Paediatric high-grade gliomas (pHGG), particularly Diffuse Midline Glioma (DMG), present a formidable challenge in oncology. Due to the aggressive biology and location of pHGGs the implementation of adult treatment modalities (surgery, chemotherapy, and radiotherapy) is severely limited resulting in pHGG remaining a terminal disease. Thus, there is a pressing and urgent unmet clinical need for the development of innovative therapeutics. We will demonstrate the identification of cell surface targets using mass spectrometry proteomics. We have developed novel binders and engineered them into second-generation CAR T cells. We will present pre-clinical in vivo intracranial mouse models examining the efficacy of novel CAR T cell therapies. We have recently reported effective CART cell targeting of HER2 in DMG, and presently we will also demonstrate superior efficacy of additional CAR T cell targeting, both in vitro and in vivo in pre-clinical PDX models of DMG. We will present new data that CAR T cells targeting EphA3 show curative efficacy in pre-clinical models of DMG. Recognising that single targeting CAR T cells are unlikely to provide long-term clinical benefit, we will also present our combination approaches using CAR T cells targeting antigens such as GD2, EGFR, HER2, EphA3, CD276 and CD63. We are expanding our research to other CAR T combination strategies focusing on immune-modulating drugs such as checkpoint inhibitors or co-stimulatory agonists. We are committed to advancing CAR T cell therapies for pHGG patients and their families with an aim to aid in the development of much-needed new therapeutic options.