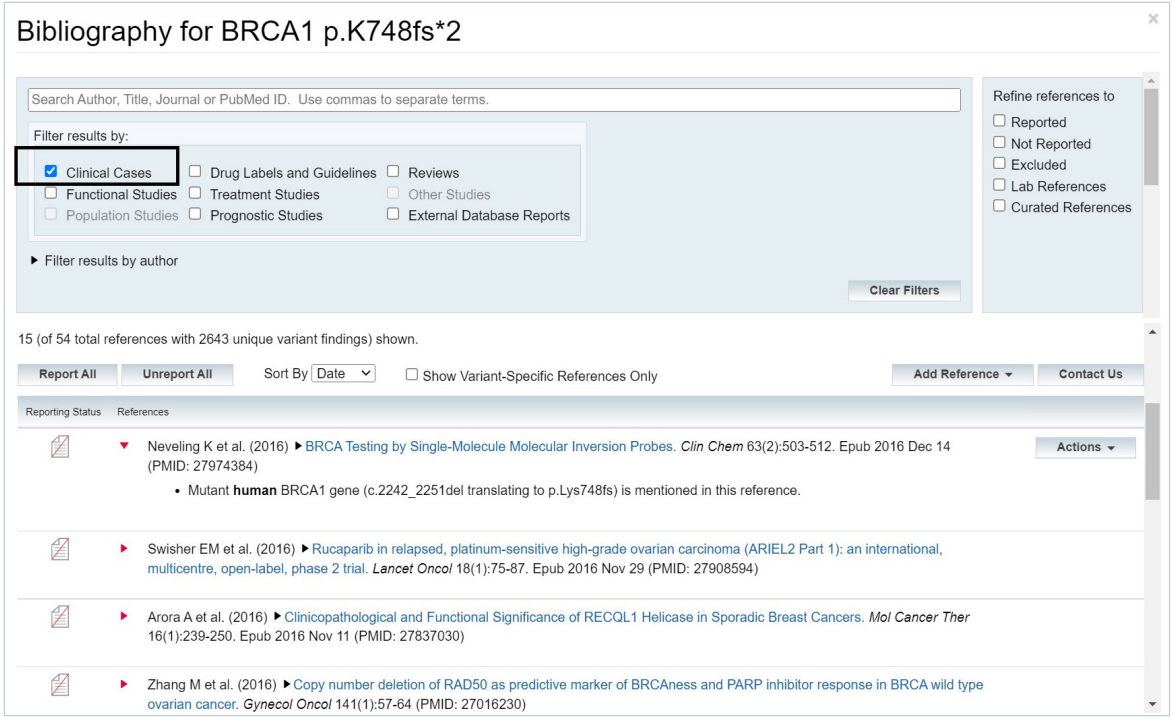


Figure 4.
The user can further manually inspect the literature to understand if the variant has been detected in other affected unrelated individuals

