Development of *In Vitro* and *In Vivo* Primary Tumor Models of Glioblastoma (GBM) to Preclinically Validate Combinatorial Approaches

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ABSTRACT

The blood-brain barrier (BBB) is a critical obstacle to developing brain-penetrant therapies for glioblastoma (GBM) research and treatment. Orthotopic intracranial GBM models are a next-generation platform that provides accurate preclinical data to inform lead candidate progression into the clinic.

Here, we use patient-derived GBM tumors with no approved therapies, to rapidly develop 3D spheroid models for high-throughput *in vitro* drug testing followed by drug gene network analysis to identify potentially efficacious standard-of-care (SOC) treatments and synergies. This information was used for the development of subcutaneous and orthotopic intracranial patient-derived xenograft (PDX) models for proof-of-concept pharmacological testing and reverse translation for confirmatory testing. This combinatorial approach provides a path forward for clinically relevant GBM model generation and personalized treatment decisions.

METHODS

Tumor samples were taken from a GBM patient using surgical resection at 0 days (BRRH-001), 60 days (BRRH-001B), and post-radiation treatment (BRRH-001C). Tumor biopsies were disaggregated, depleted of red blood cells, and propagated to form 3D spheroids. Genomic biomarkers were identified with whole exome sequencing. Patient-derived spheroid cultures were screened using a high-throughput, automated screening workflow against a collection of compounds managed by Scripps Florida.

Biomarker analysis identified specific genomic alterations for which there is no approved therapy, including a methylated MGMT gene promotor, *BRCA2* variant of unknown significance (VUS), missense mutation in *TP53*, and *EGFR* amplification.

Drug gene network analysis identified 5 standard-of-care treatments with potential efficacy and synergies (Figure 1). *In vitro* 3D spheroid models were used to establish subcutaneous and orthotopic *in vivo* GBM models for pharmacology testing. The engraftment rate for both subcutaneous and orthotopic models was 100%. Tumor volumes were monitored over time using MRI. Following *in vivo* treatment, tumors were reverse translated to *in vitro* models and tested further to validate synergistic effects seen *in vivo*.

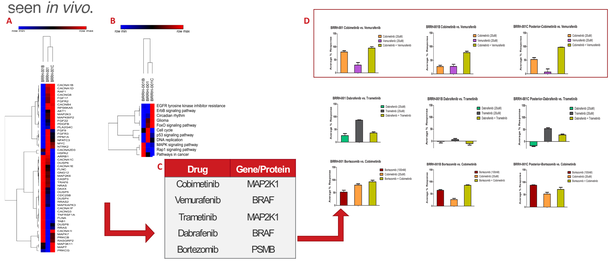
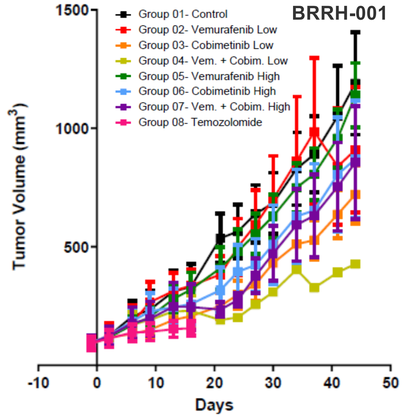


Figure 1. Methodology for Identifying Treatments for *In Vivo* Pharmacology Testing. Comparative analysis of the patient derived GBM RNA transcripts to the healthy brains subset of the GTEx database identifies significantly enriched pathways (A) as well as modulated genes in the MAPK pathway (B). Potential drug combinations were identified based on Drug-Gene Network analysis (C). *In vitro* synergy studies (D) show apparent differences in the response of the combinations of drugs tested across the different GBM samples isolated from the same patient.

RESULTS

Group	N	Treatment	Dose (mg/kg)	Dose Volume (mL/kg)	Dose ROA	Dose Schedule	Body Weight	Caliper Measurements	MRI
1	6	Vehicle	0	10	PO	QD x 4wks	BIWx4	BIWx4	Pre/Post Tx
2	6	Vemurafenib	10	10	PO	QD x 4wks	BIWx4	BIWx4	Pre/Post Tx
3	6	Cobimetinib	2.5	10	PO	QD x 4wks	BIWx4	BIWx4	Pre/Post Tx
4	6	Vemurafenib + Cobimetinib	10 2.5	5 5	PO	QD x 4wks QD x 4wks	BIWx4	BIWx4	Pre/Post Tx
5	6	Vemurafenib	20	10	PO	QD x 4wks	BIWx4	BIWx4	Pre/Post Tx
6	6	Cobimetinib	5	10	PO	QD x 4wks	BIWx4	BIWx4	Pre/Post Tx
7	6	Vemurafenib + Cobimetinib	20 5	5 5	PO PO	QD x 4wks QD x 4wks	BIWx4	BIWx4	Pre/Post Tx
8	6	Temozolomide	40	10	IP	QD x 4wks	BIWx4	BIWx4	Pre/Post Tx

