Background: Atypical teratoid rhabdoid tumor (ATRT) is a highly aggressive pediatric brain tumor driven by *SMARCB1* loss, a key component of the chromatin remodeling complex, with limited therapeutic options and a median survival of less than 2 years. CD44, a cell-surface adhesion receptor involved in cell–cell interactions and migration, undergoes alternative splicing to generate isoforms with distinct oncogenic potential. The CD44v6 isoform—containing exon v6—facilitates receptor tyrosine kinase signaling and immune evasion. Preliminary transcriptomic data suggests that CD44v6 splicing is involved in the aggressive nature of several cancers, including gliomas, colorectal, and head-and-neck cancers but remains uncharacterized in ATRTs.

Methods: A surface proteomic screen was performed using the Human Cell Surface Marker Screening Panel (BioLegend, 271 antibodies) on five ATRT cell lines (BT12, BT16, CHLA-05, CHLA-06, MAF794) that were SHH or MYC-classified. Cells were stained per manufacturer protocols and analyzed via flow cytometry to identify differentially expressed antigens relative to isotype controls. For molecular validation, total RNA was extracted from the same lines, followed by cDNA synthesis and RT-PCR using primers spanning the *CD44v6* exon junction. Amplicons were resolved on agarose gels and sequenced to confirm variant inclusion. Protein expression was also quantified by flow cytometry using a CD44v6-specific monoclonal antibody (clone VFF-18) and compared with total CD44 staining. To assess in-vivo relevance, RNA-seq data from 13 primary ATRT tumors were analyzed using Integrative Genomics Viewer (IGV). Sashimi plots were generated to visualize exon-level splicing across the *CD44* locus, focusing on junction reads spanning exons v5–v7.

Results: The 271-antibody screen identified CD44v6 as a consistently enriched surface antigen across the five cell lines. Hierarchical clustering revealed CD44v6 among the top-ranked markers. RT-PCR confirmed *CD44v6* exon inclusion in all lines, with transcript levels highest in CHLA-05 and CHLA-06 and lower but detectable expression in BT12, BT16, and MAF794 compared with negligible expression in normal brain. Flow cytometry validated concordant surface protein expression, demonstrating a rightward fluorescence shift in all lines compared to normal brain tissue. RNA-seq sashimi plots from 13 primary ATRTs showed distinct exon-spanning junctions confirming *CD44v6* inclusion, particularly in MYC subgroups.

Conclusions:
CD44v6 is recurrently expressed in ATRT cell lines and patient tumors through alternative exon inclusion, defining a distinct and targetable surface phenotype. Its restricted expression in normal brain and functional relevance support further investigation of

CD44v6-directed antibody or CAR-macrophage immunotherapies to enhance tumor recognition and overcome immune evasion in ATRT.