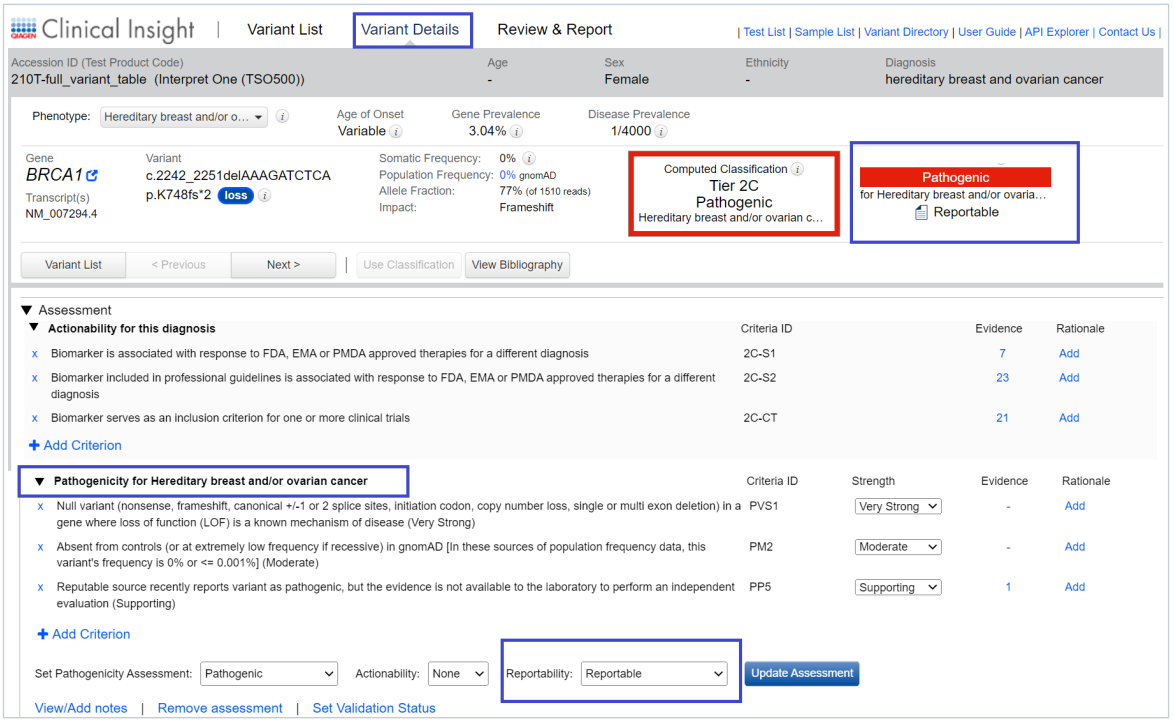


Figure 5.
QCI Interpret supports all 28 ACMG criteria, which can be transparently reviewed in the assessment section (backed up by evidence that can be reviewed)

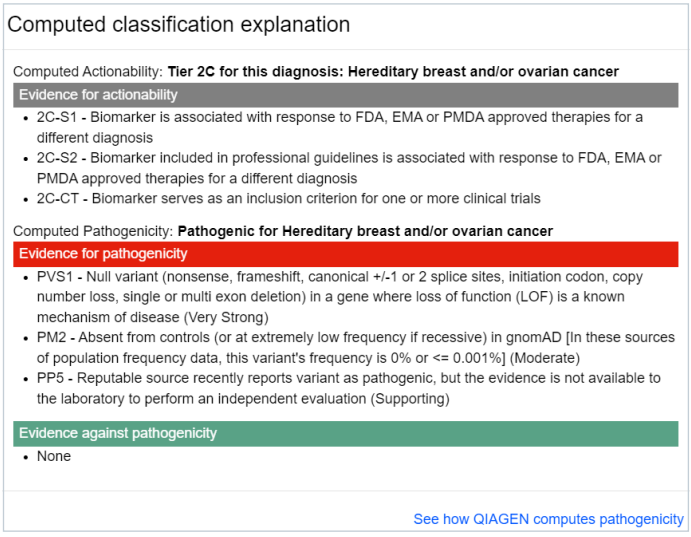


Figure 6.
Computed classification explanation in QCI Interpret

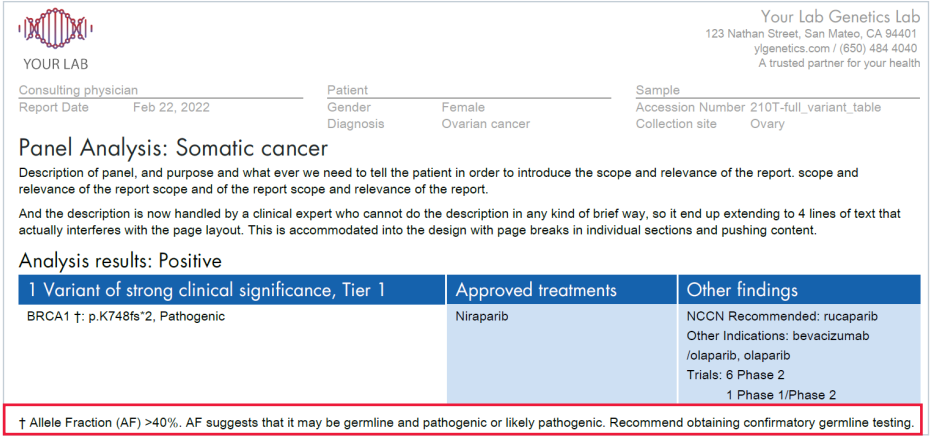


Figure 7.
QCI Interpret clinical report (for Europe) indicating the potential germline origin of a detected *BRCA1* mutation

Conclusion:

The detected BRCA1 variant is a pathogenic variant located in a high-penetrance cancer susceptibility gene. A high VAF of 77% indicates its potential germline origin, which is suggested in the QCI Interpret clinical report (Note: this report is configured for European regions. However, QCI Interpret supports labs worldwide).

