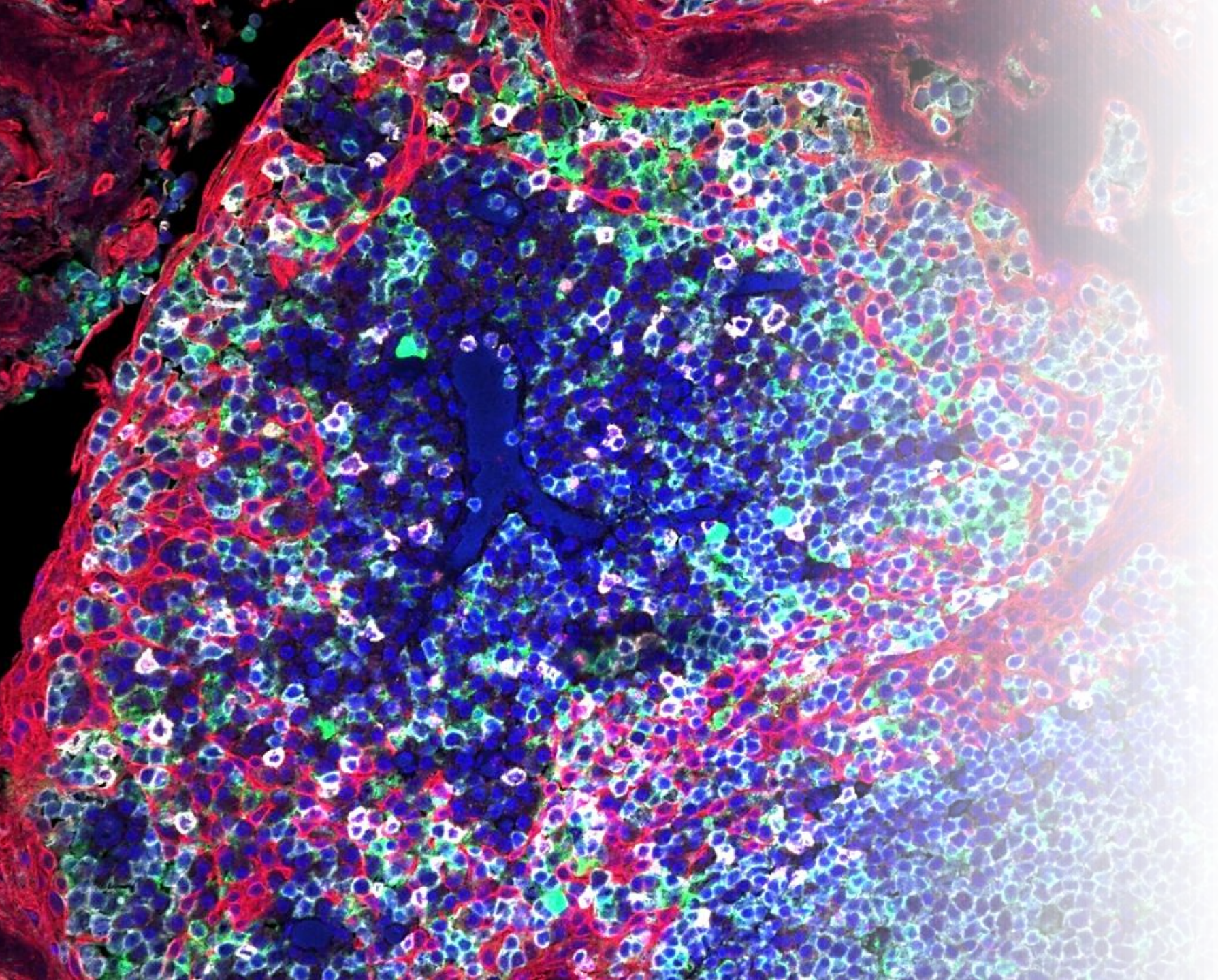


Classifying Immune Disposition in Neuroblastoma Using Optical Microscopy Techniques and Gene Expression Profiling

