

Detection of the EGFRvIII mutation in *Glioblastoma Multiforme* samples using a digital PCR platform

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Glioblastoma multiforme (GBM) is the most common primary malignant tumor of the central nervous systems in adults. It is extremely fatal: the median overall survival post-diagnosis ranges from 12-15 months. Approximately 30% of GBM tumors contain a subset of cancer cells with a mutated form of the epithelial growth-factor receptor, EGFRvIII. This mutation can confer increased proliferation of these tumor cells, and represents a cancer neo-antigen that can potentially be targeted by emerging anti-cancer therapies. **Thus, there is an urgent need for efficient, sensitive, accurate, and minimally-invasive diagnostic methods that identify GBM patients with EGFRvIII positive tumors. To this end, we tested the performance of a digital PCR (dPCR) platform to detect EGFRvIII in a variety of samples.** We assembled various ratios of EGFRvIII and EGFR plasmids, which we then analyzed via dPCR to determine our assay's limit of detection. We extracted EGFRvIII and EGFR RNA transcripts from both preserved and flash-frozen tumor tissue, as well as from mouse serum, from which we synthesized cDNA for analysis by dPCR. Finally, we tested the possible application of our assay for circulating tumor cells (CTCs) by extracting EGFRvIII and EGFR transcripts from single GBM cells spiked into and individually picked from mouse blood. We demonstrate our assay's sensitivity to 0.0008% EGFRvIII plasmid. We were also able to detect EGFRvIII in tumor tissue. We are currently refining our assay's ability to detect EGFRvIII in blood serum or in cells isolated from blood. By refining our assay, we hope to increase the feasibility of using dPCR to diagnose EGFRvIII in GBM patients from a variety of tissues including circulating tumor material extracted from blood. Ultimately, we hope to further develop minimally invasive techniques to stratify patients in the age of personalized-medicine.