CASE STUDY

HOW DO HUMAN GBM-DERIVED TUMORSPHERES TRANSLATE TO AN ORTHOTOPIC INTRACRANIAL MODEL SYSTEM?

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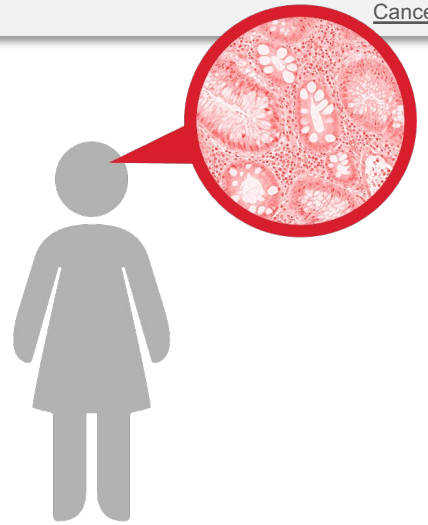
The Need for GBM Therapeutics Remains Unmet

SURVIVAL STATISTICS

Overall survival from diagnosis averaging **<20 mos**.

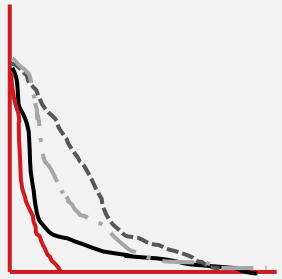
Five-year survival outcomes of **~7%.**

Cancer.org



RESEARCH FACTS

After decades of research, no significant change in survival.



Despite advances with immune checkpoint inhibitors, no significant improvement in survival compared to historical controls.

C.G. Brahm *et al. Cancers.* 2020.

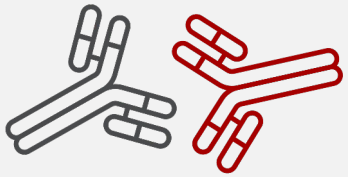
ONE MAJOR CHALLENGE: BBB

Blood brain barrier (BBB) which protects the brain from potential toxins has limited adequate drug penetration to brain.

D. Wang *et al*, *Drug Deliv.* 2019.

PROBLEMS FOR IO

The brain is considered "immune privileged" resulting in "cold" tumors.



M.G. Harris *et al*, *Nature*, 2014.

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CASE STUDY: Patient Derived GBM Spheroids

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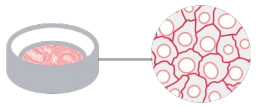
Research Goal: Developing Clinically Relevant Research Models for Personalized Medicine

Boca Raton Regional Hospital
BAPTIST HEALTH SOUTH FLORIDA

Tumor Biopsy

UF Scripps



GBM Cell Expansion & Spheroid Production

UF Scripps

In Vitro Automated Screening of 5K Compounds

- FDA-approved drugs
- Japan & EU-approved drugs
- Experimental drugs
- Pharmacologically Active
- Clinically-relevant

Shortlist of Patient-Specific Efficacious Drugs for In Vivo Testing

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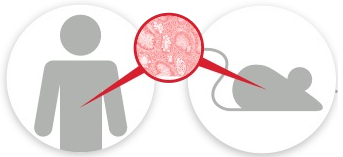
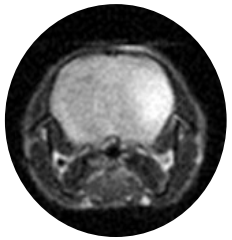


Development of In Vivo Mouse Models Using SubQ & Intracranial Engraftment



Growth & Tumor Volume Measured by MRI

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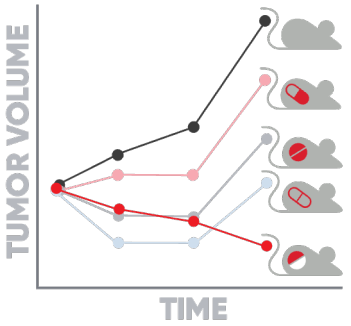
In Vivo Pharmacology of Scripps List of Patient-Specific Drugs



Re-Derivation of GBM Spheroids from In Vivo Tissue, & In Vitro Analysis

PART 1

In Vitro Pharmacology of Sequential GBM Biopsies From a Single Patient

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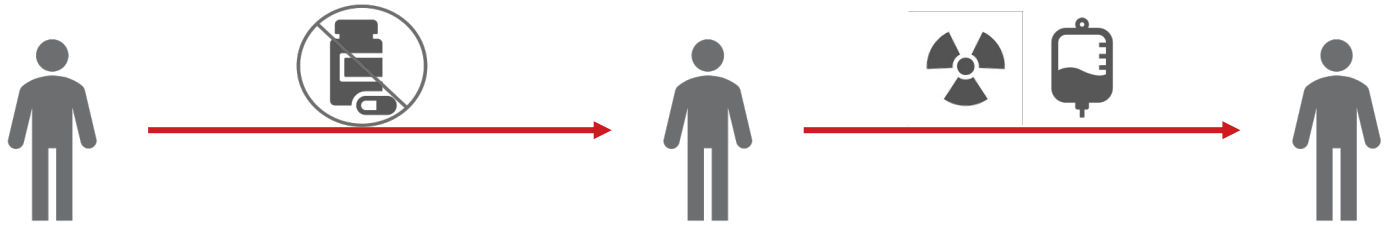
Case Study: 1 Patient, 3 Resections, A Real-World Model Of GBM Evolution

