# Case Study

## CSF cell free DNA (cfDNA) and RNA NGS (Next-Generation Sequencing) Profiling in CNS Tumors

- Non-invasive CNS tumor profiling
- Comprehensive analysis of cfDNA and RNA from a CSF sample
- Monitoring response to therapy and tracking of tumor evolution/resistance with serial CSF sampling

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## Background

A 66-year-old patient presented with progressive neurologic decline, imaging revealing a diffusely infiltrative lesion in the right temporal lobe with radiographic features suspicious for high-grade glioma. Cerebrospinal fluid (CSF) liquid biopsy was performed for genomic characterization. Both cfDNA and RNA NGS were applied to detect somatic mutations, structural alterations, and viral RNA.

### Possible Germline Mutation

- FANCI heterozygous truncating variant detected at high variant allele fraction.
  - Suggestive of possible germline origin.
  - Currently classified as a variant of uncertain significance (VUS).
  - Role in glioblastoma is not defined; warrants germline confirmation and genetic counseling.

### Somatic Mutations

- TP53 (two pathogenic variants): Tumor suppressor inactivation.
- CDKN2A/B homozygous deletion (9p−): Consistent with aggressive glioblastoma biology.
- PIK3CA mutation: Activating mutation in PI3K pathway.
- ARID2 mutation: Chromatin remodeler gene, associated with DNA repair defects.
- BLM mutation: DNA helicase, limited glioblastoma-specific data.

### Other Genomic Results

- Tumor Mutation Burden: Low (6 Mut/Mb).
- IDH1/2, H3 K27M, PTEN mutations: Not detected.
- EBV, HPV, TTV viral RNA: Not detected.
- HLA Genotype:
  - HLA-A: A31:01/A31:01
  - HLA-B: B15:01/B08:01
  - HLA-C: C03:03/C07:01

### Copy Number Alterations

- EGFR amplification (7p+): Hallmark of glioblastoma, IDH-wildtype.
- MYC gain (8q+).
- Combined +7/−10 signature: Classic genomic hallmark of glioblastoma.
- CDKN2A/B deletion (9p−): Recurrent glioblastoma lesion.
- Additional imbalances: 2p+, 5p+, −10, 13q+, +20, 21q−.

## Interpretation

The integrated molecular profile is most consistent with glioblastoma, IDH-wildtype, CNS WHO Grade 4.

- PIK3CA mutation supports possible use of PI3K/mTOR inhibitors.
- ARID2 mutation suggests enhanced sensitivity to PARP inhibitors and possibly to radiation therapy.
- TP53 mutations indicate potential for investigational therapies such as eprenetapopt (APR-246), Aurora kinase A inhibitors, or Wee1 inhibitors.
- EGFR amplification supports glioblastoma biology; EGFR-targeted therapies are under investigation, though clinical efficacy remains limited.
- FANCI truncating mutation may represent a germline finding; however, its role is uncertain and should prompt confirmatory germline testing.

