Supplementary Methods

Detection of somatic mutations

First, all bases were subjected to Phred quality filtering using a threshold Q of 30, and only positions where total depths were above 500× were considered for variant identification. The error suppression method using unique molecular identifiers was carried out to select highly confident reads supporting a non-reference allele as described in a previous study [1]. Non-reference alleles present at a frequency greater than 1% in the matched germline DNA were removed. Otherwise, non-reference alleles were subjected to the binomial test to determine if a non-reference allele was significantly more abundant in plasma DNA than the matched germline DNA (Bonferroni adjusted p-value < 0.01). To minimize false-positives due to cross-contamination among multiplexed samples, we also excluded non-reference alleles if they were found as germline single nucleotide polymorphisms in other samples processed in a capture reaction or the same lane of a sequencing flow cell. Variant candidates with a high strand bias (90% if supporting reads ≥ 20; Fisher exact test, p-value < 0.1 if supporting reads < 20) were removed. Next, we performed a Z-test to identify variants that were present at a significantly higher frequency than the corresponding background errors in the normal samples (Bonferroni adjusted p-value < 0.05). We further applied the following threshold: allele frequency ≥ 0.5% and alternative allele count ≥ 20. For biopsy specimens, single nucleotide variant profiling was performed with different thresholds: total depth ≥ 100×, allele frequency ≥ 2%, and alternative allele count ≥ 10. Indels were called in all samples with Somatic Strelka2 and MANTA using default parameters [2,3]. Variants that passed the filter in Strelka2 were further considered.

References

1. Grommes C, DeAngelis LM. Primary CNS lymphoma. J Clin Oncol. 2017;35:2410-8.
2. Thiel E, Korfel A, Martus P, Kanz L, Griesinger F, Rauch M, et al. High-dose methotrexate with or without whole brain radiotherapy for primary CNS lymphoma (G-PCNSL-SG-1): a phase 3, randomised, non-inferiority trial. Lancet Oncol. 2010;11:1036-47.
3. Hoang-Xuan K, Bessell E, Bromberg J, Hottinger AF, Preusser M, Ruda R, et al. Diagnosis and treatment of primary CNS lymphoma in immunocompetent patients: guidelines from the European Association for Neuro-Oncology. Lancet Oncol. 2015;16:e322-32.