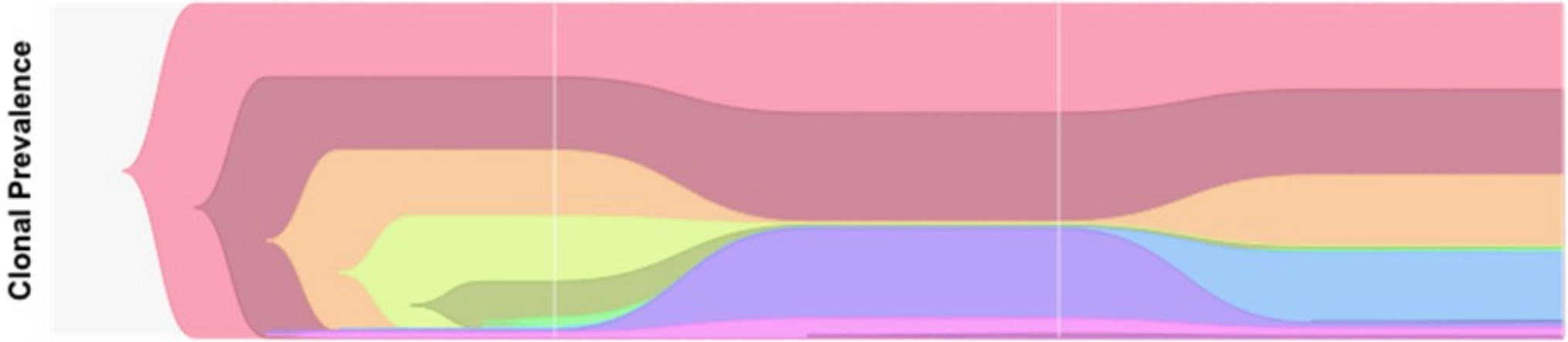
Clonal Phylogeny

BRRH-001:
1st Surgery — Dec. 6, 2018
Naive

BRRH-001B:
2nd Surgery — March 26, 2019
Naive

BRRH-001C:
3rd Surgery — Dec. 6, 2019
Pretreated with TMZ and IRT





Data courtesy Certis Data Science Team

Glioblastoma Tumor Gene Network Modeling Approach

No therapies have been approved for these specific alterations in GBM

Methylated MGMT Gene Promoter:

- Most likely to respond to chemotherapy (TMZ)

Tumor Has Mutation in BRCA2

- Pathogenic BRCA2 approved drugs in breast and ovarian cancers

but

- VUS (variant of unknown significance)

Tumor Has Missense Mutation in TP53

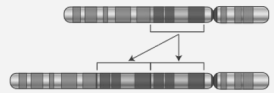
- TP53 is a tumor suppressor gene in most cancer types
- May have oncogenic activity in GBM
- Drugs targeting downstream of TP53 include CDK4/CDK6 inhibitors

Tumor Has EGFR Amplification

- There are many EGFR inhibitors

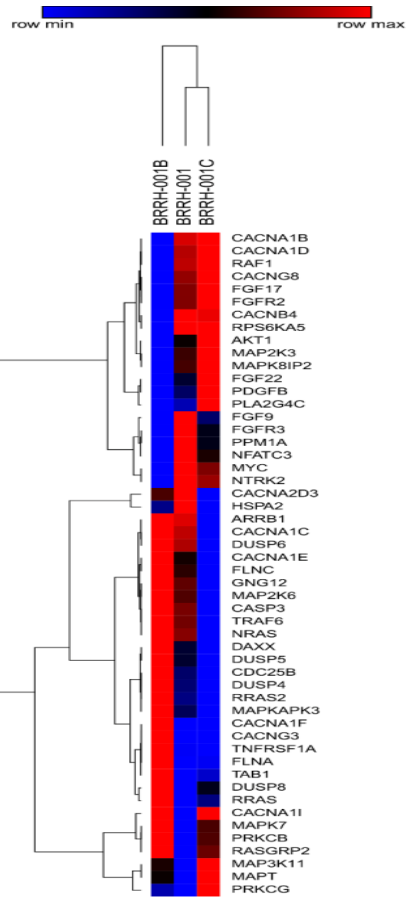
but

- GBM patients generally do not respond to them

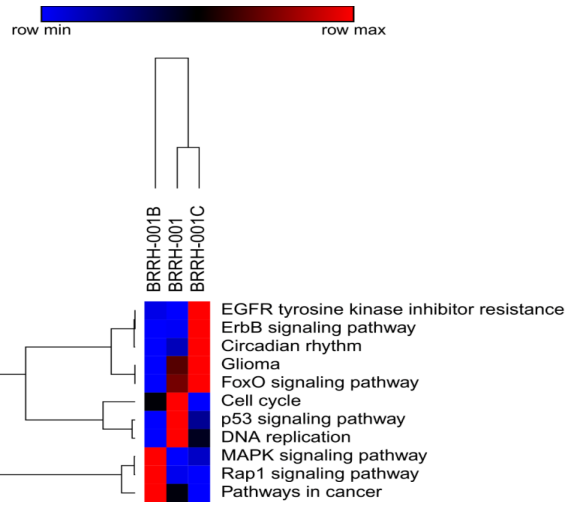


Drug Gene Network Analysis Identified 5 SOC with Potential Efficacy + Synergies

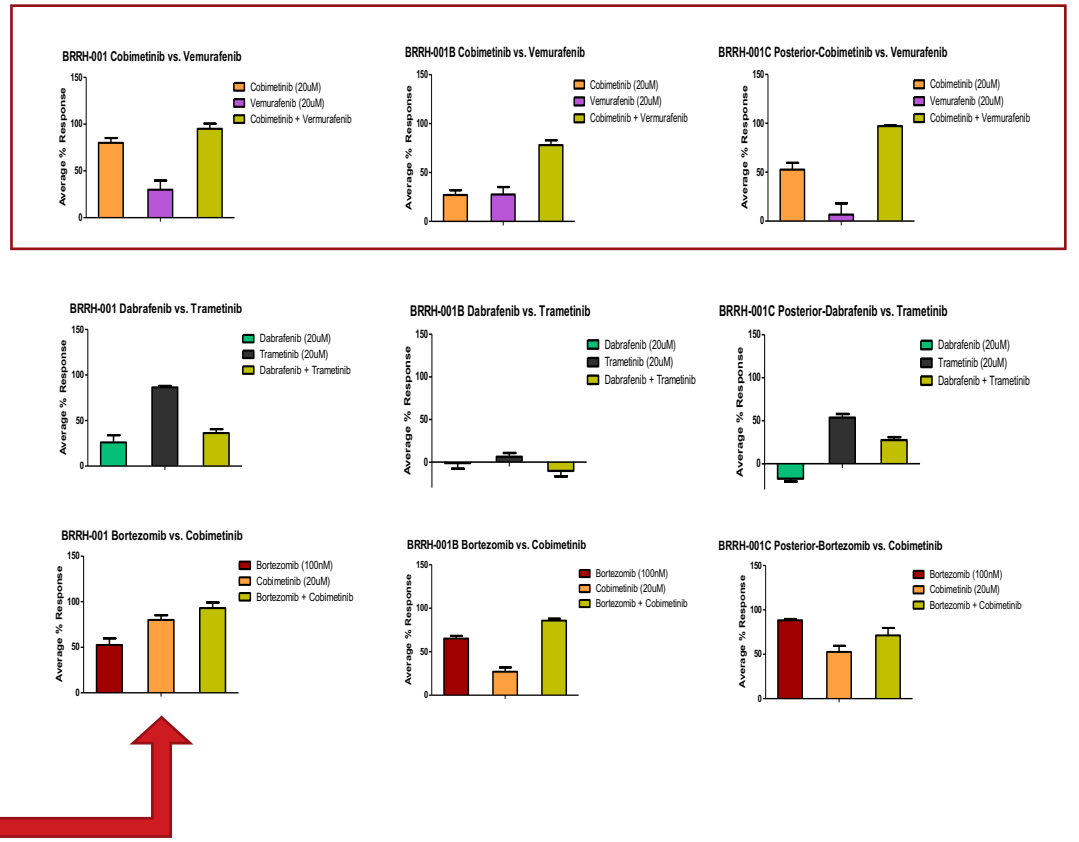
Pathways of Relative Significance



MAPK signaling pathway-Relative Significance



Drug	Gene/Protein
Cobimetinib	MAP2K1
Vemurafenib	BRAF
Trametinib	MAP2K1
Dabrafenib	BRAF
Bortezomib	PSMB