## Discussion

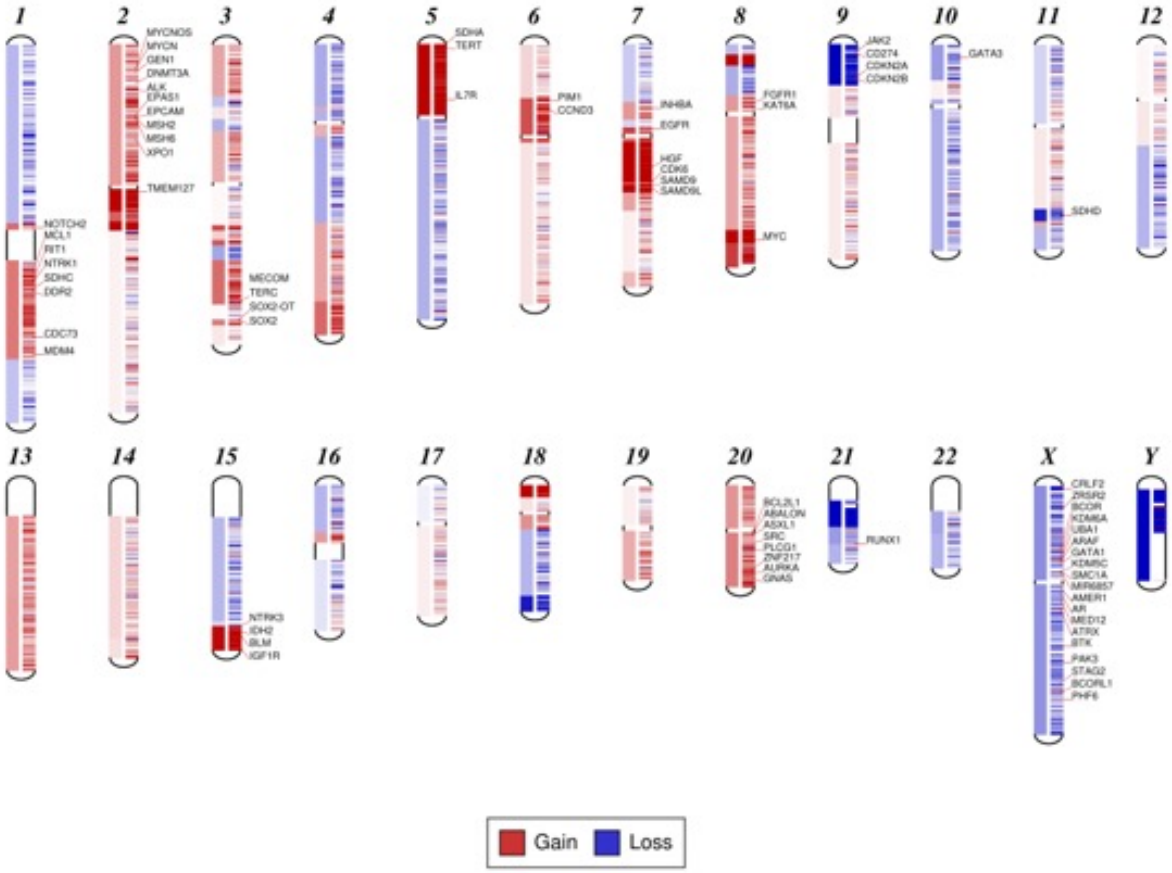
The Role of CSF cfDNA and RNA NGS

Traditional classification of CNS (Central Nervous System) tumors relies on tissue biopsy, which may be limited by anatomical inaccessibility of certain lesions and risk of complications from repeat biopsies.

