

UF Scripps

High-Throughput Molecular
Screening Center
BIOMEDICAL RESEARCH

“Chemoresponse Profiling of Patient Derived GBM for Precision Oncology”

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The Herbert Wertheim UF Scripps Institute

UFHCC Cancer Targeting and Therapeutics Retreat

February 22nd, 2024

<https://hts.UF Scripps.ufl.edu/>

Introduction to UF Scripps Molecular Screening Center

Where **robotics, chemistry and biology** join forces to help discover **new drugs**



- Our institute and screening center began in 2005
- Biologists, Biochemists, Programmers, Chemists & Engineers (>500 employees)
- Industrial scale HTS lab with Kalypsys/GNF automated platform
 >666K Proprietary (largest in academia, ~30k unique compounds, focused sub-libraries, professionally curated) and a >360K Public Domain (NIH)
- Funding is driven by NIH grants and Collaborations with Pharma and Biotech



HTS Robotic Platform



1.7m Arm

Incubators

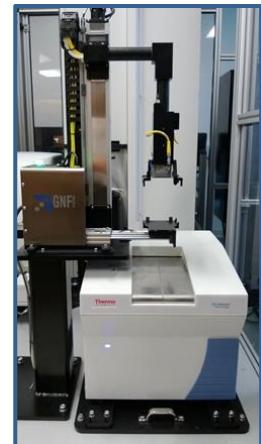
Transfer

Dispense/Wash

PE Suite

FLIPR

- Built specifically for 1536-well plate screening
- Patented lid prevents evaporation of plate contents
- Long (>96 hr) plate incubation protocols possible
- Plate capacity: 2.3 million wells when in 1536-well plate format
- Plate incubation from 4° to 50°C, 0-100% RH, any gas (CO₂, N₂, Ar, etc.), hypoxic
- 1536-well plate dispenser/ washer enable heterogeneous assays/fixing steps
- Luminescence, BRET, Absorbance, Fluorescence Intensity, FP, TRF, FRET, TR-FRET, AlphaScreen, AlphaLISA, FLIPR, HCA/High-Content...

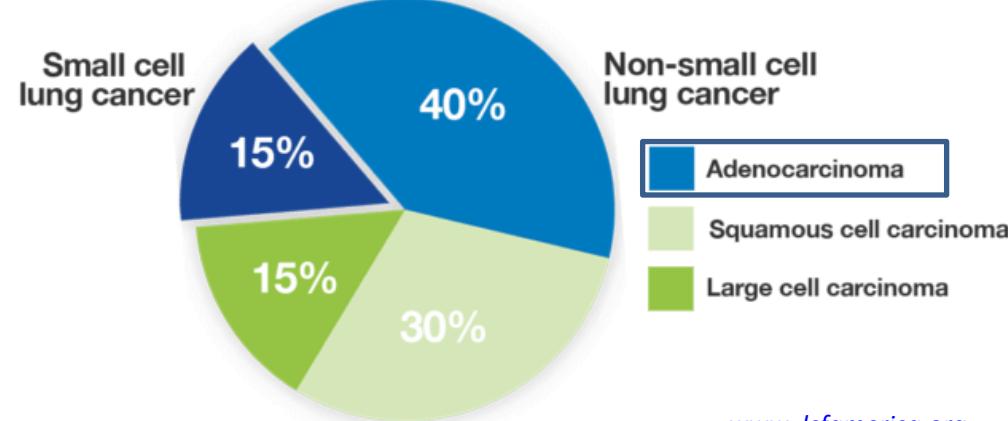
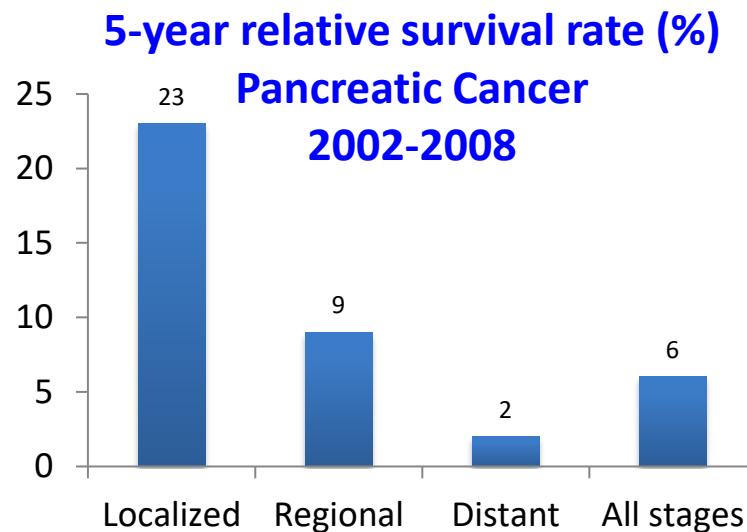