Dr. Abbirami Sathappan

سدرة للطب
Sidra Medicine

Sidra Medicine, University of Genova, and Dokuz Eylul University (DESG collaboration)



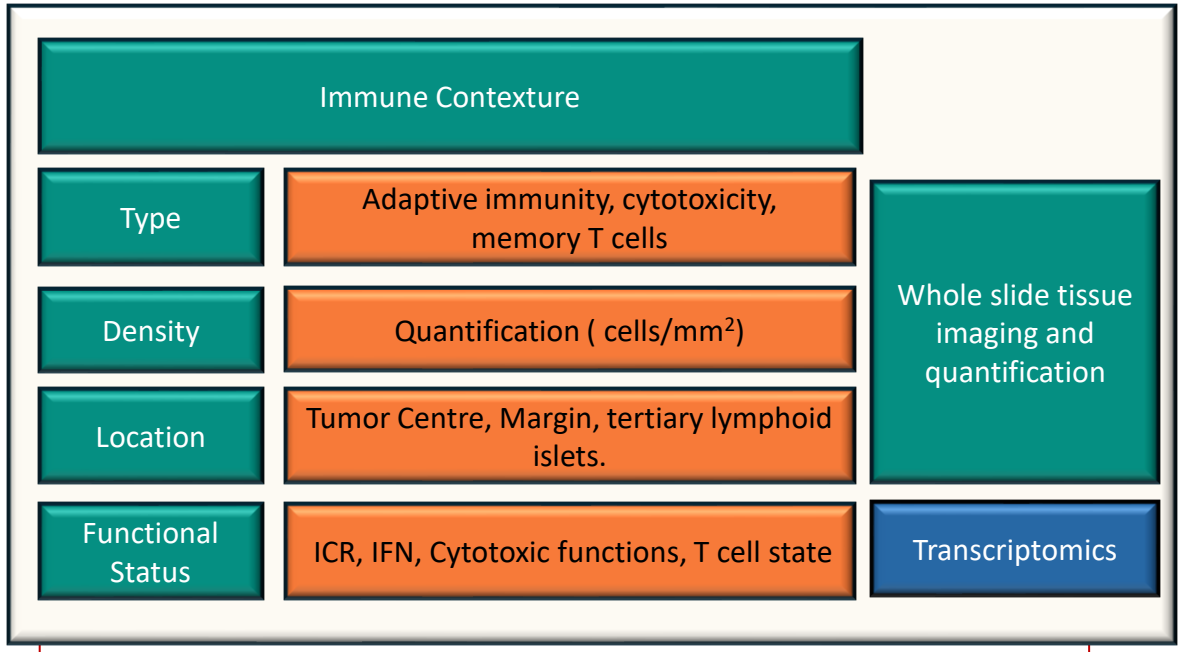
Sidra Medicine, Women and Pediatric Hospital

- Only dedicated pediatric research center in Qatar
- National Wide - Pediatric Biobank (Dr. Bedognetti, Dr. Wouter Hendriks) Enrolled more than 170 patients)



Dokuz Eylul University Hospital, Turkey

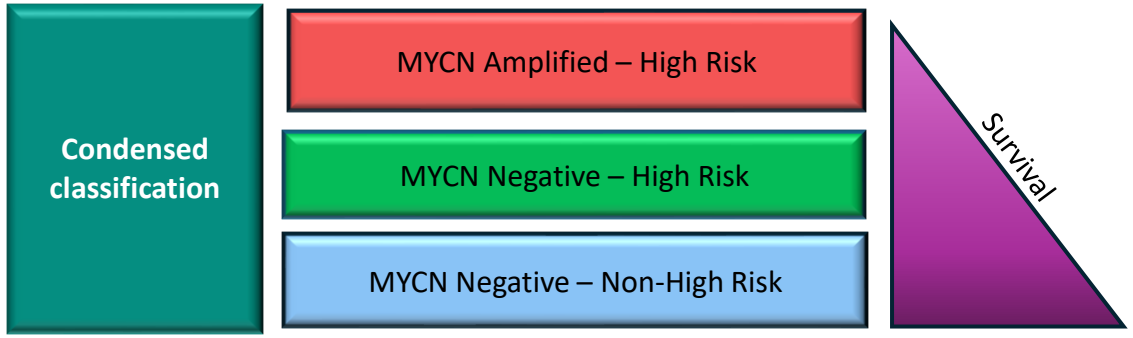
- 1900 Samples collected and classified by Turkish Pediatric Oncology Group from 1992 to 2021.



