# Proteogenomic Spatial Profiling of Pediatric and Adult Diffuse Midline Gliomas, H3 K27-altered

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## Introduction

- Diffuse midline gliomas (DMG), H3 K27M-altered are highly aggressive malignancies of the central nervous system that affect both pediatric and adult populations
- The genetic changes within the tumor microenvironment are thought to play an integral role in the phenotypic differences in tumor presentation and clinical course between both populations.
- Comparative spatially resolved high-plex proteogenomics (transcriptomics and proteomics on a single cell population) between pediatric and adult DMG may lead to eventual therapeutic targets

## Methods

- Spatial proteogenomics (RNA and protein analytes detected together on a single spatially resolved cell population, **Figure 1a**) on the NanoString GeoMx® Digital Spatial Profiler platform was used to investigate a cohort of both pediatric and adult DMG, H3 K27-altered tissue samples (5 patients each within each cohort in triplicate – 30 samples total)
- Target specific DNA tags for both RNA and protein analytes are photocleaved with UV light and sequenced to determine spatially resolved gene and protein specific expression levels. (**Figure 1a**)
- Each sample was interrogated with a combination of the GeoMx Human Whole Transcriptome Atlas (18k genes) with 147 proteins (GeoMx NGS Human Protein portfolio) simultaneously. (**Figure 1b**)
- Region of interest (ROI) selection on each sample was guided by staining with 3 fluorescently labeled antibodies targeting immune cells (CD45), epithelial cells (PanCK), tumor cells (H3 K27M) and a nucleic acid stain (SYTO-13). (ROI, **Figure 2**).

**Figure 1a**

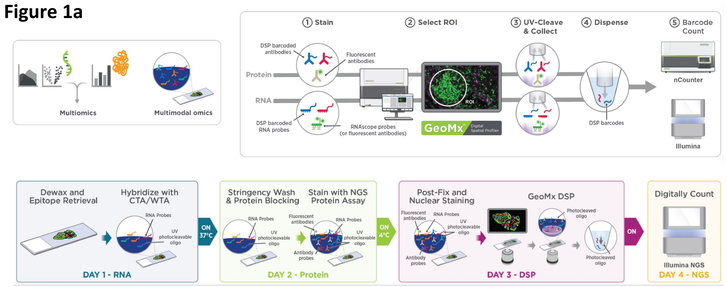
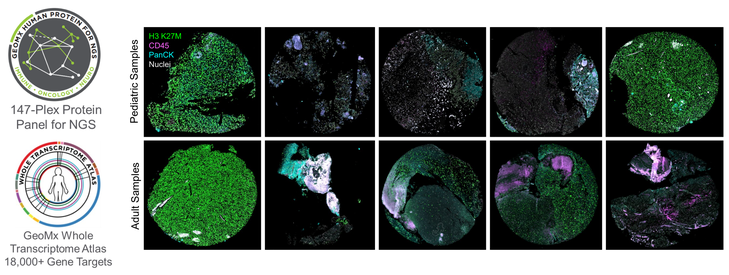



**Figure 1b**




## Acknowledgments

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## H3 K27M expression in guides targeted ROI selection

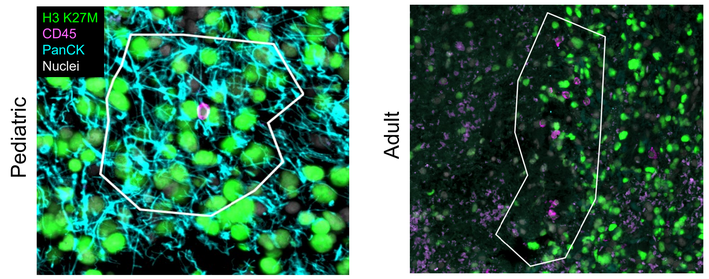


**Figure 2. Sample ROI** Each tissue sample was labeled with antibodies targeting immune cells (CD45 – *purple*), epithelial cells (PanCK – *cyan*), tumor cells (H3 K27M – *green*) and a nucleic acid stain (SYTO13 – *gray*). Specific ROIs were selected for further proteomic and gene expression analysis utilizing the NanoString GeoMx® platform and Illumina sequencing system.

