Plenary symposium 6: precision medicine meets immunotherapy (immuno-monitoring)

06.03 INTEGRATIVE ANALYSIS OF NEUROBLASTOMA BY SINGLE-CELL RNA SEQUENCING IDENTIFIES THE NECTIN2-TIGIT AXIS AS A TARGET FOR IMMUNOTHERAPY

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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 collected pre-treatment (n=10) or during resection surgery after induction chemotherapy (n=15). Samples 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 neuroblastomamodels (Neuro2a, N1E-115, N18) and one chemotherapy-resistant syngeneic model *(Th-ALK*F1174L*/MYCN* 129/SvJ).

Results Neuroblastomas were infiltrated by various immune cells, including dendritic cells, monocytes and four populations of macrophages. The latter showed an M2-like differentiation, associated with immunosuppressive and pro-tumorigenic features. Lymphoid cells consisted of NK, B, and different populations of T cells including highly suppressive Tregs. Of the two identified $CD4^+$ non-Treg clusters, one cluster likely contained tumor-reactive cells and was significantly enriched for genes associated with T cell dysfunction in tumors, such as *TIGIT* and *CTLA4*. $CD8^+$ T had significantly increased *LAG3* and *PDCD1* (PD-1) expression, also associated with T cell dysfunction. Overall, T cells showed increased signs of dysfunction/exhaustion particularly post-chemotherapy, with enhanced expression of immune checkpoint receptors. NK cells had impaired cytotoxic function (*GZMB*, *PRF1*, *GNLY*), particularly in pre-treatment tumors, which correlated with TGF-β1 signaling and a disbalance between inhibitory receptor genes *TIGIT* and *CD96* and activating receptor *CD226*. To identify functionally relevant targets for reinvigorating T/NK cell function, 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*, whichsignificantly 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 chemotherapy-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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06.04 SYNERGISTIC ANTITUMOR ACTIVITY OF PAN-PI3K INHIBITION AND IMMUNE CHECKPOINT BLOCKADE IN BLADDER CANCER

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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 (PI3K) pathway. The goal of this project was to determine the synergistic effects and mechanisms of action of PI3K inhibition and ICB combination in aUC.

Materials and Methods Alterations affecting the PI3K 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-PI3K inhibition, ICB and combination.

Results Alterations along the PI3K pathway occurred in 57% of aUCs in TCGA. CRISPR knockout of *PIK3CA* induced

pronounced inhibition of cell proliferation (p = 0.0046). PI3K inhibition suppressed cancer cell growth, migration and colony formation *in vitro*. Pan-PI3K inhibition, anti-programmed death 1 (PD1) therapy and combination improved the overall survival (OS) of syngeneic mice with PTEN-deleted tumors from 27 days of the control to 48, 37 and 65 days, respectively. In mice with tumors not containing a PI3K pathway alteration, OS was prolonged by the combination, but not single treatments. Pan-PI3K inhibition significantly upregulated CD80, CD86, MHC-I and MHC-II in dendritic cells, and downregulated the transforming growth factor beta pathway with a false discovery rate (FDR)-adjusted q-value of 0.001. Interferon alpha response was significantly upregulated with anti-PD1 therapy (q value: < 0.001) and combination (q value: 0.027). Compared to the control, combination treatment increased $CD8^+$ T cell infiltration (p = 0.005), decreased T_{reg} cell infiltration (p = 0.036), and upregulated the expression of multiple immunostimulatory cytokines and Granzyme B (p< 0.01). Secondary resistance was associated with upregulation of the mammalian target of rapamycin (mTOR) pathway and multiple *Sprr* family genes.

Conclusions The combination Pan-PI3K inhibition and ICB has significant anti-tumor effects in aUC with or without activated PI3K pathway and warrant further clinical investigation. This combination creates an immunostimulatory tumor milieu. Secondary resistance is associated with upregulation of the mTOR pathway and *Sprr* family genes. Base on this study, a Phase II clinical trial has been designed.

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Plenary symposium 8: 'lost in translation'

08.03 MHJC-BASED LARGE-SCALE SCREENING OF ANTI-TUMOR T CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA REVEALS $CD8^+$ T CELLS WITH SPECIFICITY AGAINST THE CLONOTYPIC B-CELL RECEPTOR IMMUNOGLOBULIN

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Background Chronic lymphocytic leukemia (CLL) remains incurable, indicating a need for novel strategies towards disease eradication, including reinvigoration of anti-tumor immune responses. T cells in CLL appear selected by restricted antigens, with recent evidence suggesting that the selecting epitopes may lie within the clonotypic B-cell receptor immunoglobulins (BcR IGs). Here, we present a large-scale evaluation of T cell recognition towards BcR IGs. We predicted MHC-I binding peptides from such clonotypic regions and determined the presence of T cell recognition towards such sequences, using DNA-barcoded multimers of peptide-major histocompatibility complexes (MHC).

Materials and Methods We evaluated 653 peptides derived from the clonotypic BcR IGs of 25 CLL patients across 13 MHC-I alleles based on the MHC-I typing of the patient. We constructed patient-specific peptide-MHC dextran multimers labeled with a unique DNA barcode and a fluorochrome. MHC-multimer binding T cells from PBMC samples where sorted and evaluated through amplification and sequencing of the MHC-attached DNA barcode, to determine the presence of neoepitope reactive T cells.

Results and Conclusion Across the 25 patients we observe T cell reactivity towards 3 peptide-MHC specificities, among the 653 evaluated. The T cell responses observed are listed below:

Peptide sequence	MHC-I allele association	Peptide-associated region in somatically hypermutated clonotypic BcR IG	Somatic hypermutation (SHM) position
VTVADTAVYY	A03*01	IGHV4-34 FR3	A to V at position 96
INLNPSLKRR	A03*01	IGHV4-39 FR2-FR3	T to I at position 65, Y to L at position 67, S to R at position 74
YSFTSYWINW	A24*02	IGHV5-10-1 CDR1-FR2	S to N at position 40

These response where further validated using conventionally fluorescence labelled pMHC tetramers. This demonstrates that cancer-specific somatic mutation in the BcR IG can be targets of T cell recognition of CLL, and hence serve as targets for novel immunotherapeutic strategies. The level of such T cell recognition was sparse in the cohort evaluated, but could potential be boosted with immunotherapy.

The data to be presented, was in-part presented at the European Hematology Association (EHA) annual meeting.

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Plenary session 9: young researcher session

09.01 ARMORING ANTI-HER2 CAR-T CELLS WITH C-C-MOTIVE RECEPTOR 8 (CCR8) AND A DOMINANT NEGATIVE TGF-B RECEPTOR (DNR) TO ENABLE EFFICACY IN SOLID TUMOR MODELS

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Background Chimeric antigen receptor (CAR) T cells have shown great efficacy in treating hematological malignancies. Nonetheless, in solid tumors CAR T cells have yet to demonstrate significant clinical efficacy. In solid tumors, CAR T cells are frequently prevented access to tumor tissue and face profound suppression at the tumor site. To overcome this issue,