Background High-risk neuroblastoma (NB) with amplified MYCN oncogene is a developmental aggressive disease in children of age less than 5 years. NB patients are generally given intensive treatments in consecutive phases involving multi-agent chemotherapy, surgery, radiation therapy and immunotherapy. Over recent years, immunotherapy using checkpoint inhibitors has shaped new combinatorial approaches in pediatric oncology, however, they have shown only minimal clinical responses in pediatric clinical trials. About 50% of high-risk NB patients either have refractory disease or relapse within 3 years of diagnosis with very poor treatment outcomes (Kriessman SG et al., 2013). Therefore to attain durable remissions in NB patients from novel combination therapies using checkpoint inhibitors, a deeper understanding of their mechanisms of synergy upon tumor cell encounter is needed. Here, we sought to understand how the efficacy of a-PD1 checkpoint therapy is modulated by NB tumor cells and their suppressive/escape mechanisms to achieve immune resistance.

Materials and Methods In this study, we employed CRISPR-Cas9 genome-wide screening integrated with a tumor-immune co-culture system (TICS) to allow interaction of immune interactions in the presence (or) absence of PD-1 blocking antibody nivolumab. For this, a MYCN-amplified NB cell line pre-transfected with a library of guide RNAs was used to assess the efficacy of immune activation. Next-Generation Sequencing (NGS) analysis was performed on NB cells that survived/resisted immune-mediated killing. Further selective hits were validated in in vitro assays and in vivo studies using syngeneic tumor models to assess any significant improvements in anti-tumor immunity.

Results Analysis of our NGS data from CRISPR-TICS screens revealed significant functional genes that modulate critical pathways that confer resistance and sensitivity to a-PD1 therapy. The data also unveils the immunosuppressive cues in the tumor microenvironment (TME) that can be reversed to enhance immune cell recruitment and induction of immunogenic cell death. Moreover, our approach provides a platform technology for robust discovery and validation of genes and associated pathways by interrogating the immune interaction with a pool of NB tumor cells knocked-out for 20,000 different genes all at once.

Conclusions Hence validation of target hits from our CRISPR screens, including novel epigenetic regulators and transcription pathway modulators, could serve as a potential alternative to develop targeted therapies that synergise with checkpoint blockade therapy to improve overall anti-tumor response rates in NB patients.