Performance and in vivo validation of an optical uPAR-targeted probe, ICG-Glu-Glu-AE105 (FG001), for surgical guidance in an orthotopic human xenograft glioblastoma model

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Introduction

Surgery in combination with radiation and adjuvant chemotherapy is standard of care for patients with Glioblastoma Multiforme (GBM). There is no Class I evidence for the benefit of surgery alone or the extent of resection, however a positive correlation between the radicality of the surgery and patient survival has been established. Currently, the only approved optical imaging agent guiding GBM surgery is 5-aminolevulinic acid (5-ALA).

uPAR is a glycosyl phosphatidylinositol (GPI) anchored protein highly expressed in GBM. Further, it is in particularly expressed where the tumor invades normal tissue. uPAR is at the same time minimally present or absent in adjacent healthy tissue and is therefore very specific for GBM.

AE105 is a 9-mer peptide that binds to uPAR with high affinity. AE105 coupled to ICG, ICG-Glu-Glu-AE105 (FG001), was developed by our group as a uPAR-targeting fluorescent optical probe and has been shown to delineate tumors in several studies.

Hypothesis

The primary aim of the study was to determine the optimal dose and time of administration of ICG-Glu-Glu-AE105 (FG001) in an orthotopic glioblastoma model. In addition, FG001 was used in a proof-of-concept case study to help guide a surgeon to navigate for removal of an orthotopic human xenograft brain tumor.

Material and methods

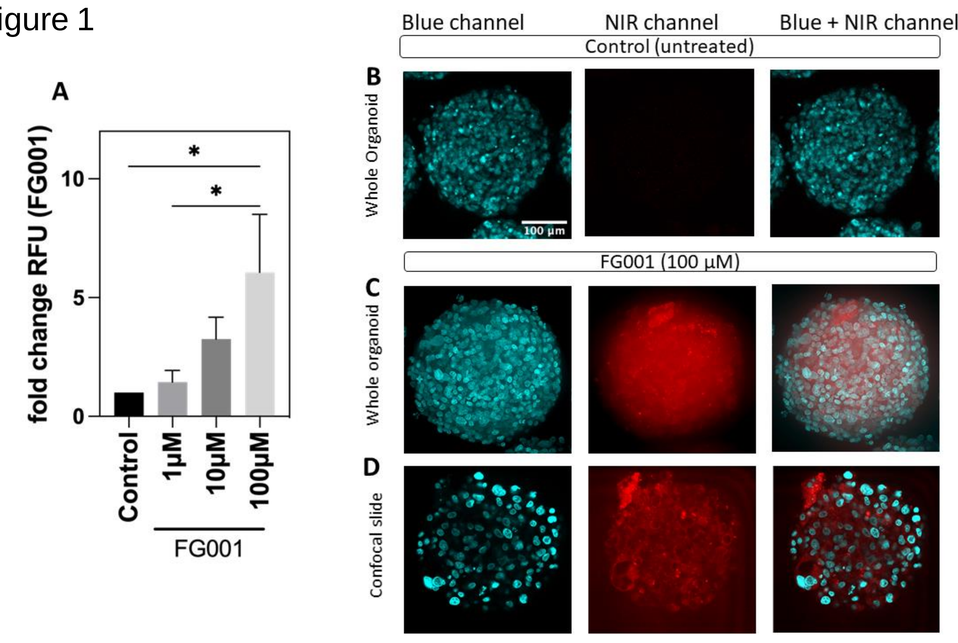
Blood-brain barrier (BBB) organoids were assembled as previously describe [1]. Briefly, 50µL of 1% agarose (w/v) in Milli-Q water is added to each well of low-binding 96 well plates (Thermo Fisher Scientific) and allowed to cool prior to the addition of cells. $1x10^3$ of each cell type is added sequentially. BBB-working media was added to each well to a final volume of 200µL per well. Organoids were allowed to self-assemble over 48-72 hours in a humidified incubator (37℃/5% CO_2). Organoids were co-incubated with 1µM, 10µM or 100µM FG001 overnight in a humidified incubator. Organoids were washed twice in PBS/FBS 5%, washed twice with sterile Milli-Q water, then organoid nuclei were stained for 10 minutes with DAPI. The organoids were analyzed with Zeiss LSM 780 confocal microscope.

Orthotopic xenograft glioblastoma model was established as previously described [REF]. FG001 was administered i.v. in four doses (0.07, 0.18, 0.36 and 0.72 mg/kg) and were imaged after 1, 3, 6 and 12 h with the ElevisionIR camera. Tumor and background intensity was measured from were Tumor to background ratio (TBR) was calculated. PK samples was collected at same timepoints and analyzed for FG001.