Adapted from: **Galon J, Bedognetti D, Marincola FM.** *The Continuum of Cancer Immunosurveillance: Prognostic, Predictive, and Mechanistic Signatures.* Immunity. 2013;39(1):11-26.

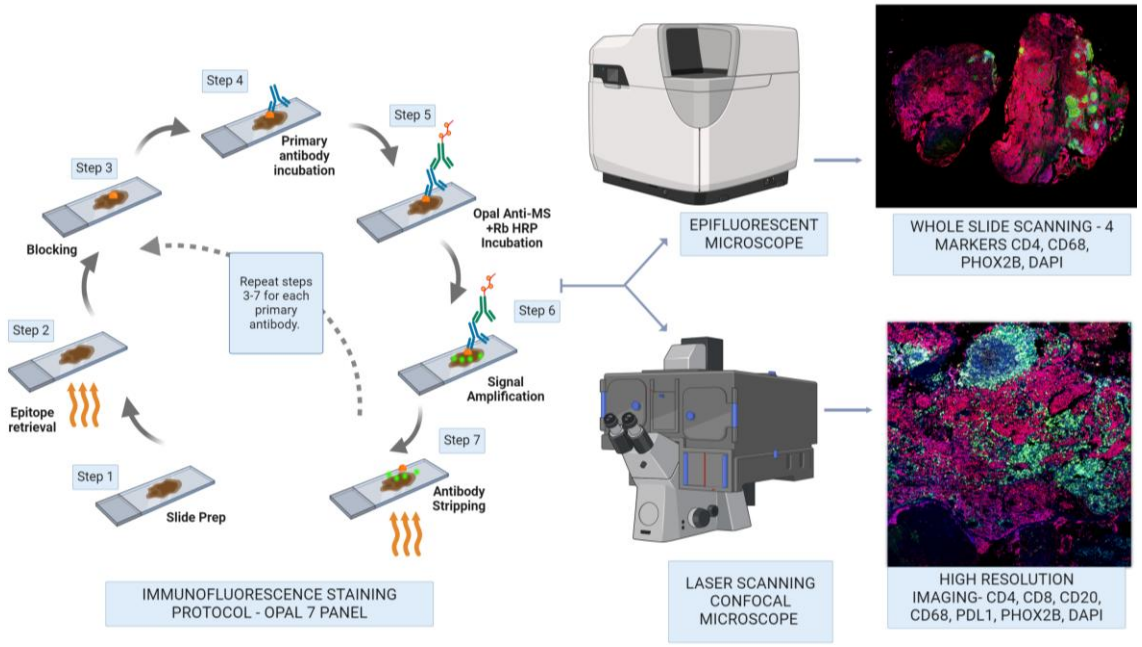
Transcriptomics

Whole Tissue Imaging





Multiplex Staining & Imaging

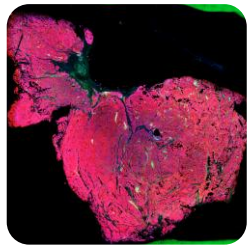


Name	Company	OPAL color
CD4	Dako	520
CD8	Dako	570
CD20	Abcam	540
CD68	Abcam	620
PDL1	Cell signaling technology	650
Phox2b	Cell Marque	690

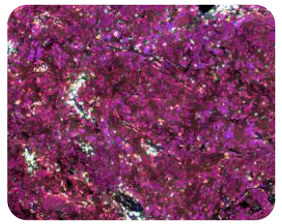
Challenges Faced:

- **Severe background/ autofluorescence**
- **Tissue detachment**
- **Identification of different cell population**
- **Tissue- severe bleeding : segregating RBCs from immune cells**

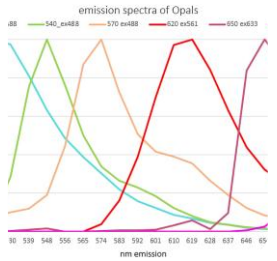
7 - PLEX



Opal staining
- Staining
- (4 days)
- 8 samples in one run



Imaging PhenoImager HT & confocal
- Choose ROI
- Scan with DAPI to identify tissue
- Tile scans of multiplex data.



