INTEGRATIVE ANALYSIS OF NEUROBLASTOMA BY SYNERGISTIC ANTITUMOR ACTIVITY OF PAN-PI3K A2

Background
Children with high-risk neuroblastoma have poor survival rates and urgently need more effective treatments with less side effects. Novel and improved immunotherapies may fill this need. However, despite their success in various adult cancers, CAR-T cells and immune checkpoint blockade show limited clinical efficacy in neuroblastoma. We aimed to provide a comprehensive overview of neuroblastoma’s immune environment and relevant immunoregulatory interactions, to identify strategies for improving immunotherapy efficacy.

Materials and Methods
25 tumor samples from 20 patients (17 with high-risk disease, 6 with MYCN amplification), were enzymatically digested, single-cell FACS sorted and sequenced by Cel-Seq2 protocol. In vitro killing assays were performed with luciferase-transduced patient-derived neuroblastoma organoids and adult healthy donor PBMCs. Checkpoint inhibition was tested in vivo in three syngeneic neuroblastoma models (Neuro2a, N1E-115, N18) and one chemotherapy-resistant syngeneic T cell model. We constructed an unsupervised interaction network. This analysis predicted an abundance of immunoregulatory interactions in the tumor microenvironment affecting T/NK cell function, which included, amongst others, CLEC2D-KLRB1, PD1-PDL1 and NECTIN2-TIGIT. Since also in T cells the TIGIT/CD226 balance proved disturbed, we tested combined TIGIT/PD-L1 blockade in vitro, which significantly increased killing of patient-derived neuroblastoma organoids. Moreover, TIGIT/PD-L1 blockade in vivo in three syngeneic models induced complete remissions in a subset of animals and significantly improved survival. Lastly, addition of TIGIT blockade to the standard backbone treatment for relapse/refractory neuroblastoma patients significantly improved survival in a chemotherapymodified T2-resistant model mimicking relapse/refractory tumors.

Conclusions
We provided a comprehensive atlas of neuroblastoma’s immune environment and identified TIGIT as a promising target for (combination) immunotherapy in neuroblastoma.

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SYNERGISTIC ANTITUMOR ACTIVITY OF PAN-P13K INHIBITION AND IMMUNE CHECKPOINT BLOCKADE IN BLADDER CANCER

Background
Immune checkpoint blockade (ICB) induces durable response in approximately 20% of advanced bladder urothelial cancer (aUC) patients. Over 50% of aUCs harbor genomic alterations along the phosphoinositide 3-kinase (P13K) pathway. The goal of this project was to determine the synergistic effects and mechanisms of action of P13K inhibition and ICB combination in aUC.

Materials and Methods
Alterations affecting the P13K pathway were examined in The Cancer Genome Atlas (TCGA) and the Cancer Dependency Map databases. Human and mouse cells with PTEN deletion were used for in vitro studies. C57BL/6 mice carrying syngeneic tumors were used to determine in vivo activity, mechanisms of action and secondary resistance of pan-P13K inhibition, ICB and combination.

Results
Alterations along the P13K pathway occurred in 57% of aUCs in TCGA. CRISPR knockout of PIK3CA induced