Fingerprint imaging was performed by slicing the entire brain in 10 µm and image these slides for FG001 on the Amersham Typhoon imager. Thereafter HE staining of the same slide was performed and an overlay of the two images was done to investigate the co-localization of FG001 and tumor.

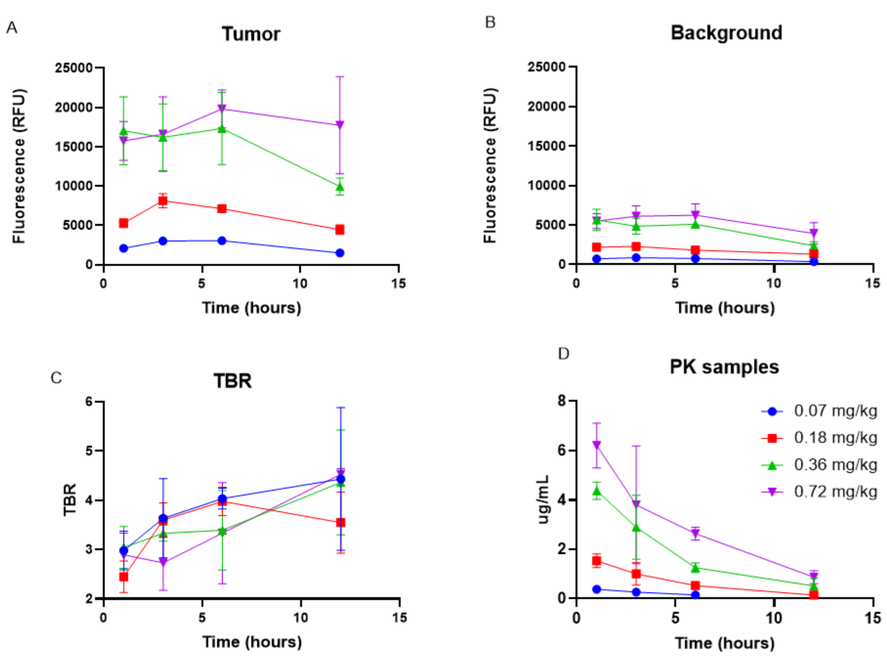
Results

Figure 1



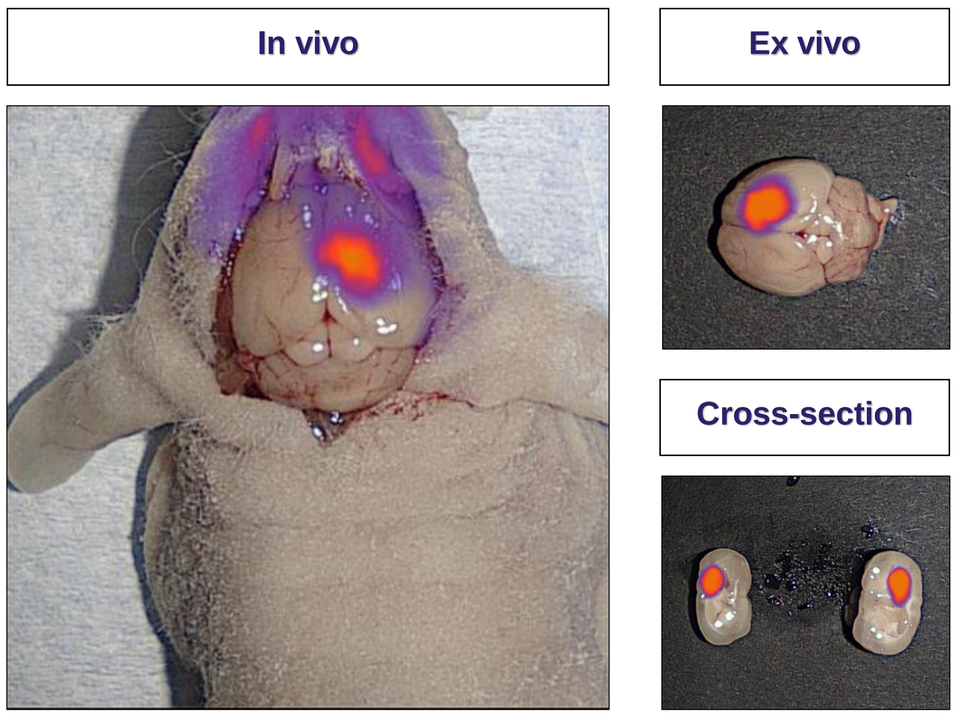
FG001 enters blood-brain-barrier organoids. A) Bar chart plotting the fold change in FG001 signal within organoids compared to untreated controls. B) Representative images of organoids (Z-projection of whole organoid). DAPI staining of healthy nuclei are seen in the blue channel while no signal is seen in the NIR channel C) Representative images of organoids (Z-projection of whole organoid) after incubation with FG001. DAPI staining of healthy nuclei are seen in the blue channel. Signal in the NIR-channel, from FG001 cellular uptake is seen in all cells. D) Single slice representing 45µm within organoid. In NIR-channel FG001 signal showing voids where cellular nuclei should be. FG001 staining within all visible cells strongly suggesting uptake is transcellular and not paracellular. Blue channel showing DAPI stained nuclei.