Several other characteristics are noted: this mutation is rare in somatic databases; it is a pathogenic variant identified in ovarian cancer ('on-tumor'); and it is presented as an important prognostic and predictive biomarker in ovarian cancer. Based on this, additional germline testing was recommended. Confirmatory germline genetic testing was positive, and the germline origin of mutation was confirmed. The patient was referred to genetic counselling and additional predictive testing for blood relatives was recommended.

Case 2

The patient is a 70-year-old white man, smoker, diagnosed with advanced-stage lung adenocarcinoma. Tumor genomic profiling identified a variant in BRCA2, p.L1357fs*8. The tumor is microsatellite stable, and the tumor mutational burden is low. The patient has no family history of cancer.

Interpretation by QCI Interpret:

TThe detected BRCA2 variant is a frameshift mutation classified as LP, with Tier 2C actionability and with a detected VAF of 25%. It is a rare mutation with a scarcity of data related to its prevalence in lung adenocarcinoma. This mutation is not present in COSMIC. The user can manually inspect the literature to understand if the variant has been detected in other affected unrelated individuals.

Besides the fact that this variant is not present in COSMIC, it is not present in hereditary breast or ovarian cases as well. The AF of 25% is not suggestive of a germline origin and QCI Interpret does not indicate the germline origin of this variant (Figures 8-12).

Conclusion:

- Reasons for suggesting germline origin: It is a LP variant detected in the high-risk cancer susceptibility gene;
- Reasons for suggesting somatic origin: The lack of family history of cancers; The lower VAF than recommended for germline origin; Tumour is microsatellite stable and the variant is detected in the off-tumour setting;

To confirm the origin of the detected mutation, germline testing was offered and performed for the patient. The patient was shown to be negative for the germline *BRCA2* mutation.

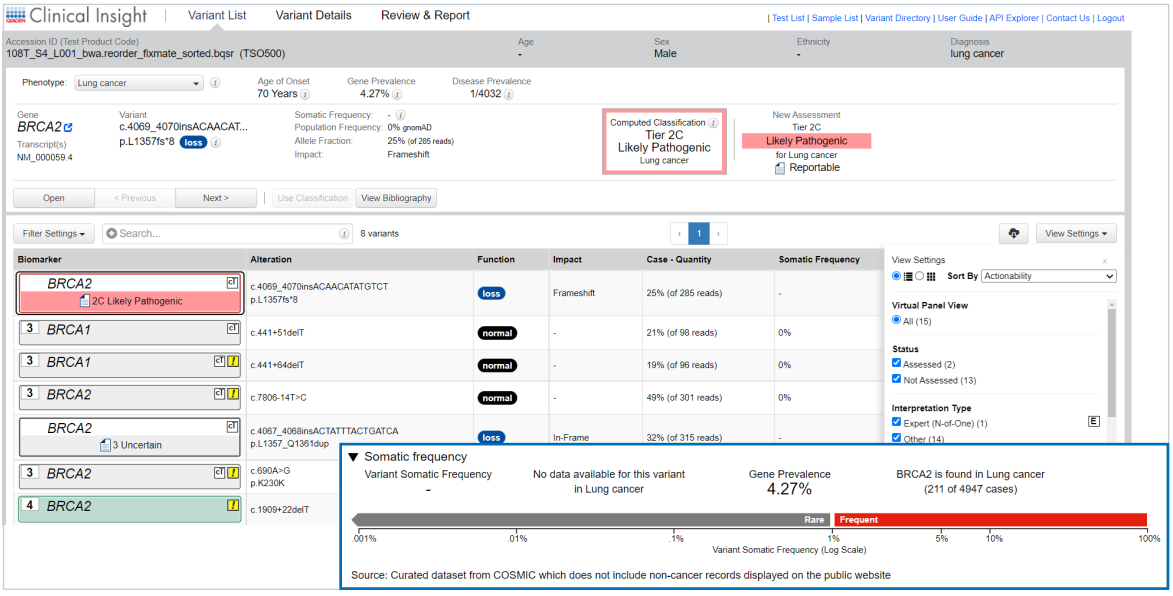


Figure 8.
The list of detected variants and the somatic frequency of *BRCA2*, p.L1357fs*8

