Translating Molecular Profiles into Clinical Biomarkers Using Spatially Defined RNA Sequencing *in situ*

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Abstract: Histologically similar diffuse lower-grade gliomas (WHO grades II and III) can have variable clinical outcomes. Recently, large molecular profiling studies of low-grade gliomas (LGGs) revealed specific genetic, transcriptional, and epigenetic signatures that predict clinical outcomes better than histological features. For instance, the status of *IDH* mutation separates LGGs into tumors with favorable prognosis (*IDH* mutated) versus highly aggressive tumors similar to glioblastomas (*IDH* wildtype). Additionally, *TP53*, *ATRX*, and co-deletion of *1p/19q* can further separate *IDH* mutated LGGs into clinical subtypes. For these and many other types of tumors, however, intratumoral heterogeneity complicates attempts to develop biomarkers and to target the tumor cells since phenotypic diversity can result also from transcriptional, epigenetic, or stromal diversity. In such cases, a combination of genetic, transcriptional, and epigenetic signatures may be necessary to define tumor subgroups along with the histologic classification. The goal of this proposal is to spatially define the intratumoral transcriptional heterogeneity of *IDH* mutated grade II and III oligodendrogliomas (*IDH R132H*; *1p/19q*-del) and astrocytoma (*IDH R132H*; *TP53*; *ATRX*) tumor sections using Fluorescent *In Situ* RNA Sequencing (FISSEQ), to validate methods for staining clinical tissue sections using mutation, transcription, and methylation probes *in situ*, and to quantify their predictive power using the known patient cohort at MSKCC. If successful, our work will establish unprecedented multidimensional understanding of the spatial organization and intratumoral diversity of IDH mutant tumors.