# Tumor surveillance using liquid biome in diffuse intrinsic pontine glioma

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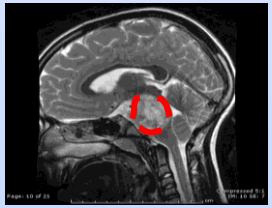
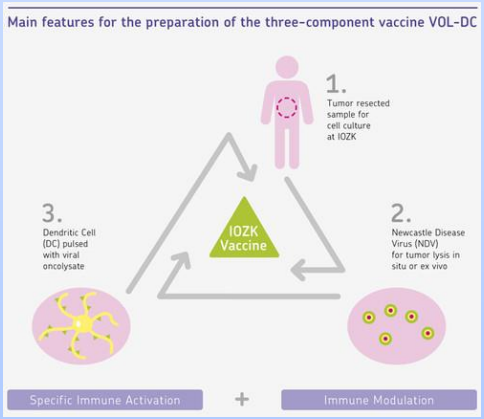
## Introduction

Immunotherapeutic approaches are being developed to treat diffuse intrinsic pontine glioma (DIPG), the deadliest childhood brain tumor. MRI is the gold standard for monitoring tumor response to therapy, but is limited by pseudoprogression: when transient inflammation of the tumor microenvironment falsely resembles progression on MRI. Thus, it is critical to develop alternative methods of monitoring tumor response, for use alongside MRI. Here, we use our digital droplet PCR liquid biopsy platform to detect *H3F3A* mutation – the most common DIPG driver mutation – in circulating tumor DNA (ctDNA) derived from plasma of DIPG patients at diagnosis. We then serially monitor *H3F3A* mutation allelic frequency (MAF) in plasma collected throughout the course of treatment, in order to correlate changes in MAF to tumor response. Patients received multimodal immunotherapy aimed at inducing immunogenic cell death selectively in tumor cells; if successful, this treatment would decrease tumor burden, which we expect to correlate to lower *H3F3A* MAF in plasma collected post-treatment. Thus, longitudinal MAF monitoring may provide a surrogate biomarker for non-invasively monitoring tumor response, as a complementary method to traditional MRI.



- DIPG tumors are inoperable
- Lack of tumor tissue for diagnosis of mutations
- MRI for monitoring response is limited

### DIPG Immunotherapy trial

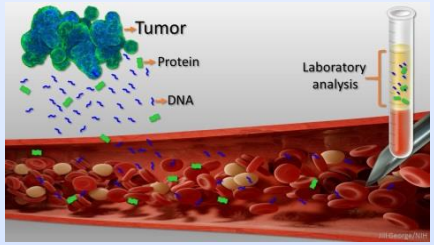



Van Gool et al., 2017. *Immun-Okologisches Zentrum Koln*. http://www.iozk.de/en/topics/dendritic_cells_oncolytic_virus

### Hypotheses

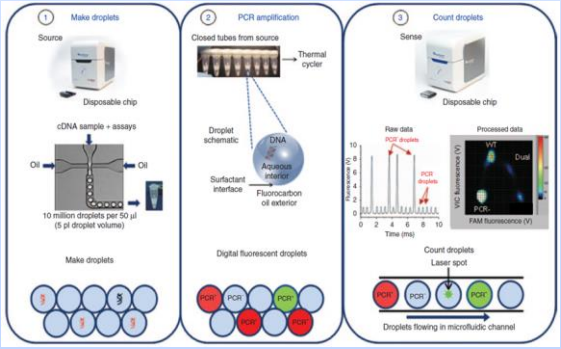
- Circulating tumor DNA in plasma can be screened for key driver mutations, including *H3F3A* (somatic mutation found in 80% of DIPG tumors)
- Longitudinal analyses of *H3F3A* mutation allelic frequency in plasma collected throughout treatment may provide a surrogate biomarker for tumor response

## Methods




NIH, 2018. https://directorsblog.nih.gov/2018/01/30/new-liquid-biopsy-shows-early-promise-in-detecting-cancer/

- Extract circulating tumor DNA from plasma collected at diagnosis, and throughout course of immunotherapy
- Screen for hotspot *H3F3A* mutation allelic frequency (MAF) using ddPCR
- Monitor changes in *H3F3A* MAF and correlate to clinical outcome




Chen et al., 2013. Figure 2, *BEAMing* and droplet digital PCR analysis of mutant IDH1 mRNA in glioma patient serum and cerebrospinal fluid extracellular vesicles. *Mol Ther Nucleic Acids* 2(7): e109.