Figure 2



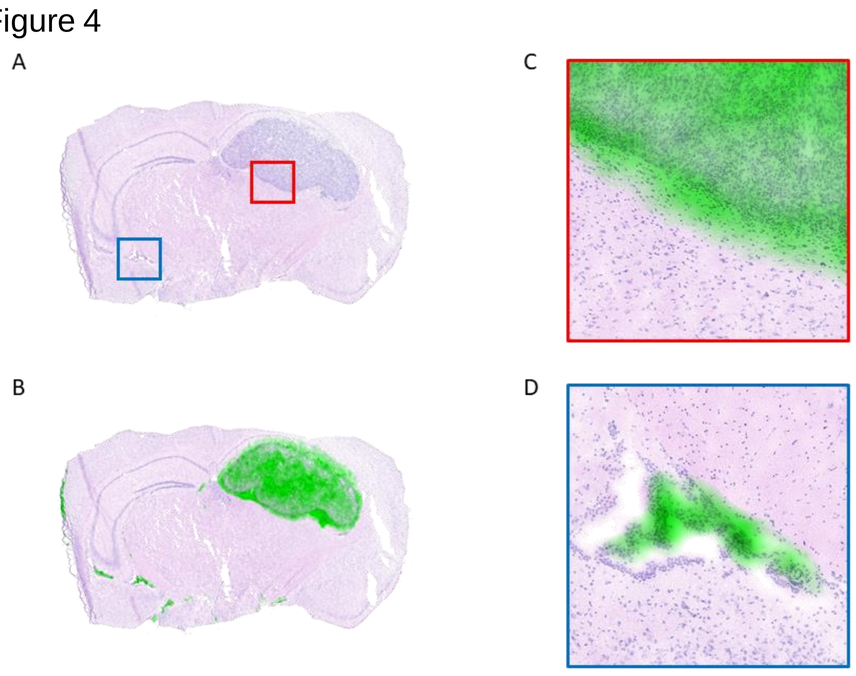
Fluorescence intensities, TBR values and PK profiles. A) and B) Fluorescence intensities obtained from the in vivo images using the EleVision™ IR camera. C) TBRs for each dose covering the 12-hours period post administration. All measured TBRs were above 2.5. D) Pharmacokinetic profile of FG001 in mice after i.v. administration of FG001. A dose-dependent increase in exposure is seen. Data are presented as mean ± standard deviation.

Figure 3



Fluorescence images. The three different images recorded by the EleVision™ IR camera (in vivo: with open skull and exposed brain and tumor, ex vivo: on whole excised brain and cross-section: cross-section through the tumor). Here a merge of a white light image and fluorescent image is seen.

Figure 4



Fingerprint histological slides. Histological section of a mouse brain comprising the orthotopic glioblastoma tumor. The tissue section, comprising normal brain, tumor tissue and regional tumor spread to the ventricles is depicted. A) HE staining. B) Merge of fluorescence image and HE staining. Pictures presented in the panel C and D show the border between tumor and healthy tissue by zooming in on the two areas (highlighted in red and blue) in panel A. Picture in panel C demonstrates a clear intersection line between malignant and healthy tissues for the main tumor, and panel D shows distinct spread of tumor cells to the ventricles

Conclusions

FG001 has been proven a valuable optical imaging agent in a preclinical model of GBM and has the potential for guiding cancer surgery. These results lead to initiation of a now completed Phase I trial in GBM patients.

- BBB model indicate that FG001 may penetrate the BBB
- In vivo optical imaging shows useful guidance with TBR values above 2.5
- PK profiles show dose dependent exposure
- Fingerprint imaging shows precise delineation between tumor and healthy tissue

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