# Using cell free DNA (cfDNA) and RNA (cfRNA) in the diagnosis and monitoring of primary and metastatic CNS tumors - Abstract Code: INNV-08

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## Background

In the current study, we expand our prior study and present results of sequential CSF samples from patients with suspected CNS neoplasm submitted for testing by LBx to rule out or confirm CNS tumor.

## Methods

Each sample was centrifuged to isolate the cells. From the supernatant, cell-free DNA(cfDNA) and cell-free RNA (cfRNA) were extracted. From the cell pellet, cellular RNA (cRNA) was extracted. cfDNA was sequenced by next generation sequencing (NGS) using a targeted DNA panel of 302 genes. The cfRNA and cellular RNA were sequenced independently using a targeted RNA panel of more than 1,600 genes. From January 2024 to August 2025, 1397 consecutiv samples were analyzed, 6 of which yielded inadequate cfDNA or cfRNA for analysis and excluded from further analysis. ( the negative cases **35% were submitted with various diagnos related to CNS including malignant neoplasm of unspecified brain or secondary neoplasm of central nervous system. 15% of negative cases were submitted with a diagnosis of lung cancer, 6% with a diagnosis of breast cancer and 4% with lymphoma.**

## Results

Characteristics of the tested samples and the detected abnormalities.

| Clinical Diagnosis | Number of samples | Number of detected SNV |
|---|---|---|
| ALL | 14 (1.3%) | 14 |
| AML | 10 (0.9%) | 22 |
| Brain | 325 (30.2%) | 1885 |
| Breast | 138 (12.8%) | 965 |
| Carcinoma | 62 (5.8%) | 225 |
| Colorectal | 3 (0.3%) | 13 |
| Endometrial/Ovarian | 9 (0.8%) | 72 |
| Lung | 138 (12.8%) | 715 |
| Lymphoid | 80 (7.4%) | 447 |
| Melanoma | 6 (0.6%) | 74 |
| Neuroendocrine | 7 (0.7%) | 33 |
| Sarcoma | 5 (0.5%) | 25 |
| Other | 278 (25.9%) | 539 |
| Total | 1075 | 5029 |

| Detected Abnormalities | Number | Percentage |
|---|---|---|
| Total samples | 1391 | 100.0% |
| Negative for SNV, CNV and clonality | 231 | 16.6% |
| Positive for CNV | 535 | 38.5% |
| Pos for CNV only | 14 | 1.0% |
| B-cell clonality | 108 | 7.8% |
| B-cell clonality only | 2 | 0.1% |
| Fusion genes | 68 | 4.9% |
| CAR-T cells | 5 | 0.4% |
| Cases with Germlines | 445 | 32.0% |
| CHIP only | 69 | 5.0% |
| 17p(TP53) deletion | 167 | 12.0% |
| TP53 Mutation+Deletion | 96 | 6.9% |
| EGFR Amplification | 30 | 2.2% |
| ERBB2 amplification | 31 | 2.2% |

Detection of B-cell clonality in CNS lymphoma

Heavy chain

Heavy chain

| CDR3 | Counts |
|---|---|
| **IGHV3-7** | **7197** |
| IGHV3-62 | 54 |
| IGHVII-51-2 | 2 |
| IGHV3-63 | 2 |
| IGHV3-65 | 1 |
| IGHVII-65-1 | 1 |

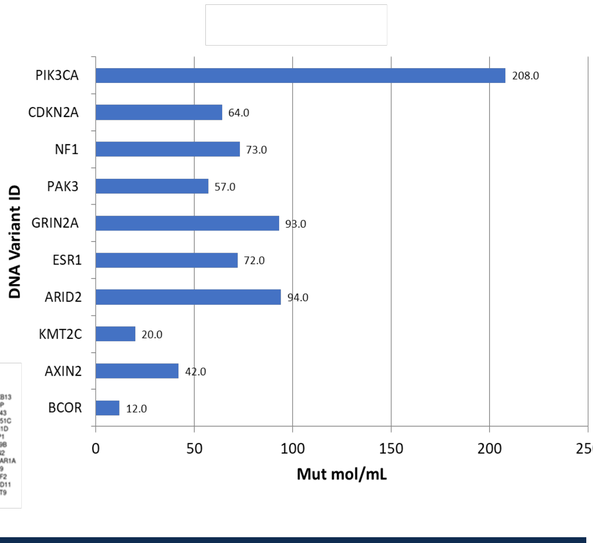
Kappa

| CDR3 | Counts |
|---|---|
| **IGKV1-8** | **1040** |
| IGKV2-26 | 143 |
| IGKV1-12 | 1 |
| IGKV7-3 | 1 |
| IGKV1D-16 | 1 |
| IGKV3-31 | 1 |

