Intracranial, intratumoral drug-releasing microdevices in patients with high grade gliomas identify biomarkers of drug activity and predict tumor response to systemic chemotherapy

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Abstract

The lack of reliable predictive biomarkers to guide effective therapy is a major obstacle for the advancement of therapy for high grade gliomas (HGG), and particularly glioblastoma (GBM), one of the few cancers whose prognosis has not improved over the past several decades. With this pilot clinical trial we provide first in human evidence that drug-releasing intratumoral microdevices (IMD) can be safely and effectively used to obtain patient-specific, high throughput molecular and histopathological data to inform selection of drugs based on their observed antitumor effect *in situ*. The use of IMD is seamlessly integrated in standard surgical practice during tumor resection. None of the six enrolled patients experienced adverse events related to the IMD, and the retrieved tissue was usable for downstream analysis for 11 out of 12 retrieved specimens. Molecular analysis of the specimens provided, for the first time in humans, preliminary evidence of the robustness of the readout, with strong correlation between IMD analysis and clinic- radiological responses to temozolomide. We also identified novel transcriptomic and metabolomic biomarkers of response and resistance to a range of targeted and cytotoxic agents used on the IMD. From an investigational aspect, the amount of information obtained with IMD allows unprecedented characterization of tissue effects of any drugs of interest, within the physiological context of the intact tumor.

Background

- Gliomas are a particularly aggressive type of cancer with dismal outcomes.
- Gliomas comprise a diverse group: Though knowledge of the genomic landscape of glioblastoma has increased, these findings have yet to result in improved outcomes for GBM patients.
- Clinical decision making currently lacks predictive biomarkers that allow us to reliably identify responders for optimal drug treatment.
- Developing new therapies for gliomas is a major challenge, as the field lacks methods for developing rational combination regimens.
- The ideal way to bypass this problem is to investigate drug pharmacodynamics directly in the patient.

Technical Approach

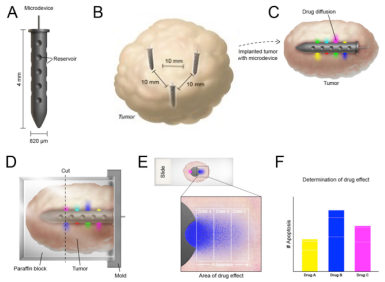

Figure 1: Concept for in-vivo drug sensitivity assay: Device is implanted directly into tissue. During implantation, drugs diffuse into confined regions of tumor. Each such region can be assayed independently to assess the tumor-specific response of a given drug. Following incubation, the device/tissue specimen is retrieved surgically or by biopsy. This tissue contains the regions of drug diffusion and is sufficient for determination of efficacy of drugs.

Clinical workflow

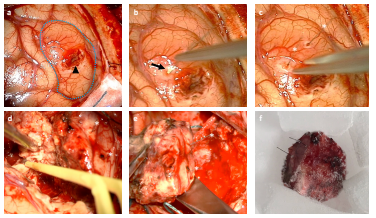

Figure 2: Surgical phases of IMD insertion/retrieval. a: Lesion biopsy (black arrowhead). **b** and **c:** serial implantation of two IMDs (black arrow), and localization of "tails" (white asterisks). **d:** Resection of the tumor region away from IMDs. **e:** Removal of the part of tumor containing the IMDs. **f:** Flash-freezing of specimen on dry ice. The dotted blue line in panel **a** represents the superficial projection of the tumor on the brain cortex.

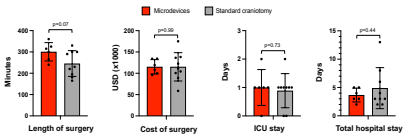

Figure 3: IMD integration in surgical care of patients with HGG. Comparison of common healthcare metrics between the group of patients receiving IMD implantation (red, n=6) and a cohort of patients receiving standard surgery for HGG operated during the same period of time (grey, n=9). Reported are mean and standard deviation for each group.

Results

Consistent, localized release of drug microdoses in the TME

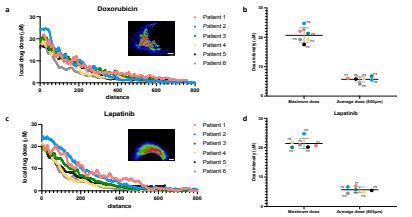

Figure 4. Drug release profiles from each patient for Doxorubicin (**a**) and Lapatinib (**c**). Inset shows typical 2-dimensional spatial profile of drug distribution. Inset scale bar is 200mm. The variation in maximum and average dose for each drug between patients is shown in (**b**,**d**). Each dot on the graph represents the mean of triplicate measurements of drug release profile values (maximum or average dose) from biologically distinct regions of each patient's tumor. Error bars represent standard deviation.