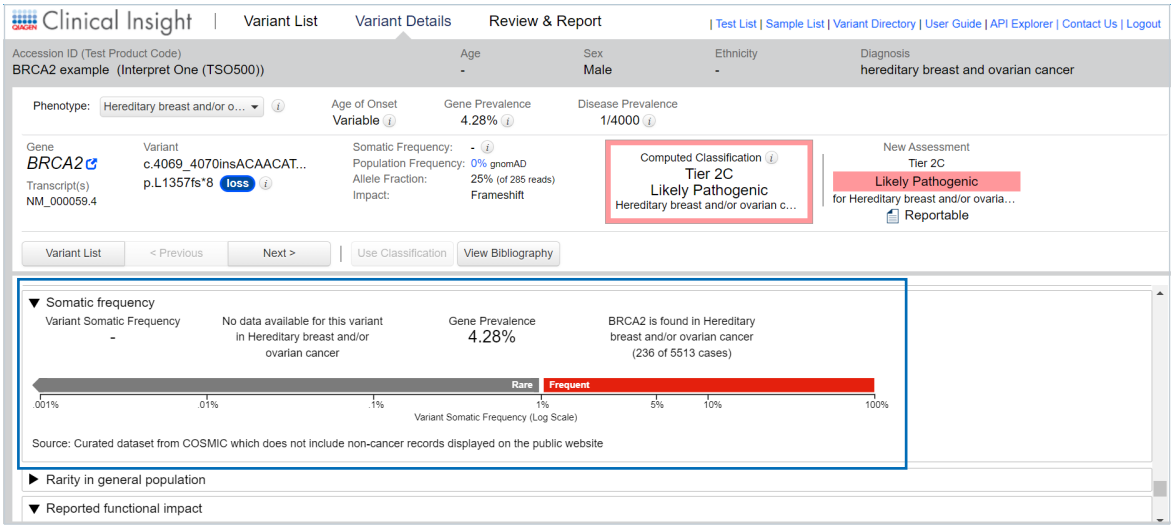


Figure 9.
BRCA2, p.L1357fs*8 is a rare mutation in both somatic and germline settings

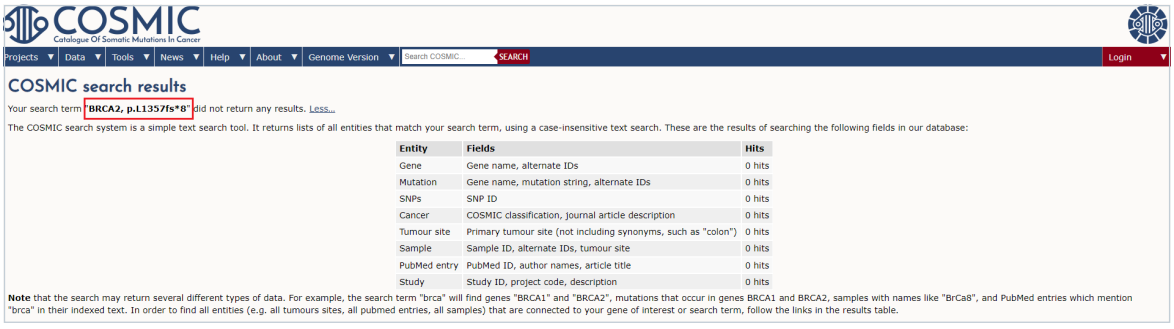
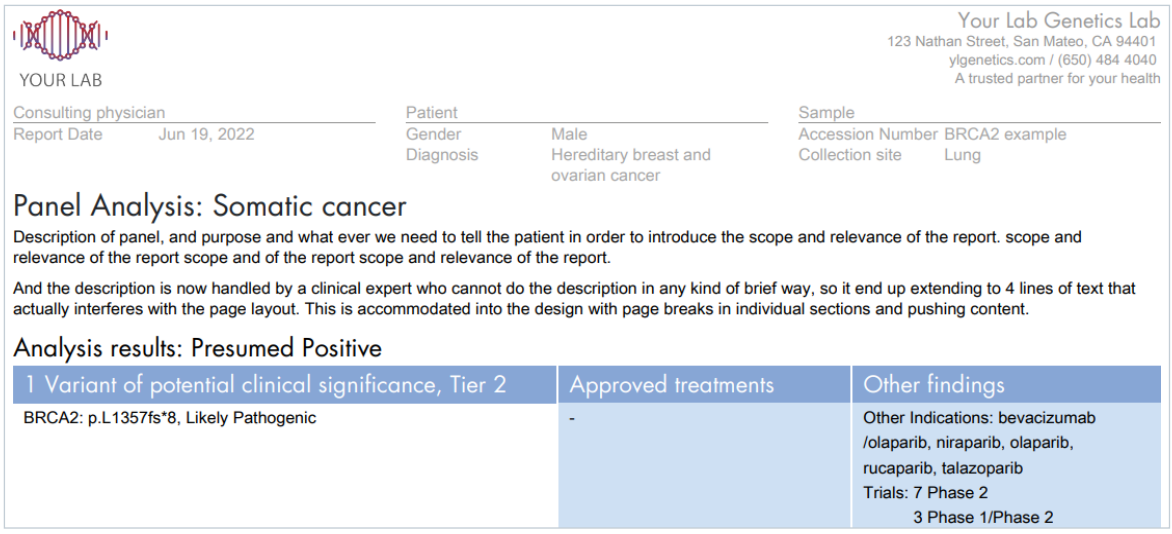