The combination of drug IC50 outcomes with genomic sequencing of the autologous tumors.
(Simina Boca, PhD, at Georgetown University)

Synergy studies following a Chou-Talalay modeling method show clear differences in the response of the combinations of drugs tested across the different samples isolated from the same patient.

UF Scripps | Biomedical Research UNIVERSITY of FLORIDA

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PART 2

Development of Sub-Q & Intracranial 3D Spheroid-Derived Xenograft Models

&

Proof-of-Concept In Vivo Pharmacology

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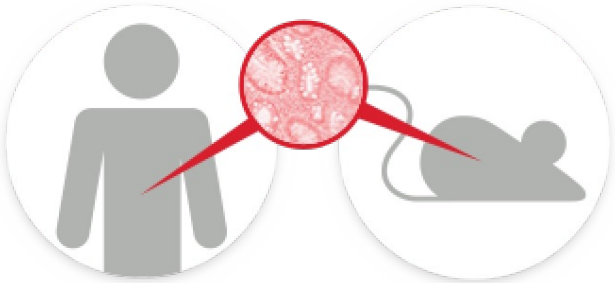
Personalized In Vivo Pharmacology Studies

STEP ONE: Initiation Study



Does the human donor tissue successfully engraft and grow in mice?

YES

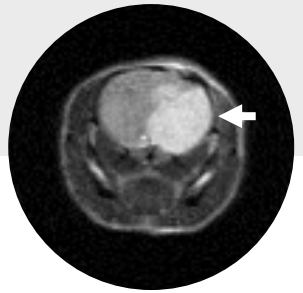
STEP TWO: O-PDX Development & Pharmacology

Mouse avatars are developed orthotopically, then multiple therapeutic options can be simultaneously tested to evaluate tumor response.