Spectral unmixing
(Inform & QuPath)
- Single controls images
- Unmixing from each control
- Stitching the tiles

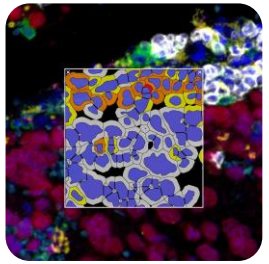
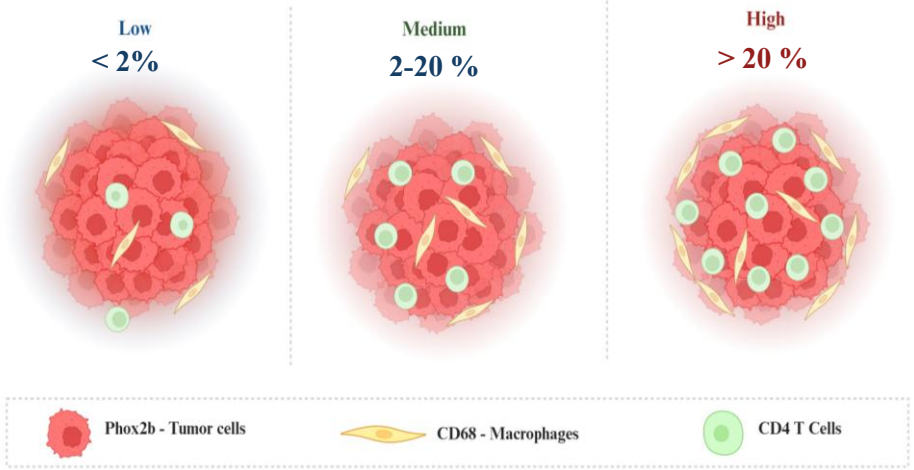


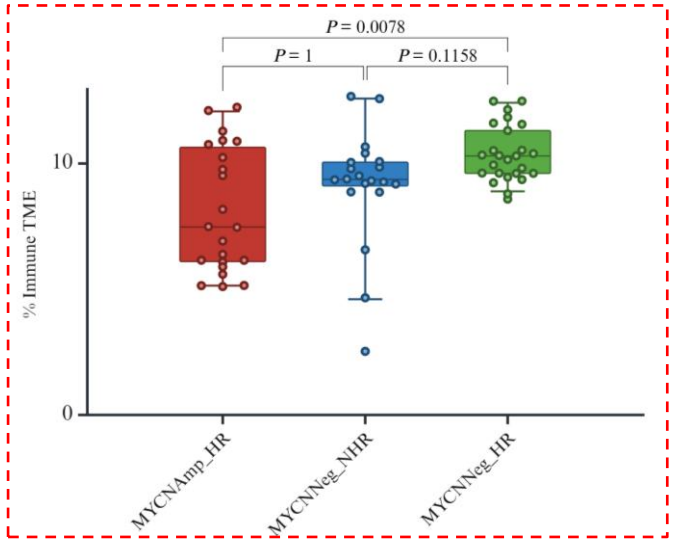
Image analysis
(Halo)