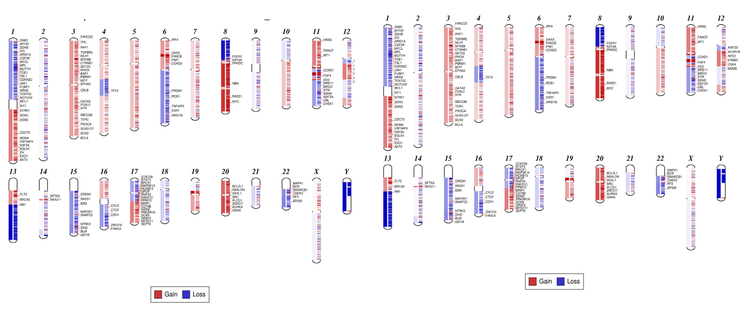
Tumor Load Vs Variant Allele Frequency

The bars represent the tumor load (number of mutant molecules in 1 mL of plasma). The VAF data suggests that the ESR1 mutation is in a subclone of that with the PIK3CA, CDKN2A and NF1. The tumor load is more representative and shows that one clone has PIK3CA, CDKN2A, NF1 and ESR1 mutations. The difference in VAF is due chromosomal copy number variation.

| Gene | VAF % | Chr |
|---|---|---|
| PIK3CA | 59.26 | 3 |
| CDKN2A | 48.12 | 9 |
| NF1 | 41.95 | 17 |
| PAK3 | 32.02 | X |
| GRIN2A | 29.81 | 16 |
| ESR1 | 24.41 | 6 |
| ARID2 | 22.93 | 12 |
| KMT2C | 12.9 | 7 |
| AXIN2 | 11.02 | 17 |
| BCOR | 4.84 | X |



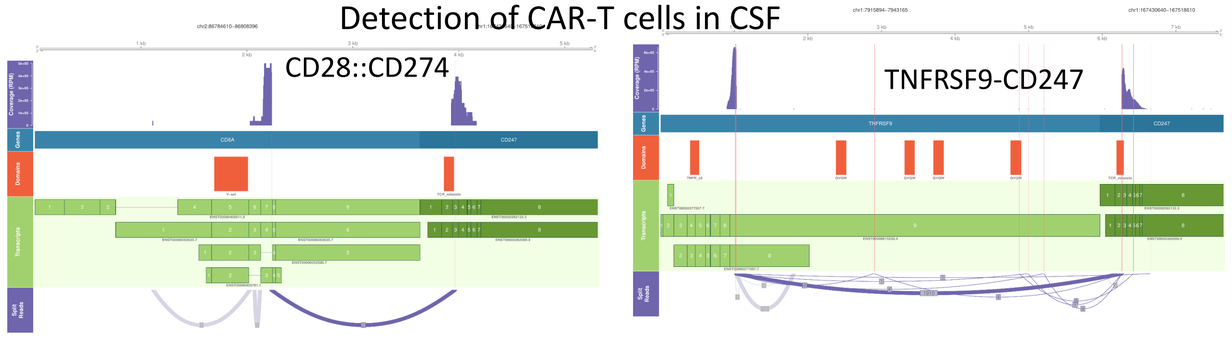

example of breast cancer with only chromosomal abnormalities and a second sample from a patient with EGFR gene amplification. Both had no detectable mutations




Most common mutations detected in various neoplasms

| | | |
|---|---|---|
| ALL | CEBPA | 14.3% |
| AML | NPM1 | 20.0% |
| Brain | TP53 | 16.9% |
| Breast | TP53 | 37.0% |
| Carcinoma | TP53 | 22.6% |
| CRC | APC | 33.3% |
| Endommetrial/Ovarian | TP53 | 55.6% |
| Lung | TP53 | 29.7% |
| Lymphoid | KMT2C | 18.8% |
| Melanoma | BRAF | 50.0% |
| Neoroendocrine | KIT | 28.6% |
| Sarcoma | CBL | 40.0% |
| Other | TP53 | 22.7% |

Detection of CAR-T cells in CSF




## Conclusions

**-Liquid biopsy of CSF is highly reliable in the diagnosis and monitoring of primary and Metastatic tumors.**

**-Clonality (B and T-cell) and chromosomal abnormalities should be a part of CSF evaluation**

**-RNA evaluation is required for detecting chromosomal translocations**

**-RNA can be used in evaluating ICAN Vs tumor in CSF.**

**-Quantifying the tumor load in CSF provides important information that are different from VAF.**