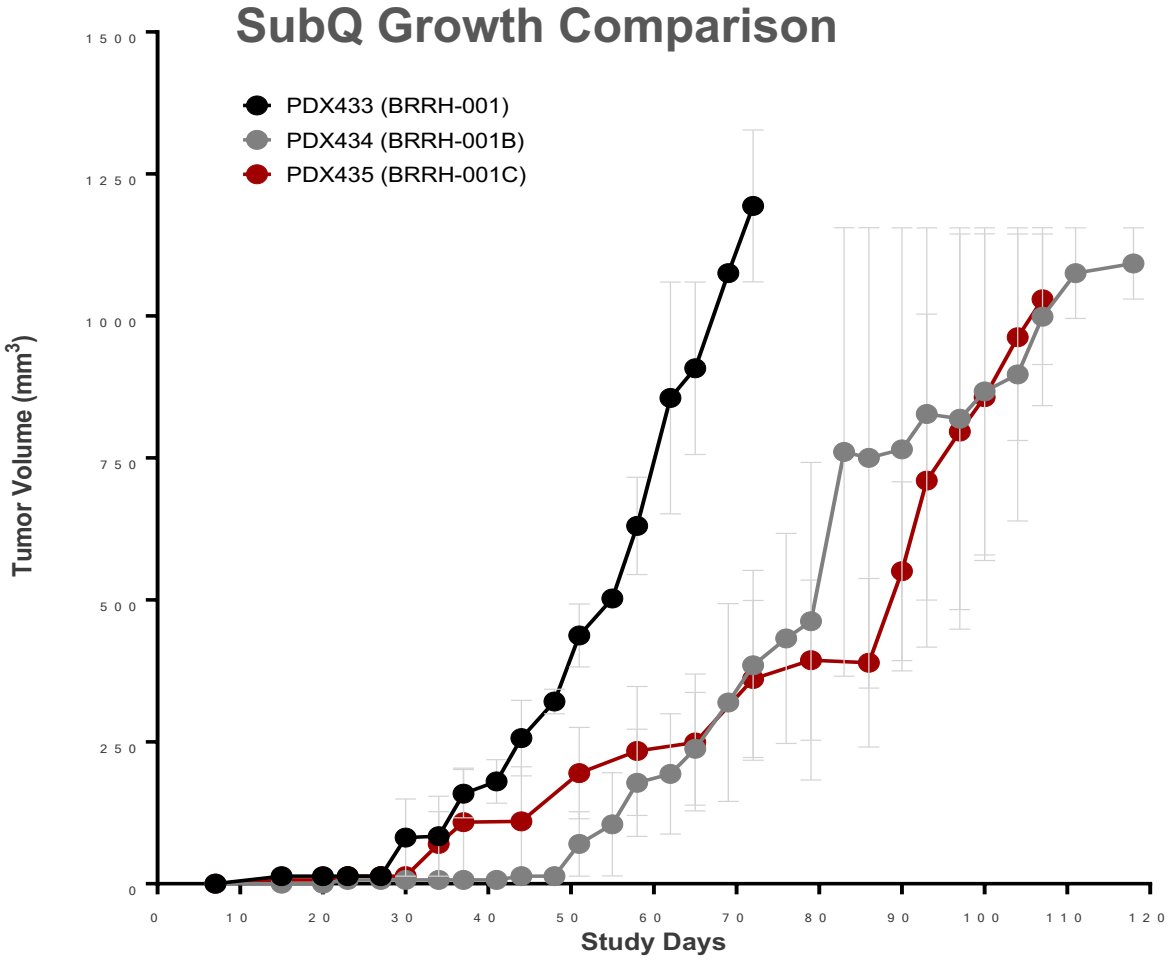
Tumor volumes are measured by MRI

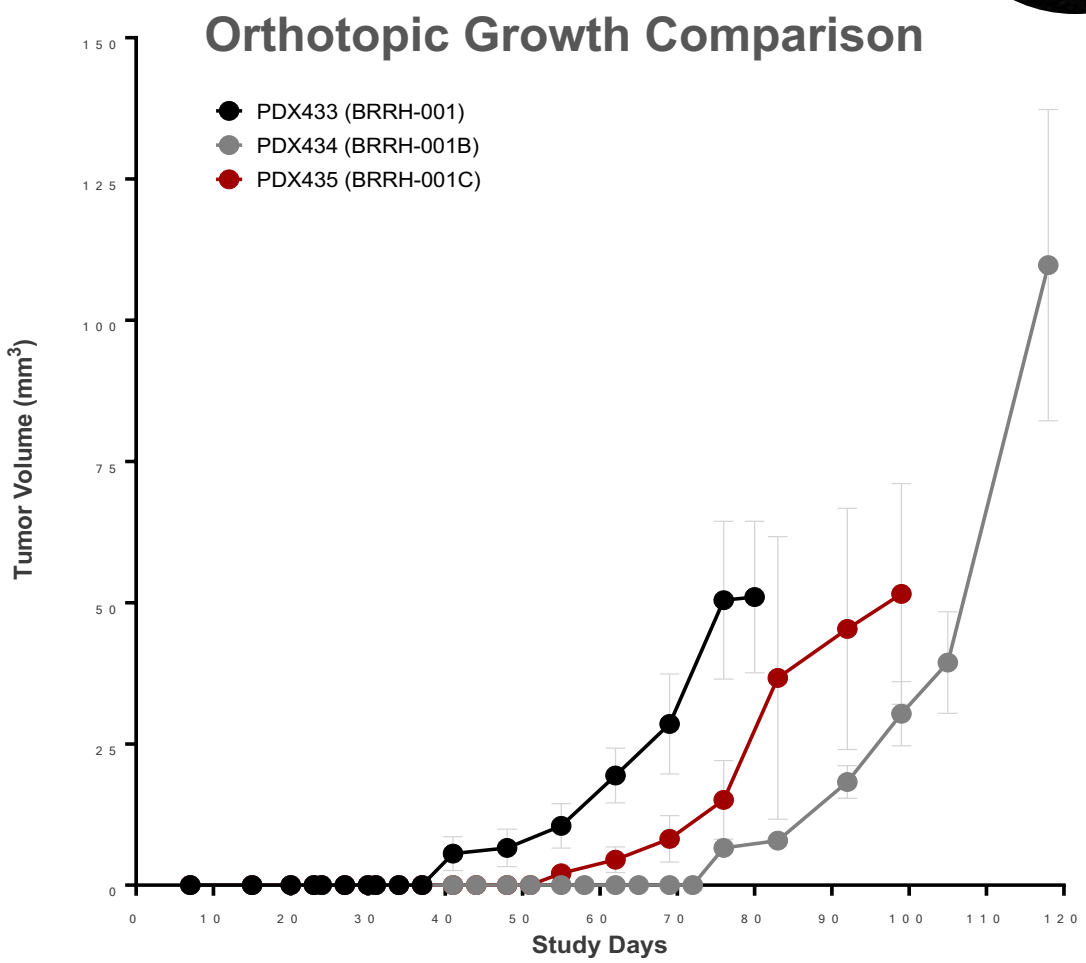
Results inform treatment decisions

Development of Sub-Q & Intracranial GBM Models



100% Engraftment Rate for Both SubQ & OT Model Builds





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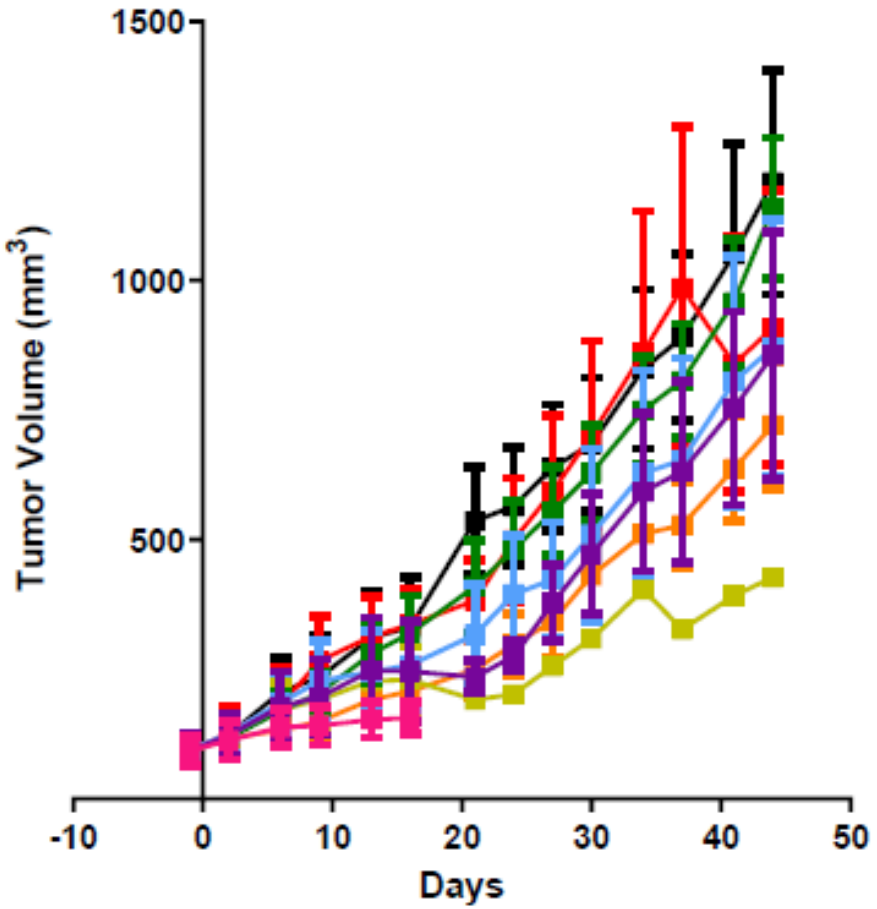
In Vivo Pharmacology - Proof of Concept

BRRH-001



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

- Group 01- Control
- Group 02- Vemurafenib Low
- Group 03- Cobimetinib Low
- Group 04- Vem. + Cobim. Low
- Group 05- Vemurafenib High
- Group 06- Cobimetinib High
- Group 07- Vem. + Cobim. High
- Group 08- Temozolomide

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PART 3

Reverse Translation of In Vivo Tumors Into 3D Spheroid Cultures & In Vitro Pharmacology Correlations