CellInsight

What's the goal?

Cancer patients need help and their providers need to be better enabled!

1. GBM brain cancer patients with <15-month survival outcome.
2. Pancreatic cancer remains a leading cause of cancer-associated death, with a 5-year survival rate less than 10%, or worse when metastatic.
3. Non-small-cell lung cancer (NSCLC), a heterogeneous class of tumors that represents approximately 85% of all new lung cancer diagnoses.
4. OMICs and Multi-Omics data is limited in its prediction purpose
5. Animal testing is slow and expensive



www.lcfamerica.org

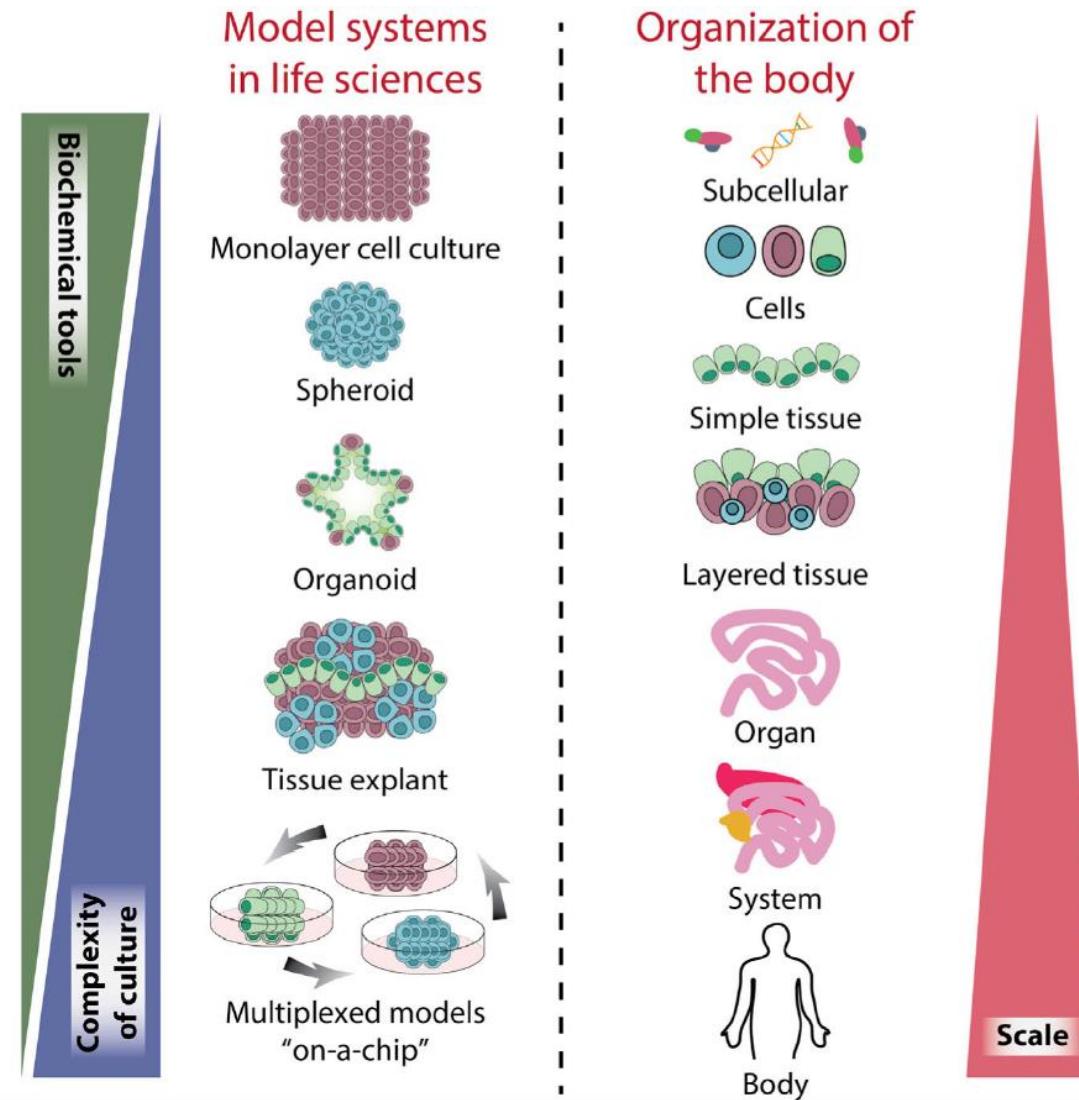
S.F. Boj, D.A. Tuveson, et.al. Cell, 2015, 160: 324