Figure 10.
BRCA2, p.L1357fs*8 is not present in COSMIC



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Report Date Jun 19, 2022

Patient
Gender Male
Diagnosis Hereditary breast and ovarian cancer

Sample
Accession Number BRCA2 example
Collection site Lung

Panel Analysis: Somatic cancer

Description of panel, and purpose and what ever we need to tell the patient in order to introduce the scope and relevance of the report. scope and relevance of the report scope and of the report scope and relevance of the report.

And the description is now handled by a clinical expert who cannot do the description in any kind of brief way, so it end up extending to 4 lines of text that actually interferes with the page layout. This is accommodated into the design with page breaks in individual sections and pushing content.

Analysis results: Presumed Positive

1 Variant of potential clinical significance, Tier 2	Approved treatments	Other findings
BRCA2: p.L1357fs*8, Likely Pathogenic	-	Other Indications: bevacizumab /olaparib, niraparib, olaparib, rucaparib, talazoparib Trials: 7 Phase 2 3 Phase 1/Phase 2

Figure 11.
QCI Interpret does not suggest a germline origin of *BRCA2*, p.L1357fs*8

Bibliography for BRCA2 p.L1357fs*8

Search Author, Title, Journal or PubMed ID. Use commas to separate terms.

Filter results by:
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Lab References
Curated References

12 (of 50 total references with 2297 unique variant findings) shown.

Report All | Unreport All | Sort By Date | Show Variant-Specific References Only | Add Reference | Contact Us

Reporting Status | References

Swisher EM et al. (2016) ▸ Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. *Lancet Oncol* 18(1):75-87. Epub 2016 Nov 29 (PMID: 27908594) — Actions

- Relapsed platinum-sensitive high-grade ovarian carcinoma treated with rucaparib (600 mg 2x per day) in female patient(s) (age: 54 - 77 years) with mutant **human** BRCA2 gene (unspecified DNA mutation) that were previously treated with platinum-based chemotherapy [platinum chemotherapy regimen] had objective response rate of 82% (calculated) (Phase 2) (population size: 9/11).
  - Experiment Type: response evaluation criteria in solid tumors version 1.1

Arora A et al. (2016) ▸ Clinicopathological and Functional Significance of RECQL1 Helicase in Sporadic Breast Cancers. *Mol Cancer Ther* 16(1):239-250. Epub 2016 Nov 11 (PMID: 27837030)

Zhang M et al. (2016) ▸ Copy number deletion of RAD50 as predictive marker of BRCAness and PARP inhibitor response in BRCA wild type ovarian cancer. *Gynecol Oncol* 141(1):57-64 (PMID: 27016230)

Figure 12.
Bibliography that can be used for manual inspection of the literature

Conclusion:

Apart from somatic mutations, NGS is capable of identifying incidental germline variants, which may influence patient management, as well as provide a rationale for timely genetic counselling. When sequencing only tumor specimens, one should either computationally predict whether a certain variant is germline, or resort to additional germline analysis.

QCI Interpret provides in-depth information on detected variants and suggests potential germline origin based on the type of the mutation, VAF, and the literature evidence. Thanks to insights provided by the software, users can further manually inspect the criteria for potential additional testing and/or genetic counselling. It is important that variant origin prediction is done on time by the computational tools so that users can manually inspect the data and direct further patient management promptly.

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