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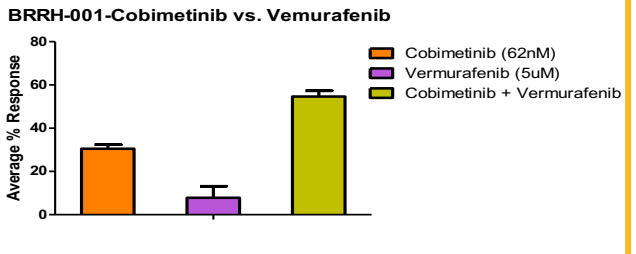
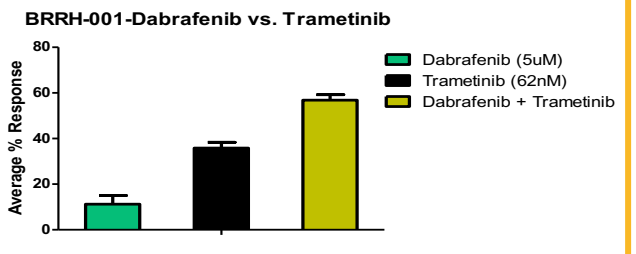
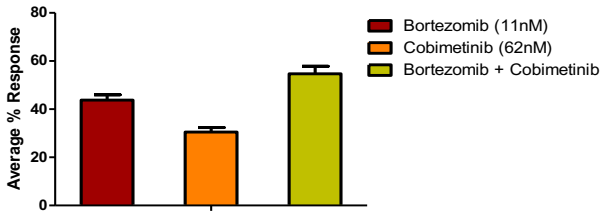
Reverse Translation of In Vivo PDX Tumors Matches Prior In Vitro Results for Some, but Not All Combinations