Single controls images
Unmixing from each control
Stitching the tiles

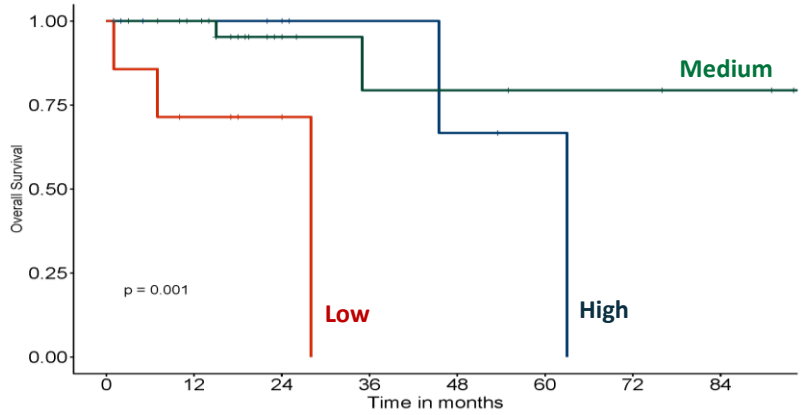
TME Profiling and spatial analysis

Cellular count is not important!

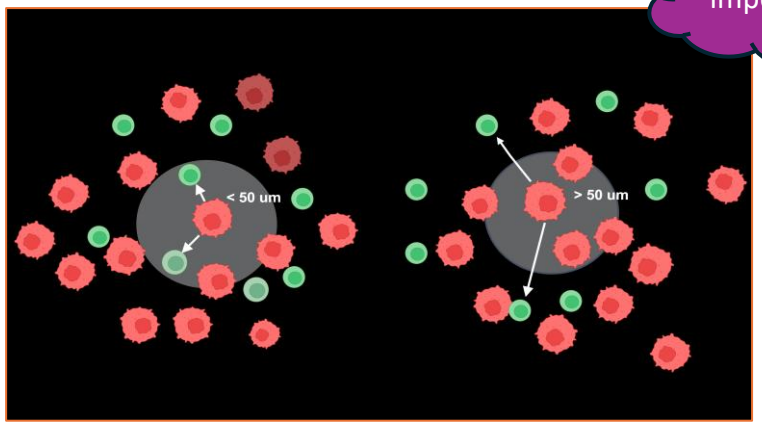




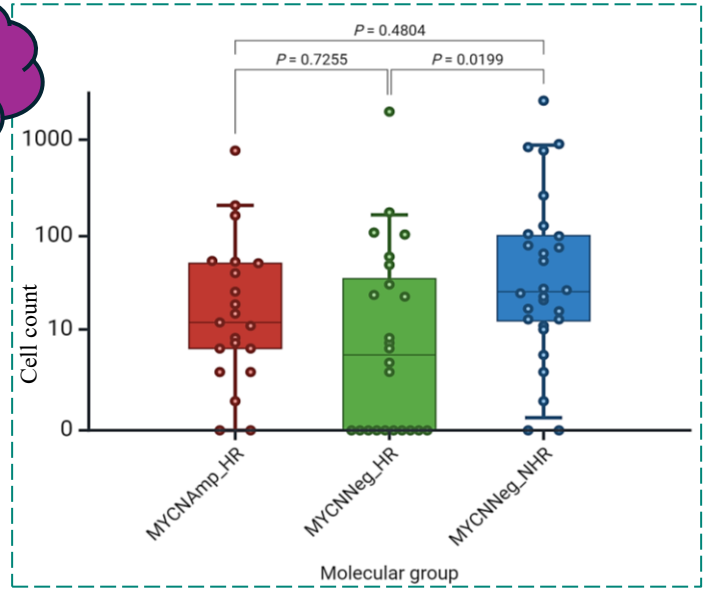
Survival Curve based on CD4 and CD68 cells