**Digital droplet PCR** involves partitioning of sample into millions of pico-liter sized droplets, and amplifying target alleles in each droplet, using probes and primers specific to the target sequence. Specific probe hydrolysis results in bright fluorescent output when target allele is present.

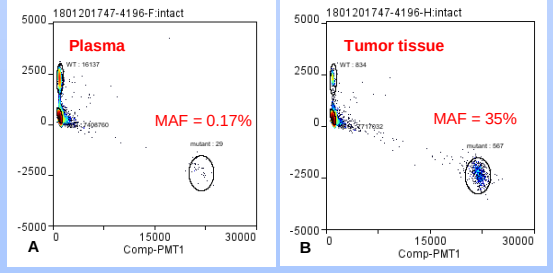



**Figure 1. RainDrop Analyst results for digital droplet PCR of circulating tumor DNA in plasma of a DIPG patient undergoing immunotherapy (A), and genomic DNA from tumor tissue of a *H3F3A* mutant patient (B).** Mutation allelic frequency (MAF) calculated as number of mutant droplets divided by number of mutant + wild type droplets.

## Results

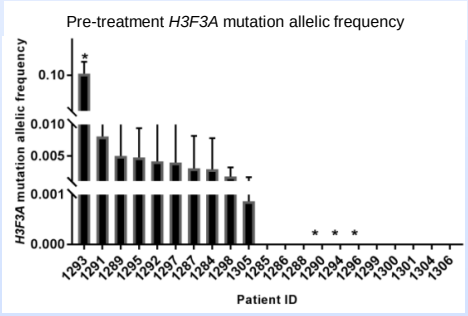



**Figure 2. Baseline *H3F3A* MAF in plasma obtained at diagnosis for all patients enrolled in trial.** Cut-off at MAF of 0.001% represents samples that are positive for the mutation. * Denotes samples known to be either variant of histone 3 mutation (*H3F3A* or *HIST1H3B*).

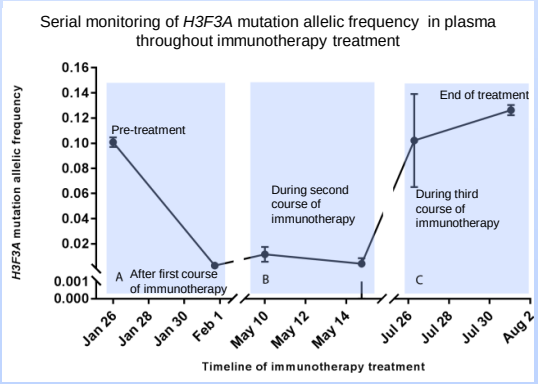



**Figure 3. Serial monitoring of *H3F3A* MAF in plasma of one patient throughout the course of immunotherapy**. **A**, **B**, and **C** represent three separate courses of vaccination with autologous tumor antigen and Newcastle Disease Virus, combined with local application of modulated electrohyperthermia. Changes in plasma MAF are detected throughout treatment.

### Conclusions

- *H3F3A* driver mutation is detectable in plasma at diagnosis
  - Non-invasive tumor genotyping when tissue is scarce
- Changes in *H3F3A* MAF can be serially monitored as a potential biomarker for tumor response, for use alongside MRI

### Future directions

- Screen for *HIST1H3B* variant, and other hotspot mutations associated with DIPG
- Integrate clinical date to validate MAF as accurate biomarker for immunotherapy response

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