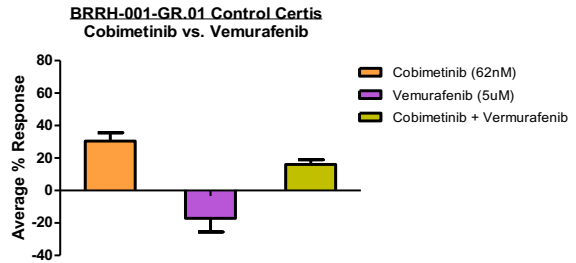
Initial Spheroids

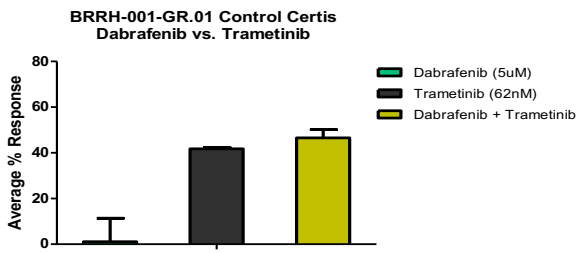


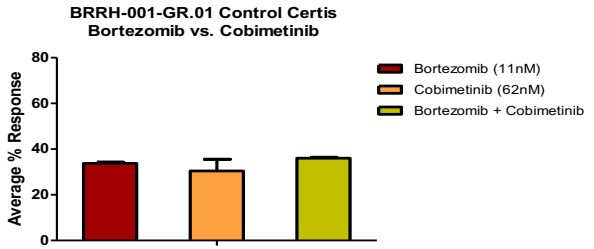




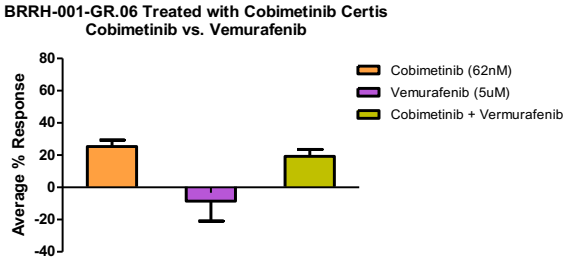
GR.01 Control

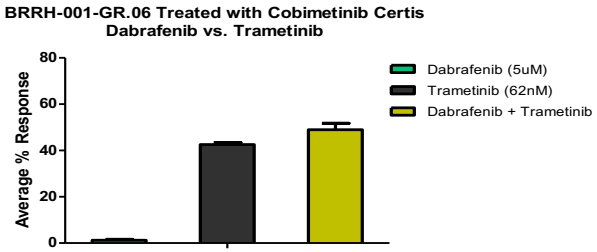


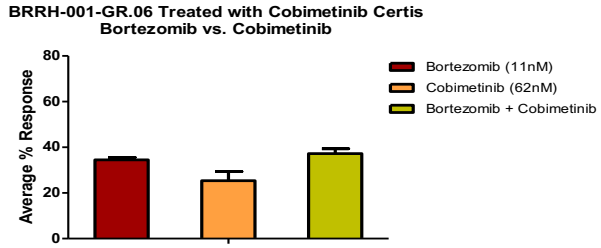




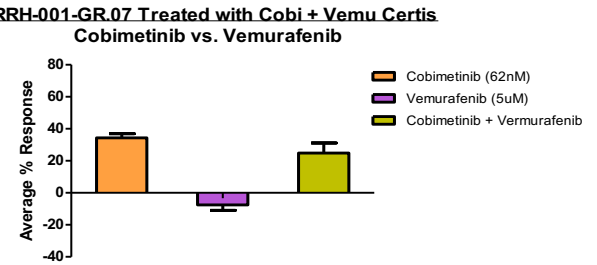
GR.06 Treated with Cobi

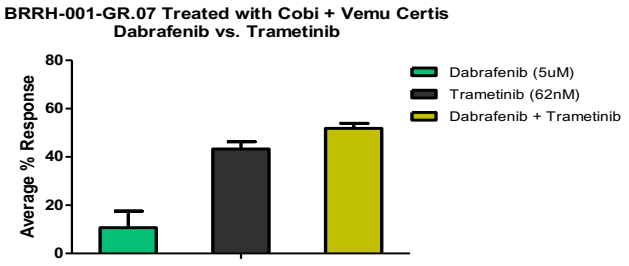


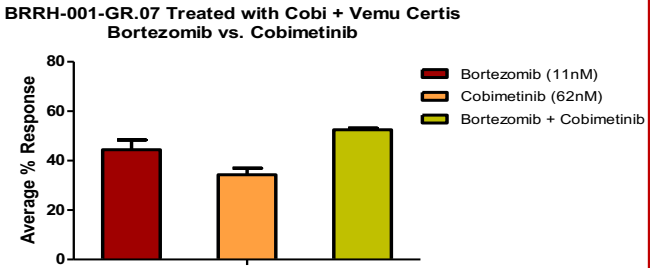




GR.07 Treated with Cobi + Vemu







FUTURE DIRECTIONS

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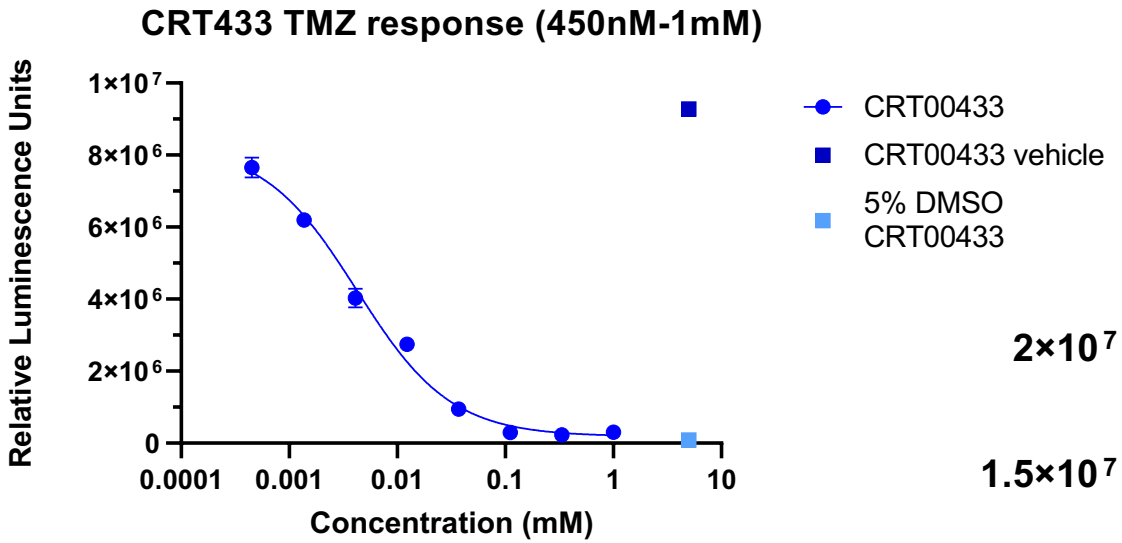
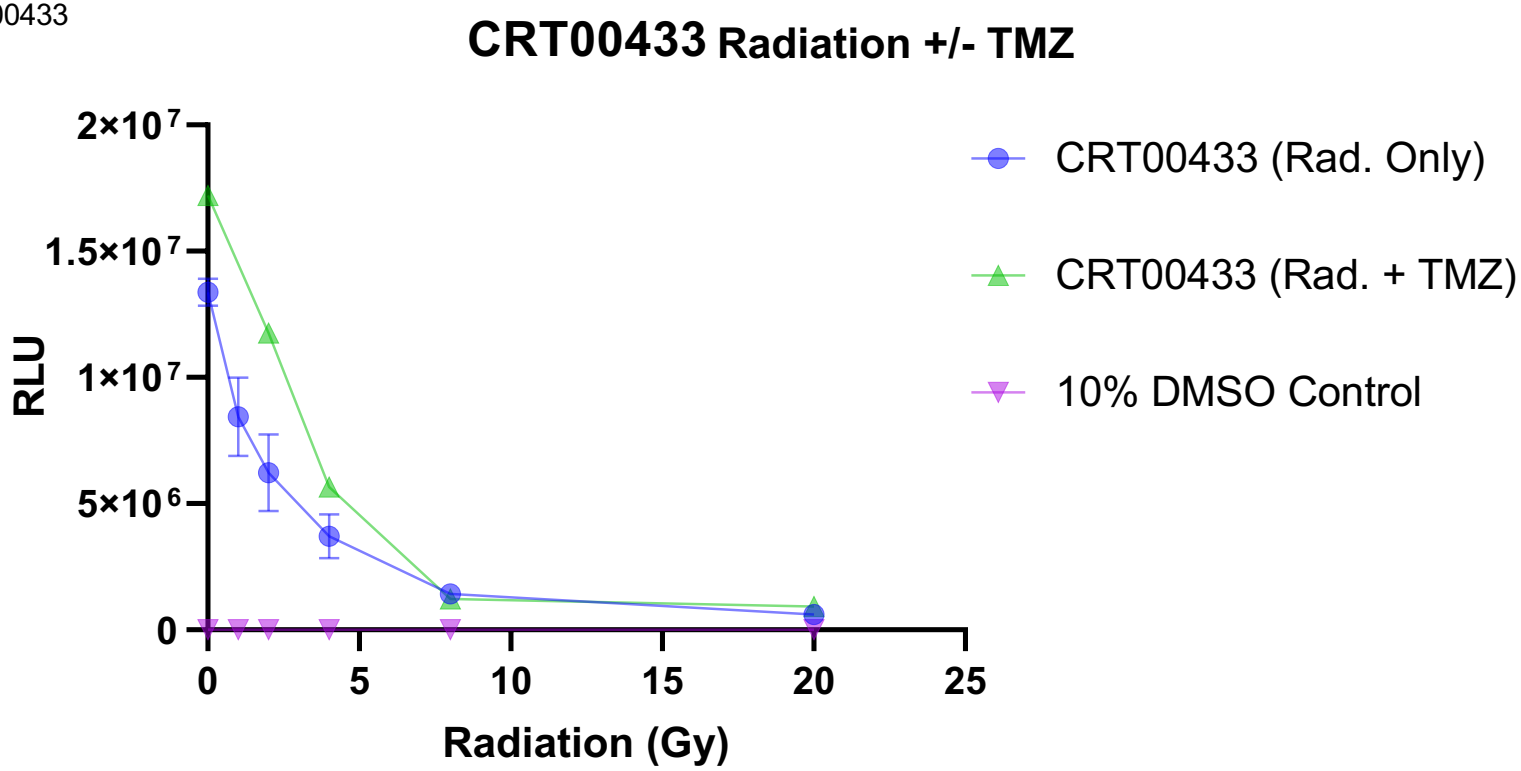
In Vitro Dose Response in Combination with Radiation



Has potential to be a fast and easy way to screen in vivo!



