sensitivity for very early recurrence, necessitating an urgent need for identifying new, robust biomarkers for the early diagnosis, prognosis, and clinical management of disease progression in EPN. We hypothesise that microRNA (miRNA) quantification in the cerebrospinal fluid (CSF) will have clinical utility in the early detection of MRD in these patients.

**PATIENTS/METHODS:** A discovery cohort of EPN CSF samples (n=12) from patients enrolled on the SIOP Ependymoma II clinical trial (trials.gov identifier: NCT02265770) and control leukaemia CSF samples (n=8) underwent RNA-sequencing (QIAGEN) after total RNA extraction using QIAGEN miRNAeasy Serum/Plasma kit. The EPN cohort consisted of infratentorial (IT; n=9), supratentorial (ST; n=1) and spinal (SP; n=2) cases taken prior to, or at, resection (primary/relapse).

**RESULTS:** Raw sequencing data entered a quality-control pipeline (Tally/Cutadapt), followed by alignment to miRBase-v22 (https://mirbase.org/) using Chimira (PMID:26093149). Two outlying (infratentorial) EPN samples were removed, leaving 10 (IT+ST+SP) versus 8 comparison. Extracted raw counts were normalised across samples using DESeq2 (PMID:25516281) and 87 differentially-expressed miRNAs identified (adj-p<0.05). EPN samples completely segregated from controls on clustering/heatmap analysis. From these 87 miRNAs, a core set of 53 were overlapping in comparisons removing spinal (IT+ST remaining) and supratentorial (IT alone) samples. These miRNAs underwent combined ranking by adjusted-p and abundance (normalised count) values, and top-ranking candidates taken forward for confirmation using highly-sensitive Taqman PCR, prior to validation in additional samples.

**CONCLUSION:** Circulating miRNA quantification offers promise for EPN diagnosis and MRD detection. Further CSF study in larger patient cohorts, and serum/plasma analysis, are now warranted.