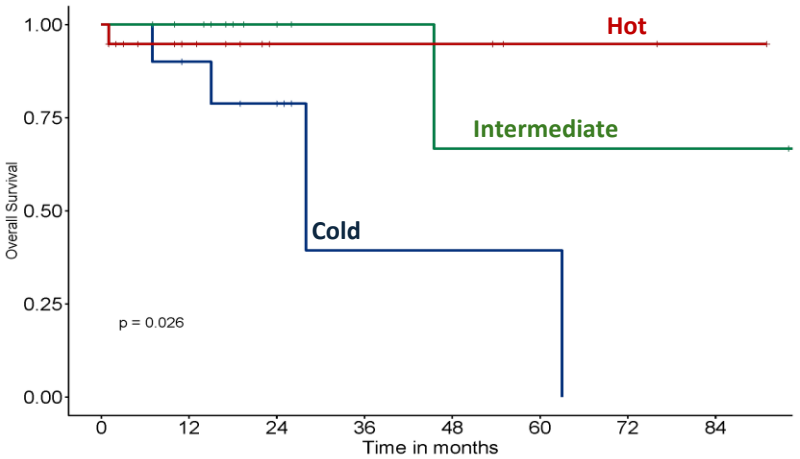
Proximity is important!



CD4 T cells (green) in 50um distance to Tumor cells (Pink)



Survival Curve based on Distance



سدرة للطب
Sidra Medicine

Dataset mining: Immune Landscape of Pediatric Solid Tumors.

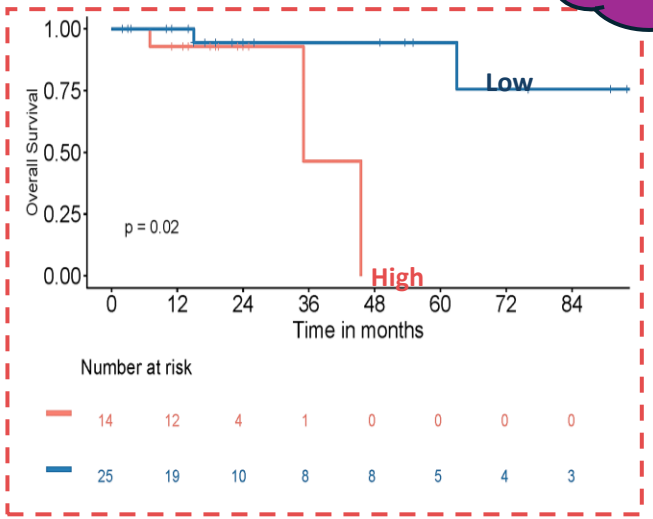
TRANSCRIPTOMIC DATA ANALYSIS

- RNA extraction: Maxwell FFPE KIT (serial sections)
- Validation cohort: TARGET DATASET
- ssGSEA – Immune cell populations between the DESG and TARGET cohort. *ssGSEA_A, *ssGSEA_B
- ssGSEA - Oncogenic pathway analysis between DESG and TARGET Dataset

single sample Gene Set Enrichment Assay (ssGSEA); ***ssGSEA_A** - Angelova M, Charoentong P, Hackl H, et al. *Characterization of the Immunophenotypes and Antigenomes of Colorectal Cancers Reveals Distinct Tumor Escape Mechanisms and Novel Targets for Immunotherapy*. Genome Biol. 2015;16(1):64.– ***ssGSEA_B** - Bindea G, Mlecnik B, Tosolini M, et al. Spatiotemporal Dynamics of Intratumoral Immune Cells Reveal the Immune Landscape in Human Cancer. Immunity. 2013;39(4):782-795. *Oncological pathways - Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, T-HO Y, et al. The immune landscape of Cancer. Immunity. 2018;48:812–830.e14.*

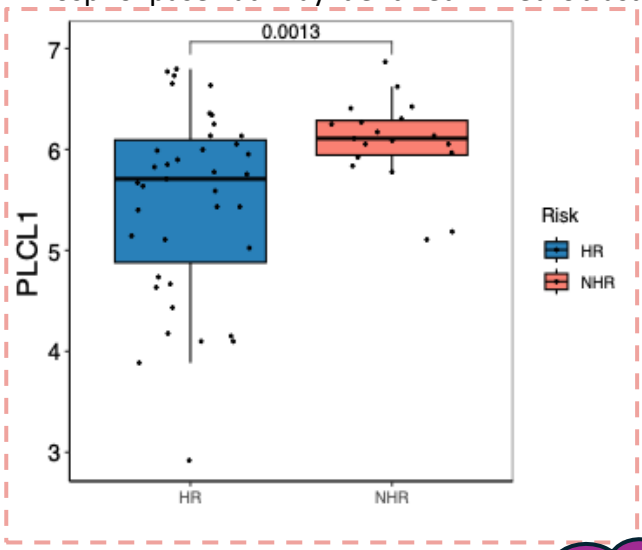
Activated CD4

Immune Exhaustion?