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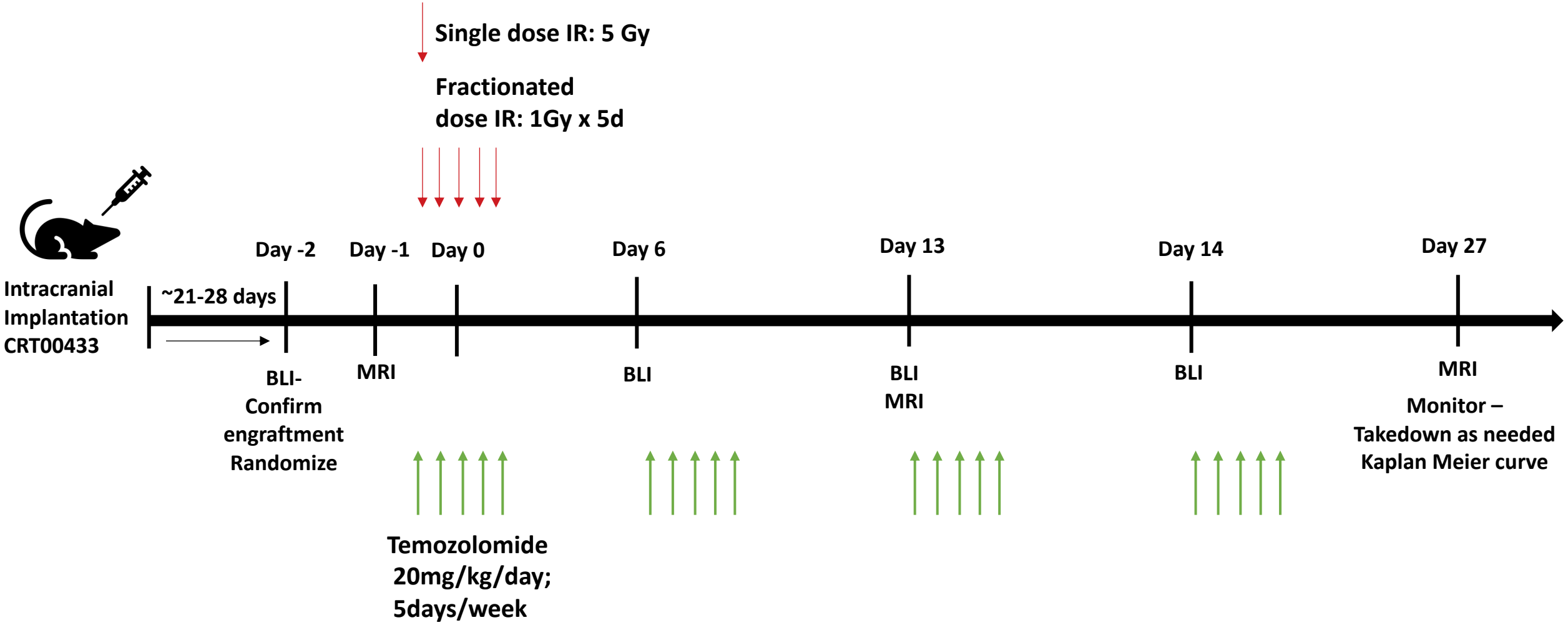
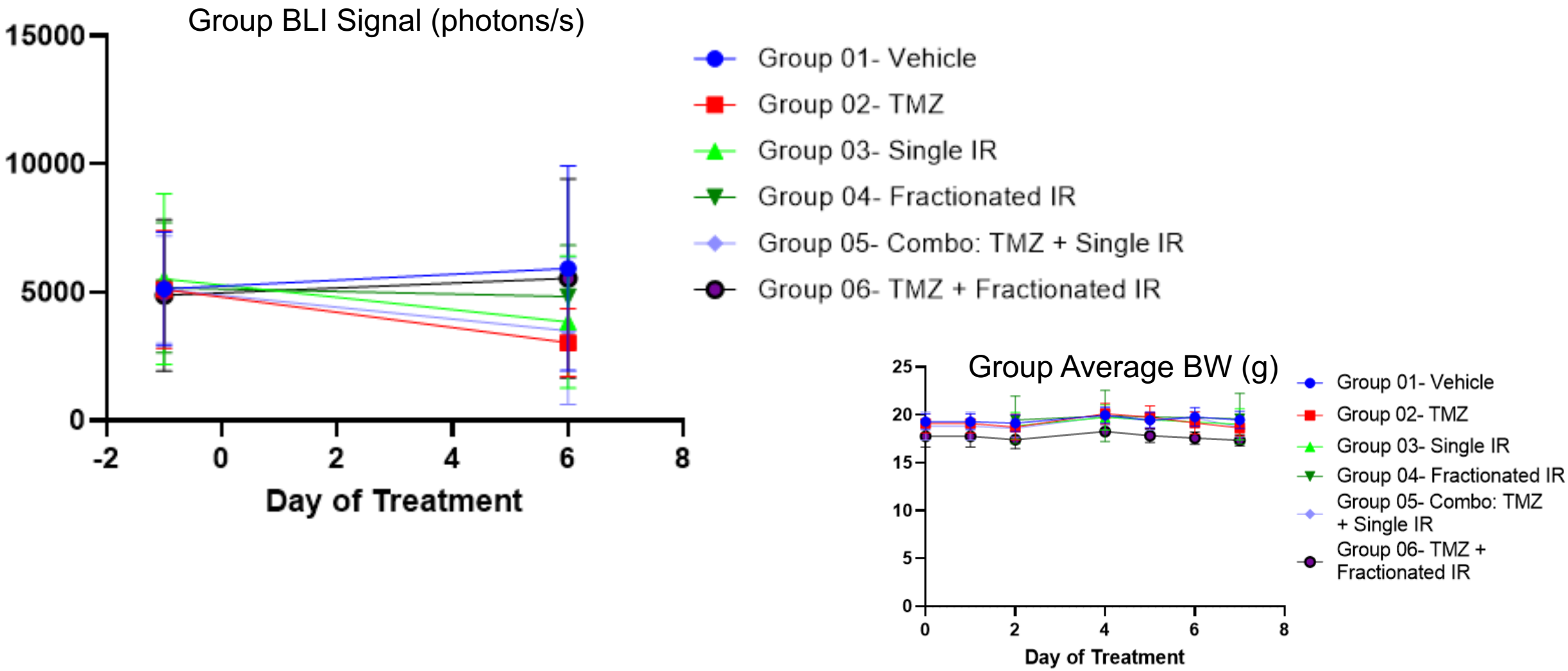
In Vivo IRT + TMZ Combination Design



Early Data… More to come!



