BACKGROUND: Medulloblastoma has been redefined into four molecular subgroups (WNT, SHH, Group 3, and Group 4), with distinct prognoses and responses to treatment. The ACNS0332 trial investigated the impact of carboplatin and isotretinoin in high-risk medulloblastoma finding a differential effect of carboplatin by molecular subgroup. An interim futility analysis showed no benefit from isotretinoin, but did not evaluate results by molecular subgroup in the primary analysis. We performed a secondary analysis to examine whether the effect of isotretinoin on event-free survival (EFS) differed by molecular subgroup. METHODS: Evaluable patients with known molecular subgroups were analyzed. Molecular subgroup analysis was conducted retrospectively in 231 patients at a central laboratory by methylation array. Kaplan-Meier survival analysis compared EFS (time from enrollment to disease progression or recurrence, second malignancy, death from any cause, or to date of last follow-up for patients without events). RESULTS: Based on data from the 2014 interim analysis, 164 patients were eligible for secondary analyses. Group 3 (n=56, 34.1%) and Group 4 (n=75, 45.7%) were the most common groups. SHH (n=22, 13.4%) and WNT (n=11, 6.7%) groups were less common. There was no difference in isotretinoin (p=0.87) or carboplatin (p=0.49) randomization by subgroup. Race was well-balanced with expected differences in age (p=0.017) and sex (p=0.016) among molecular subgroups. Based on the interim analysis, there was no evidence of a difference in EFS by isotretinoin randomization (p=0.34; one-sided log-rank test stratified by carboplatin randomization). Based on secondary analysis data, there was no difference in EFS between patients receiving isotretinoin or no additional therapy in any molecular subgroup: Group 3 (p=0.68), Group 4 (p=0.55), SHH (p=0.68), WNT (p=1) based on two-sided log-rank tests. CONCLUSIONS: Despite differential efficacy of carboplatin among molecular subgroups, no difference was found in EFS between patients receiving isotretinoin and those who did not, regardless of molecular subgroup.