Figure 2. *In Vivo* Model Generation and Proof-of-Concept Pharmacology Studies. Pharmacological testing was designed under the conditions detailed in the above table. 3D spheroids derived from BRRH-001 tumor specimens were injected subcutaneously, and tumor volumes were measured by MRI (right panel) over 44 days. High and low concentrations of drugs were tested for single and combination treatments, and body weight was measured in parallel (BW data not shown). At day 44, tumors were harvested for the reverse translation experiments described in Figure 3 below.



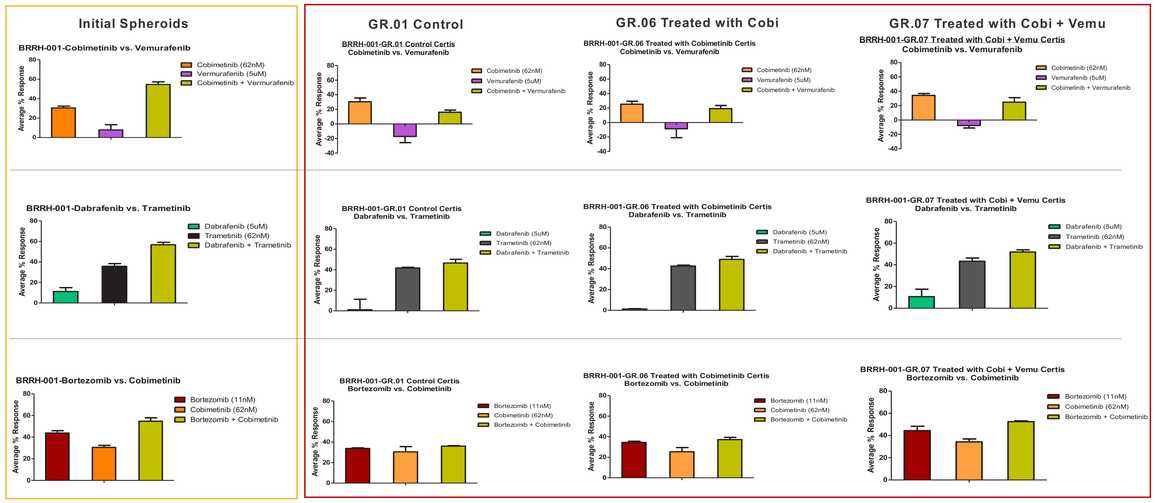


Figure 3. Reverse Translation of *In Vivo* Pharmacology. Mouse tumors were reverse translated to an *in vitro* 1536 well 3D spheroid format and drug synergies were retested and compared to initial spheroid pharmacological testing (left-most panel of graphs).

CONCLUSIONS

The treatment of GBM tumors is clinically challenging, and the need for safe and effective therapeutics is largely unmet.

Using high-throughput drug screening of patient-derived 3D spheroids in combination with whole exome sequencing, we have demonstrated that drug-gene network analysis can guide the selection of potentially efficacious drug combinations for GBM tumors for which there are no currently approved treatments. The drug-gene network predicted synergistic effects between certain SOC drugs, and those effects were observed in our *in vitro* 3D spheroid models.

Furthermore, we successfully established subcutaneous and orthotopic PDX models from 3D spheroids and performed translational proof-of-concept pharmacological testing. These PDX models were also used for reverse translation back to 3D spheroids for additional *in vitro* testing, and we observed some matching to prior results for some, but not all, drug combinations.

While these results are preliminary, the validated workflow described here illustrates a clear path forward for precision clinical decision-making in managing GBM and other solid tumors.

CITATIONS & ACKNOWLEDGEMENTS

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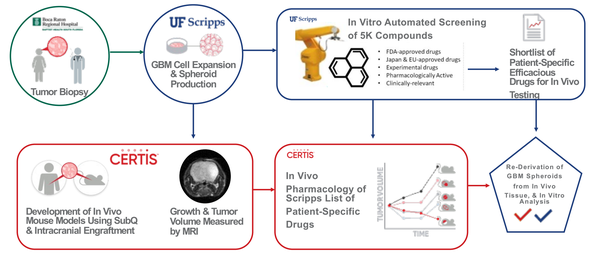

Figure 4. Institutional workflow and relationships

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