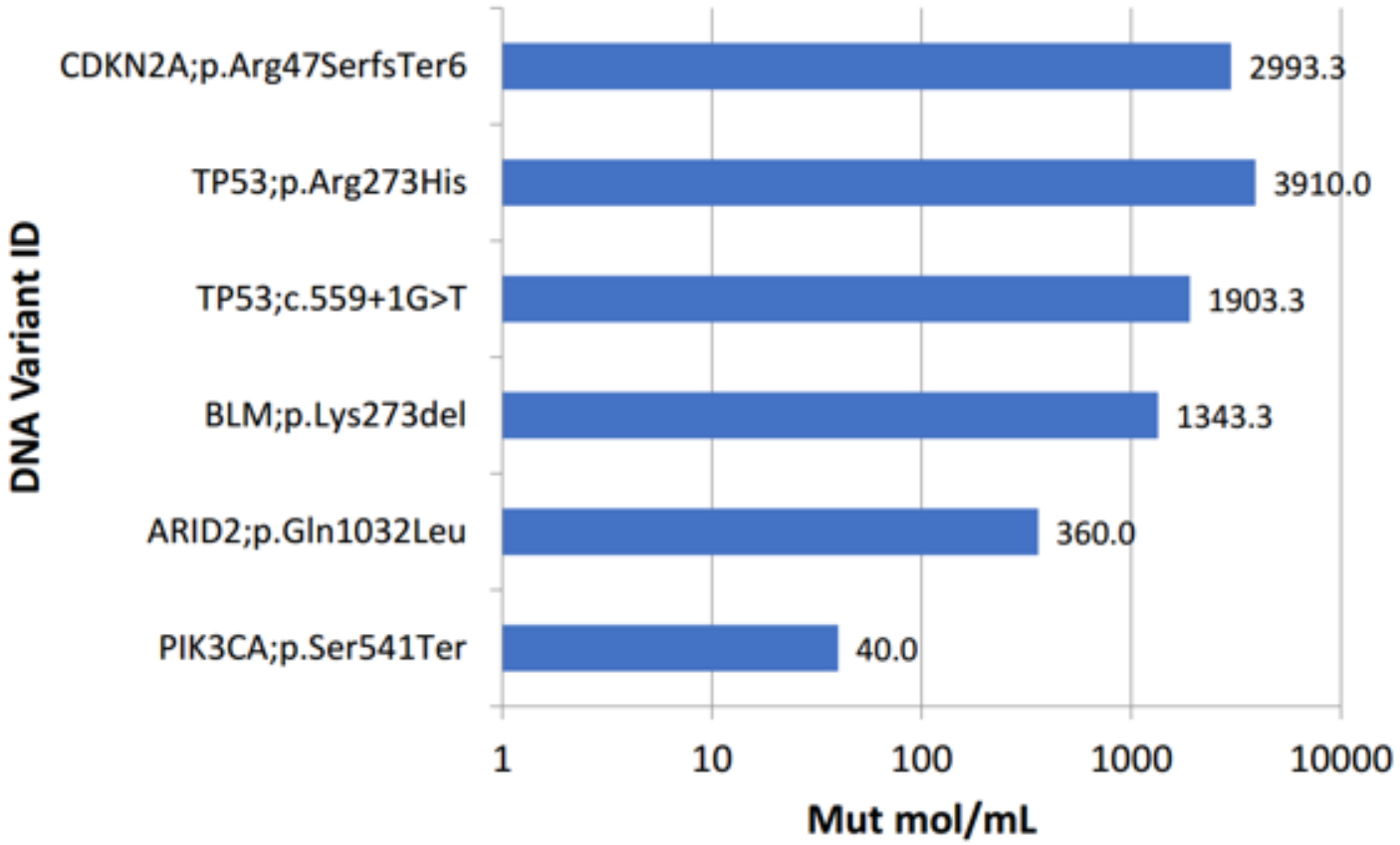
1. Non-invasive "liquid biopsy" of the CNS – captures tumor DNA/RNA shed into the cerebrospinal fluid.
2. Comprehensive profiling – allows simultaneous detection of:
   - Somatic mutations (TP53, PIK3CA, ARID2, etc.)
   - Copy number alterations (EGFR amplification, +7/−10)
3. RNA expression patterns and fusions (if present).
4. Improved sensitivity in heterogeneous tumors – avoids sampling bias from a single tissue block.
5. Diagnostic confirmation – in this case, the hallmark +7/−10 signature and EGFR amplification firmly established glioblastoma, IDH-wildtype, without requiring invasive re-biopsy.
6. Therapeutic stratification – cfDNA and RNA profiling identified PIK3CA and ARID2 mutations, opening potential targeted therapy avenues.
7. Dynamic monitoring – serial CSF sampling can track tumor evolution, therapy resistance, and recurrence in real time.

## Conclusion

This case illustrates the diagnostic and therapeutic value of CSF cfDNA and cfRNA NGS in CNS tumors. Beyond confirming glioblastoma, IDH-wildtype, the analysis uncovered actionable alterations and highlighted the possibility of germline predisposition. As molecular diagnostics evolve, CSF NGS will increasingly complement or even substitute tissue biopsy, offering a safer, more comprehensive, and dynamic method for CNS tumor classification and management.




Gene copy number and karyotyping using cfDNA in CSF




Mutation load in CSF

## Literature Context

- Miller et al., Nat Med 2019 – Demonstrated that CSF-derived cfDNA sequencing provides a more accurate representation of the glioma genome compared to plasma cfDNA, and captures mutations missed by tissue biopsy due to spatial heterogeneity.
- Pan et al., Clin Cancer Res 2019 – Showed that CSF cfDNA profiling detects EGFR, TP53, and other hallmark mutations in glioblastoma, highlighting its role in diagnosis and therapy guidance.
- Pentsova et al., Nat Commun 2016 – Pioneering study proving that CSF liquid biopsy can identify clinically relevant mutations in brain tumors, laying the foundation for its clinical utility.
- Wang et al., JCO Precis Oncol 2021 – Highlighted the utility of cfRNA in CSF for detecting gene fusions and expression profiles, further enhancing CNS tumor classification.

Genomic Testing Cooperative, LCA
25371 Commercentre Dr, Lake Forest, CA 92630
Tel: 1-949-540-9421 | Fax: 1-949-301-9719 | Toll-Free: 1-866-484-8870
Website: genomictestingcooperative.com
e-mail: gtc@genomictestingcooperative.com