Supplementary methods:

CNS9204, BBSFOP and CNS9904 trial overview:

The CNS9204 trial was designed for intracranial ependymomas in children aged up to three years (6). Treatment comprised of maximal surgical resection, followed by four courses of alternating myelosuppressive (carboplatin and cyclophosphamide) and non-myelosuppressive (vincristine, cisplatin and high dose methotrexate) chemotherapy given at 14 day intervals, which was repeated for seven cycles. Fractionated radiotherapy (50 Gray (Gy) to primary tumor in non-metastatic cases, 25 – 35 Gy with 20 Gy primary tumor boost in metastatic cases) was only given if recurrence had been identified on surveillance neuro-imaging.

Children in the BBSFOP trial were aged below five years (7). As with the 9204 trial, treatment consisted of maximal tumor resection, followed by three cycles of chemotherapy (A: carboplatin/procarbazine, B: etoposide/cisplatin, C: vincristine/cyclophosphamide). Cycles were delivered every 21 days for an intended duration of 16 to 18 months. Fractionated involved-field radiotherapy (50 Gy) was again reserved as salvage therapy for disease progression/relapse.

The CNS9904 trial was designed for patients with intracranial ependymomas aged between 3 – 21 years. In contrast to the other trials, post-operative radiotherapy (54 Gy) was administered to the primary tumor site in all patients. In cases of incomplete resection, four cycles of chemotherapy (vincristine, etoposide, and cyclophosphamide) preceded radiotherapy.

500K SNP array data analysis:

SNP array assays were performed at the Hartwell Centre for Bioinformatics and Biotechnology, St Jude Children’s Research Hospital, USA and the Almac
Biotechnology Centre, Northern Ireland, UK. Affymetrix® .cel files containing fluorescent signal intensity values were created using Affymetrix® Genotyping Analysis Software (GTYPE 4.1). Subsequent genotype calls were generated in .chp files using the BRLMM (Bayesian Robust Linear Model with Mahalanobis) algorithm (32). The mean SNP call rate across all arrays was > 95 %. Copy number analysis was performed by importing .cel and .chp files into the CNAG (Copy Number Analyzer for Affymetrix Genechip version 2.0) algorithm (33). To enable a more precise identification of tumor-specific genomic aberrations, data for 42/48 (38 primary, 4 recurrent; 86 %) tumors was normalized against 38 patient-matched constitutional DNA samples. The six remaining unpaired tumors (14P, 17P–R1, 35P–R1 and 39P) from four patients were normalized against the pool of constitutional DNA specimens used for paired analysis, conforming with manufacturer’s recommendations (Affymetrix, CA, USA). Inferred copy number values were predicted by CNAG from tumour:reference log2 ratios for each array probe using the Hidden Markov model (HMM). Genomic deletion was defined as a HMM inferred copy number value of zero or one, gain as an value of three or four, and amplification by a value of five or six. Normalized copy number data for each tumor from CNAG was imported alongside 500K probe annotation (Netaffx file build 07.12.07, Affymetrix, Santa Clara, CA) into the Spotfire® DecisionSite™ (version 9.0) programme for visualisation. Analysis of the X chromosome was not performed as both genders contributed to the constitutional reference pool used. The .cel files of 31 tumors (25 primary, six recurrent), but not the corresponding patient blood specimens, were incorporated into a preceding collaborative SNP array study of 204 adult and pediatric ependymomas (1). The microarray data generated during this study has been deposited in GEO with an accession number GSE32101.
Interphase fluorescence in situ hybridization – evaluation criteria and scoring system:

Signal visualization was performed using a Nikon Eclipse 90i fluorescent microscope (Nikon, Japan) (60x objective, oil immersion) with spectrum green, Texas red and DAPI filters and a Hamamatsu ORCA-ER camera (Hamamatsu, Japan). Resulting images were merged using Improvision Volocity 5.0 software (Perken Elmer, USA). Only tumor samples demonstrating sufficient FISH efficacy (> 80 % nuclei showing signals) were examined. A minimum of 100 intact, non-overlapping nuclei were counted per tumor sample, except for one case (CNS9904 – 39P) where only 62 nuclei were assessed due to the cellularity and quantity of tissue available. Sections of normal tonsil tissue were used as a reference control for each experiment. Chromosome 1q25 gain was identified as widespread or focal. Widespread gain was defined by at least 15 % of the counted nuclei across all cores of a tumor sample containing three or more signals of the 1q25 probe. Focal 1q25 gain was defined by at least 15 % of the nuclei counted within one core from a sample containing three or more probe signals. An independent investigator (BM) evaluated approximately one-third of the scorable cohort (55 tumors). Assessment by a third investigator (JW) was used to reach consensus in cases of scoring disparity.

Statistics:

Fisher’s exact test was used to determine associations between variables in two-way frequency tables. Comparison of parametric continuous variables was performed using an Independent sample t-test with 95 % confidence intervals (CIs) or a one-way analysis of variance (ANOVA) if more than two subgroups were assessed simultaneously. Post-hoc ANOVA comparisons were performed using the Tukey
HSD test and effect size calculated as an eta squared value. Non-parametric comparisons were performed using the Wilcoxon Rank sum test. Correlation of 1q gain results from the SNP array analysis with 1q25 gain findings by FISH was performed by Spearman’s Rank correlation coefficient. Comparison of FISH scoring between independent investigators was assessed using the Kappa measure of agreement.

Survival curves were generated by the Kaplan-Meier method with significance values established by the log rank test. Progression-free survival (PFS) was defined from the date of original surgery to the date of disease recurrence, progression or death. Overall survival (OS) was calculated from the date of initial surgery to the date of death from any cause. Patients remaining alive were censored at the date of last follow-up (for OS only). Median follow-up was estimated by the inverse Kaplan-Meier method (34). To assess the impact of studied factors on survival, hazard ratios were computed using Cox proportional hazard models. Cox models for 1q25 gain as determined by FISH were stratified on the three therapeutic trial cohorts, taking into consideration the different populations in terms of age and treatment. Variables with a p-value equal to or below 0.25 in univariate analysis were introduced into the corresponding multivariable model as described previously (7).

The final model for 1q25 gain was defined as the multivariable model in which all of the variables had a p-value below 0.05. Estimators were given with their 95 % confidence interval (95 % CI). Stability of the prognostic value of 1q25 gain on progression-free survival was also evaluated between cohorts receiving either primary adjuvant radiotherapy or chemotherapy. This was performed by estimating a prognostic heterogeneity term between the 1q25 result and adjuvant therapy
administered (CNS9204 and BBSFOP versus CNS9904/RT), which was added to the final multivariable model.

FISH results were not obtained for 42/189 patients across the three cohorts; the multivariable survival analysis was therefore performed on 147 patients. After confirming the stability of results from the univariate analyses of clinical factors between the whole population and the one with complete data, all the prognostic factor analyses were performed on the 147 patients.