Measurement of in-situ drug effect

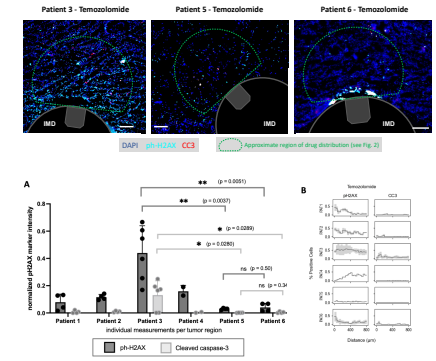

Figure 5: Differential tumor response to temozolomide. a: Quantification of IF stains for pH2AX and CC3 in IMD tissue from each numbered patient. Each point represents a measurement from a distinct tumor region comprising 800mm x 400mm exposed to drug. Bars display mean and standard deviation. Each dot on the graph represents a unique biological replicate measurement from a tumor region within the area of tumor exposed to TMZ for a given patient. Statistical comparisons are made using Repeated Measures ANOVA test, with p-values shown for each comparison (in parentheses). **b:** Distance and concentration dependent analysis of pH2AX and CC3 stains across the six patients.

Correlation of microdevice readout with clinical response to systemic treatment

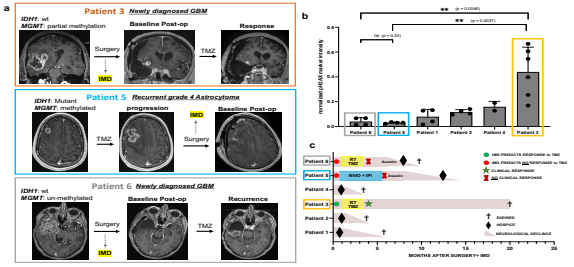

Figure 6: Clinical-molecular comparisons. a: Time-course MRIs of three representative patients who received systemic therapy after surgery and IMD analysis. **b:** Quantification of specific in-situ response to TMZ (by pH2AX immunostaining) for each patient in the study as determined by IMD analysis. Each point represents a measurement from a distinct tumor region comprising 800mm x 400mm exposed to drug. Bars display mean and standard deviation. Comparisons use a Repeated Measures ANOVA test with p-values shown per each comparison (in parentheses).**c:** Survival data for each patient in the study, including type and timing of adjuvant therapy administered. Specific patients are color-coded to better visualize the alignment among radiologic data, IMD response and survival.

In situ target validation & discovery of novel biomarkers of response

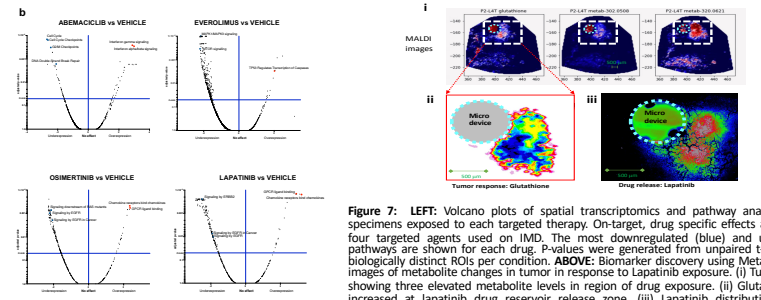

Figure 7: LEFT: Volcano plots of spatial transcriptomics and pathway analysis from tumor specimens exposed to each targeted therapy. On-target, drug specific effects are confirmed for four targeted agents used on IMD. The most downregulated (blue) and upregulated (red) pathways are shown for each drug. P-values were generated from unpaired t-tests based on 4 biologically distinct ROIs per condition. **ABOVE:** Biomarker discovery using Metabolomics: MALDI images of metabolite changes in tumor in response to Lapatinib exposure. (i) Tumor cross section showing three elevated metabolite levels in region of drug exposure. (ii) Glutathione levels are increased at lapatinib drug reservoir release zone. (iii) Lapatinib distribution measured by autofluorescence shows spatial overlap with elevated metabolite levels. Scale bar = 500mm

Key Findings & Conclusions

- Implantable microdevices harboring up to 20 different agents are implanted directly into the patient's brain tumor
- Microdoses of each agent are released into locally confined regions of the tumor in a consistent and reproducible manner. Drug concentration gradients are directly measured.
- Drug effects are measured using a range of anti-tumor markers (e.g. Cleaved-caspase-3 for apoptosis) and pharmacodynamic markers for drug activity (e.g. ph-H2AX for DNA damage). These markers show differential effects for Temozolomide and other agents across the pilot cohort of six patients.
- The degree of DNA damage and apoptosis induced by Temozolomide (TMZ) microdoses via the IMD, correlate directly with the clinical response to systemic TMZ for all three patients in the trial that received systemic TMZ. Both high responses (Patient 3) and tumor progression (Patients 5 and 6) were predicted correctly by the IMD, even in cases where the MGMT methylation status provided incorrect or ambiguous predictions.
- Using spatial transcriptomics (Nanostring GeoMx), target engagement and validation could be confirmed for several targeted agents. Systematic identification of upregulated pathways showed IFN signaling in some samples, indicating potential synergy with checkpoint inhibitors or other immunotherapies.
- Spatial metabolic profiling (MALDI) identified novel metabolic signatures of tumor response to targeted agents (e.g. glutathione response to Lapatinib)

References

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