Conclusions

High Throughput Screening of 3D Spheroids	• Rapid • Cost effective • Physiologically relevant
Drug-gene network & RNA profiling analysis	can guide us to proposed new drug combinations
DGN predictions	are remarkably effective for predicting in vitro synergy combinations
In vivo translation into animals	has been enabled as a translational proof of concept for in vitro HTS results
Reverse translation in vitro	of in vivo PDX tumors matches prior in vitro results for some, but not all combinations
HTS in vitro and in vivo tools	are validated for more testing across GBM and other solid tumors

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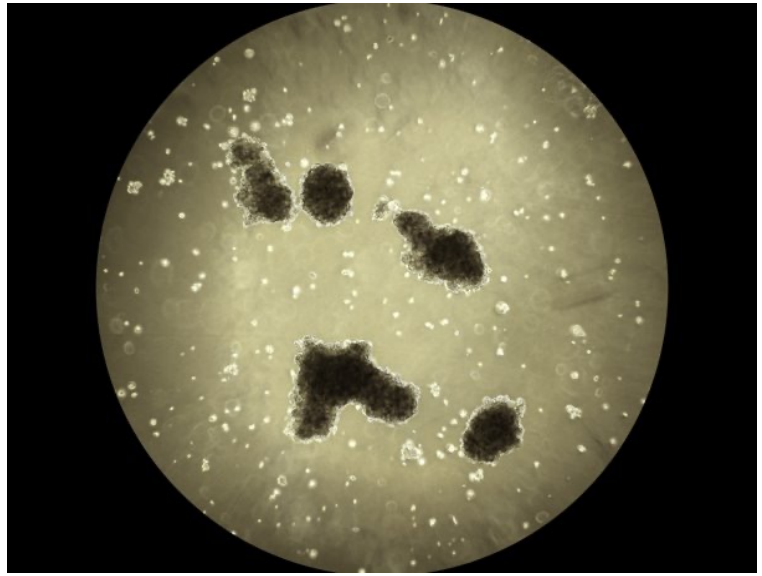
Glioblastoma 3D Spheroid In Vitro CertiScreen™

Enroll Now!

WELL-CHARACTERIZED, RICHLY ANNOTATED, LOW PASSAGE, AVAILABLE NOW



3D Spheroid Model CRT00359.
Primary brain tumor in 54-yr-old female treatment-naïve patient.

Screen Protocol:

- 3D spheroid format
- Flexible custom plate design, 384 or 96 well formats
- 4 or 7-day readout using Promega Cell Titer Glo
- Day 0 control plate upon request
- Combinations with irradiation available
- Clonogenic & migration assays, IncuCyte® imaging & analysis, etc. available

Enrollment Closes May 1!

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BarneyOI Cancer Model Database™

Registered Users Get New Customized Model Alerts!

Find the Most Clinically Relevant Model

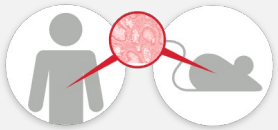
Select models from a wide range of disease states — from treatment-naïve or pretreated, primary or metastatic, orthotopic or heterotopic PDX or cell culture.

10+ terabytes
of raw characterization data

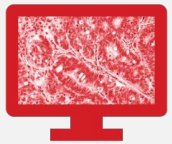
3,086,061
gene expression results compared to matched disease-free tissue

515+
growth curves

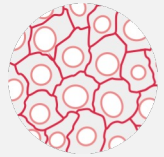
18
implantation sites



Hundreds
of highly characterized, richly annotated, low-passage, patient-consented, PDX models and matched cancer cell lines



380+
MRI and histology images



42
cancer subtypes

17
easy-to-use filters

Certis Oncology- Preclinical Oncology Services

In Vivo Studies
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Office of Laboratory Animal Welfare OLAW

PROUDLY, AN aaalac INTERNATIONAL ACCREDITED PROGRAM

CLIA Clinical Laboratory Improvement Amendments

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Acknowledgments & Publications From This Work

1. Madoux F, Tanner A, Vessels M, Willetts L, Hou S, Scampavia L, Spicer T**. A 1536-Well 3D Viability Assay to Assess the Cytotoxic Effect of Drugs on Spheroids**. *SLAS Discovery*. 2017 Jan. PMID:28346088
2. Singhera F, Cooper E, Scampavia L, Spicer T. **Using bead injection to *model dispensing of 3-D multicellular spheroids into microtiter plates***. *Talanta*. 2017 Sep. PMID:29108585
3. Wolff R, Wang-Gillam A, Alvarez H, Tiriac H, Engle D, Hou S, Groff A, San Lucas A, Bernard V, Allenson K, Castillo J, Kim D, Mulu F, Huang J, Stephens B, Wistuba I, Katz M, Varadhachary G, Park Y, Hicks J, Chinnaiyan A, Scampavia L, Spicer T, Gerhardinger C, Maitra A, Tuveson D, Rinn J, Lizee G, Yee C, Levine A. **Dynamic changes during the treatment of pancreatic cancer.** Oncotarget. 2018 Feb. PMID:29599906
4. Hou S, Tiriac H, Sridharan B, Scampavia L, Madoux F, Seldin J, Souza G, Watson D, Tuveson D, Spicer T. **Advanced Development of Primary Pancreatic Organoid Tumor Models for High-Throughput Phenotypic Drug Screening.** *SLAS Discovery*. 2018 Apr. PMID:29673279
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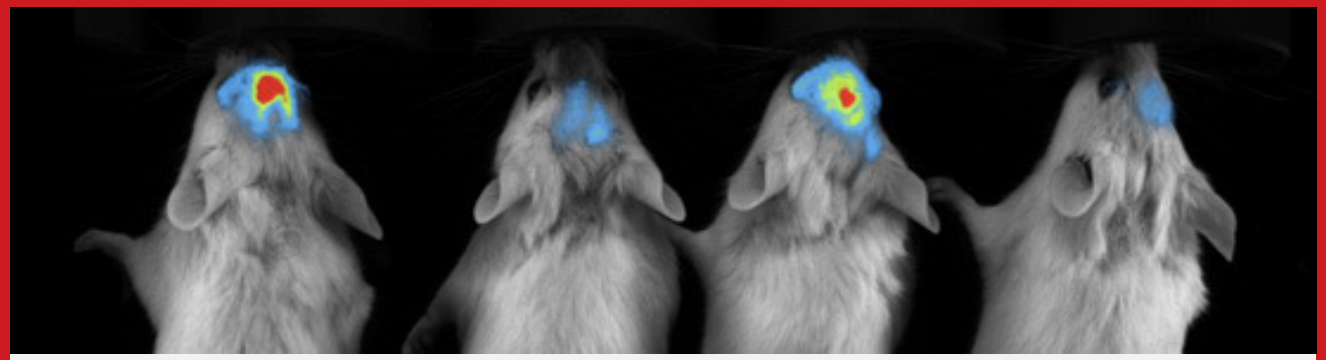

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