P08.09 GENOME-WIDE CRISPR/CAS9 SCREENING FOR IDENTIFICATION OF TUMOR-INTRINSIC MECHANISMS OF RESISTANCE TO ANTI-PD1 THERAPY IN HIGH-RISK NEUROBLASTOMA

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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 3years 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.

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P08.10 COMBINING STING-AGONIST WITH RADIOTHERAPY REMODELS TUMOR IMMUNE MICROENVIRONMENT IN HUMAN MALIGNANT PLEURAL EFFUSIONS

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Background Radiotherapy (RT) serves as a major backbone for different standard therapies for various solid malignancies. It has proven to be a double-edged sword unpredictively evoking immunogenic or immunosuppressive changes in the tumor immune microenvironment (TIME). The combination of RT with stimulator of interferon genes (STING) agonists provides the prospect of robustly remodeling the local TIME, thereby enhancing the pro-inflammatory cytokine production, as well as altering the phenotypes of the local inhabitants. In pre-clinical mouse models, it has shown to be superior to RT monotherapy. However, with a void in human test systems modeling the TIME there is little pre-clinical evidence that this is the case in the human organism.

Material and methods We developed a novel human *ex vivo* culture platform derived from malignant pleural effusions (MPE) collected from patients (n=9) with metastasized solid tumors (breast, pancreatic, gastric, ovarian and colon cancer). Upon concomitant treatment with a STING agonist (10 µM ADUS-100) and RT (1×8 Gy), changes in T cell activation and secretory cytokines were measured following 24 h of incubation. T cell activation and degranulation was determined by measuring CD69 and CD107a expression using flow cytometry (FCM). Secreted immunomodulatory cytokines (INF-α, INF-β, INF-γ, IL-6, IP-10, RANTES, TNF-α, MIP1-α, MIP-1β, MCP-1, IL-8, IL-10, IL-1rα) were measured with the Human LUMINEX Discovery Assay.

Results Treatment with RT alone lacked the ability to induce immunostimulatory changes and caused a twofold increase in death of cytotoxic (CD8+) and helper (CD4+) T cells in cultures derived from MPE. Combining RT with a STING agonist however significantly induced the activation (CD69+) and degranulation (CD107a+) of cytotoxic and helper T cells, at least doubling the respective populations. The secretion of pro-inflammatory cytokines such as INF-α, INF-β, INF-γ, IL-6, IP-10 and TNF-α was also markedly enhanced upon combinatory treatment.

Conclusion We established a novel human *ex vivo* system to monitor therapeutically induced changes in the local TIME of MPE. The STING agonist robustly remodels the TIME into a pro-inflammatory state inducing a local destructive inflammatory stimulus and chemoattractant properties. In prospect of harnessing the cytotoxic power of RT, as well as immunostimulatory effects of the STING pathway, we propose this combination as a potential therapeutic regimen for solid malignancies.

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