BACKGROUND: The immune microenvironment of pediatric gliomas is relatively understudied despite being a leading cause of childhood mortality. Pilocytic astrocytomas (PA) are the most common pediatric glioma and surgical resection is often curative. However, PAs are not always amenable to surgery and can have significant long-term morbidity and mortality. The ten-year progression-free survival for PA patients with significant residual tumor is less than 50% and other non-surgical treatment options are frequently necessary. METHODS: To identify PA patients that may be responsive to immune checkpoint inhibitors and to guide prioritization of available immunotherapeutic treatments, orthogonal immune profiling strategies were used to identify patients that may benefit from the next generation of immune therapeutics. Because the KIAA1549-BRAF fusion is expressed in 70% of PA, we prospectively collected patients to comprehensively characterize the unique immune biology of these tumors using nanostring profiling, scRNASeq, and SeqIF™ multiplex staining. RESULTS: The tumor microenvironment of BRAF-fusion PA was notable for antigen presentation and the interaction of CD11c+ cells with T cells through Lck+ immunological synapses. PA CD11c+CD206+ dendritic cells localized to vessel walls and were positive for TIM3 expression, an immune checkpoint marker. A unique highly activated microglia subcluster was identified that morphologically resembled T cells expressing TNF and TIM3. Our results are consistent with others that suggest potential benefit in targeting tumors with elevated MAPK activity. Finally, the expression of other immune checkpoints such as Lag-3, PD-1, and TIGIT are minimally co-expressed, suggesting that T cells are not in a state of exhaustion, as seen in adult high-grade gliomas. CONCLUSIONS: Analysis of BRAF-fusion PA identified a unique highly activated P2RY12+ microglia population dispersed throughout the tumor microenvironment, active antigen presentation between a CD11c+ antigen presenting cells and T cells, and a unique therapeutic opportunity for targeting TIM3.