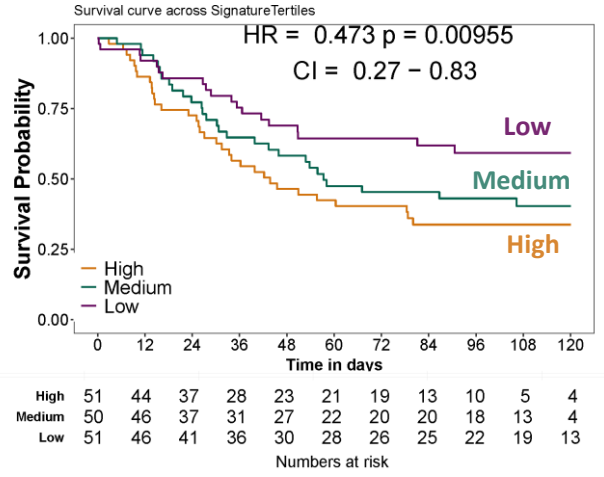


DESG Cohort:

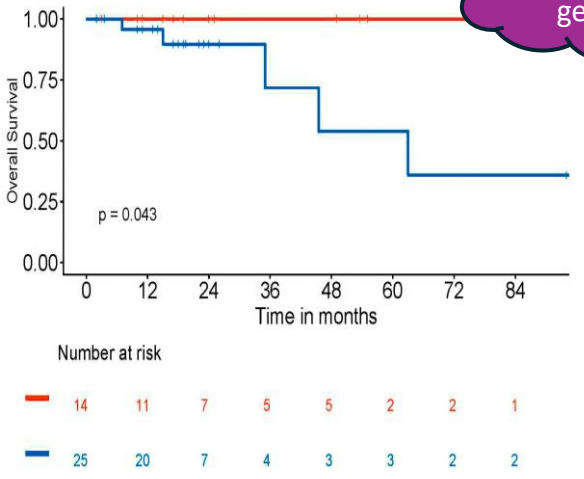
Phospholipase Pathway identified in Neuroblastoma



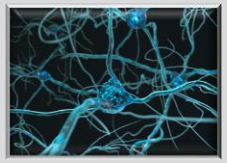
Activated CD4



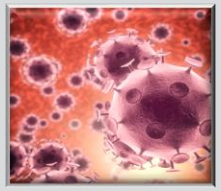
Therapeutic target gene?



Conclusion DESG Cohort



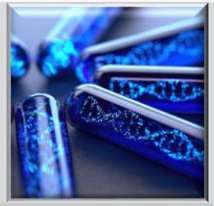
New Integrated Neuroblastoma dataset with transcriptional and phenotypic information has been assembled



We observed a differential prognostic role of the Th polarization in Neuroblastoma based on CD4 T - Macrophage quantification, distance between CD4 T cells and tumor cells, and activation of oncogenic pathways such as MYCN Amplification



Further analysis is planned to expand the cohort and deepen the analysis through interrogating relationship between oncogenic pathways and immunological disposition. We will also use orthogonal platforms such as spatial transcriptomics.



Phospholipase oncogenic pathway is associated with better prognosis in high-risk patients, making it a promising therapeutic target for high-risk neuroblastoma.

Thank you

ABRF 2025 - OPPORTUNITY

ADVANCED RESEARCH CORE

Dr. Davide Bedognetti

Dr. Chiara Cugno

❖ Pediatric Oncology Team:

Dr. Wouter Hendricks

Dr. Shimaa Sheriff,

Yarah EL-Zeini,

Farhia Abdullahi Ali.

❖ Genomics Core Facility:

Fazulur Rehman Vempalli,

Lisa, Oleksander Soloviv.

❖ Pathology

Prof. Erdener Ozer

