BACKGROUND: Pediatric patients with recurrent/relapsed diffuse intrinsic pontine glioma (DIPG), glioblastoma, or high-grade glioma (HGG) have a dismal prognosis. The present study investigated the impacts of 1) the absolute count of Wilms tumor 1 (WT1)-specific cytotoxic T cells (CTLs) at the initiation of WT-1 specific peptide vaccine (Ombipepimut-S) consisting of a killer peptide—adegramoside, a helper peptide—nelatimotide, and adjuvant Montanide™ ISA 51 VG and of 2) cumulative steroid dose on patients’ survival. We aimed to explore the optimal timing for initiating this cancer immunotherapy for them. METHODS: The absolute counts of WT1-specific CTLs and lymphocytes were examined at the initiation of vaccination. This first-in-child phase 1/2 study investigated survival (overall survival [OS] and progression-free survival [PFS]), as well as cumulative steroid dose until day 28 after vaccination initiation and immune responses according to the Kaplan-Meier method and receiver-operating curve (ROC) analysis, respectively. RESULTS: 18 patients (median age: 8.9 years [range: 2.8-18.7], 11 (61.1%), 5 (27.8%), and 2 (11.1%) had DIPG, glioblastoma, and HGG, respectively) were intradermally injected with ombipepimut-S. WT1-specific immune responders accounted for 70.6% of patients. Median OS was significantly longer (P = 0.024) in WT1-specific immune responders (9.9 months [6.0-31.4]) than in nonresponders (4.9 months [2.2-16.5]). Under a low cumulative steroid dose (< 0.66 mg/kg body weight), ombipepimut-S significantly extended both OS (P=0.0015) and PFS (P=0.007). Immune responders tended to show the higher absolute counts of WT1-specific CTLs (P=0.091) and lymphocytes (P=0.087) at the initiation of vaccination. CONCLUSIONS: Ombipepimut-S exhibited antitumor activity, and this cancer immunotherapy appeared to be preferably initiated when the patient’s immunity is relatively preserved immediately after the completion of standard therapy for primary disease or before the tumor recurs or relapses.