## Distinct RNA and Protein Profiles observed between pediatric and adult DMG samples

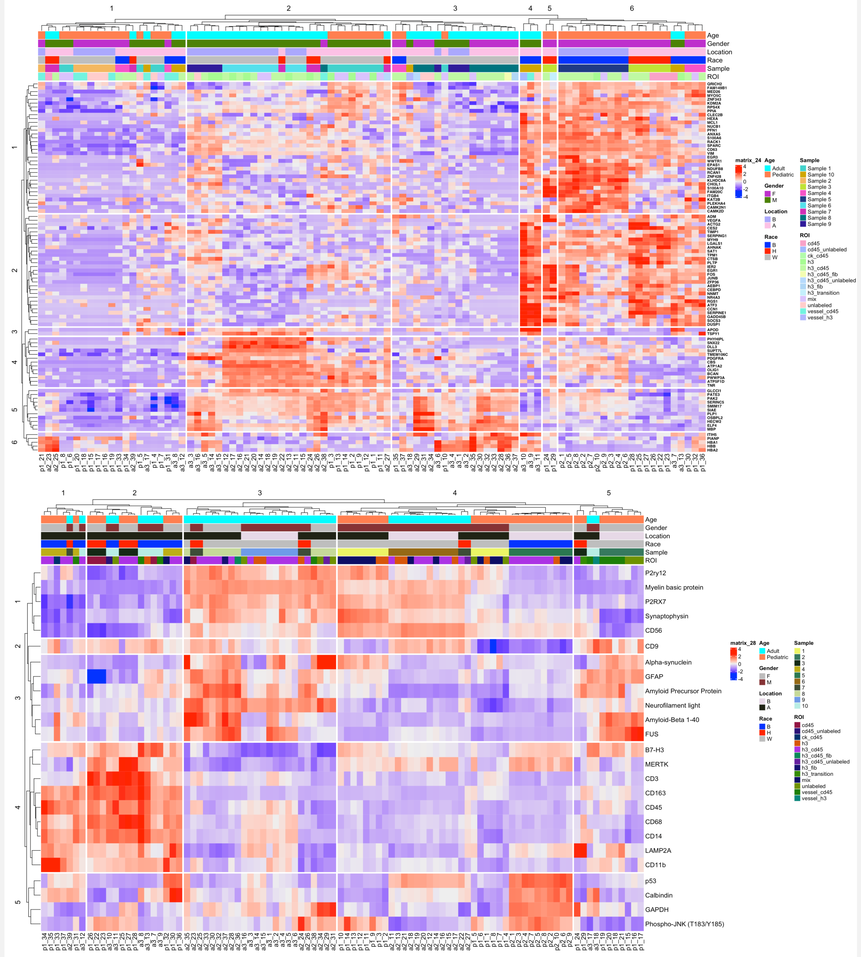


**Figure 3 (Top) & Figure 4 (Bottom) Heatmaps for the RNA and protein data from the H3+CD45 ROI cohort.** Figure 3 demonstrates increased RNA expression in multiple genes within the pediatric samples compared to the adult. These specific genes were then further analyzed via pathway analysis as demonstrated in Figure 5. Most of the observed differences appear to be sample specific and not associated with tumor anatomical location or race of the individual. Figure 4 shows the protein data for our samples with wide variability.

## Increased RTK pathway activity observed in Pediatric H3 K27M DMG

**Kinase Perturbations from GEO down**

- FGFR1 mutant 25 GDS4046
- EGFR drugactivation 30 GDS4361
- MAPK1 knockdown 45 GSE12291
- MET knockout 253 GSE30651
- HIPK2 defectivemutant 29 GDS4233

**Kinase Perturbations from GEO up**

- BRAF druginhibition 175 GSE42872
- BRAF druginhibition 38 GDS5085
- MET knockout 263 GSE8747
- MAP2K1 druginhibition 172 GSE39984
- HIPK2 overexpression 28 GDS4233

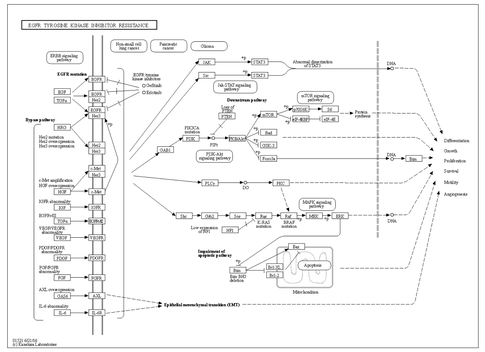


**Figure 5 Pediatric DMG Clinical Pathways:** The highest expressed genes from the H3 K27M + CD45 ROIs were plugged into pathway analysis. Expression pathway analysis done via Kyoto Encyclopedia of Genes and Genomes (KEGG).

## Results and Discussion

- The GeoMx Spatial Proteogenomic Assay allows for the ultra high-plex co-detection of proteins and RNA from a single spatially resolved cell population and the in depth characterization of limited and precious biological samples like pediatric and adult diffuse midline gliomas.
- Bioinformatic analysis revealed upregulation within various RTK pathway components along with the MAPK pathway, including EGFR, PDGFR, FGFR, and other components of the MAPK pathway. (**Figure 5**).
- These pathways are highly integrated with each other and are known to contribute to tumorigenesis in various cancers including brain tumors
- These aberrations seen in the pediatric samples may contribute to their more aggressive phenotype compared to adult tumors.
- Currently, there are on-going investigations as to targeted inhibitors of these pathways and their role in treatment of DMG
- Given the heterogenous nature of DMG tumors, likely will require multi-modal therapies with immuno-targeted therapy approach in pre-clinical studies

## Conclusion

- The spatial proteogenomic profiling of pediatric and adult DMG, H3 K27-altered shows differing profiles between adult and pediatric samples with potential for clinical relevance
- Upregulation of the RTK and MAPK pathways is more prevalent in the pediatric samples
- These observed differences may play a role in future treatments for H3 K27M-altered DMG.

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