Rubio et al., G3 (Bethesda). 2018 Nov; 8(11): 3627–3636

Baek et al., Scientific Reports volume 10, Article number: 18951 (2020)

Herrman et al., Briefings in Bioinformatics, Volume 22, Issue 3, May 2021

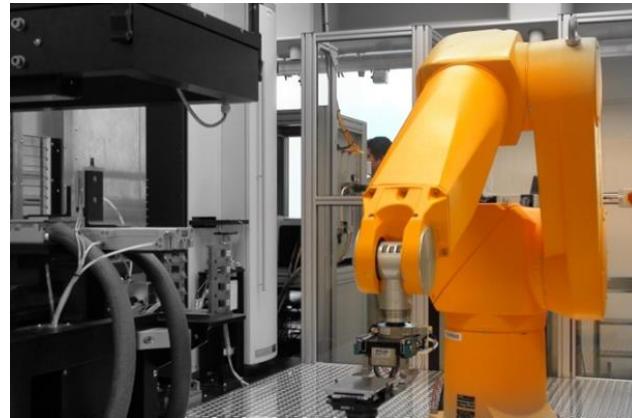
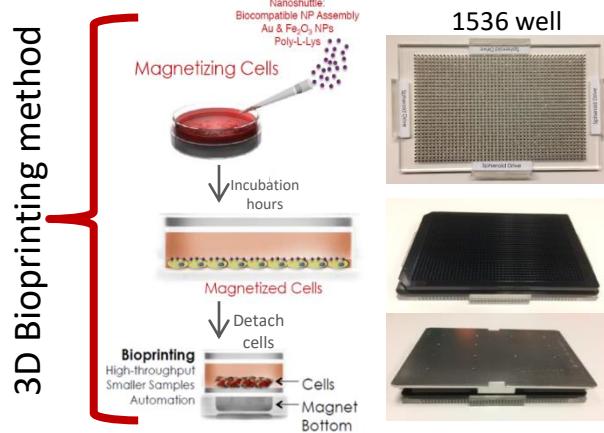
3D HTS Formats are More Physiologically Relevant



Our Approach-3D Spheroid Technologies: HTS

Plate Technology #1

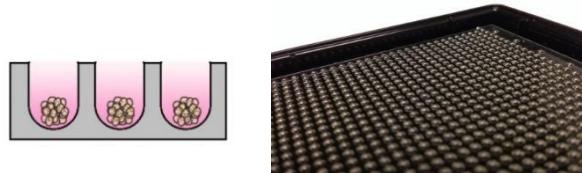
Greiner m3D Technology



- Scalable and automation friendly
- Miniaturizable to 1536-well plate format
- Single Spheroid per well and centrally located within the well
- Well-to-well uniformity of spheroid size and morphology
- Homogenous format (no transfer of spheroids or aspiration step required)

Plate Technology #2

Corning ULA spheroid Microplates

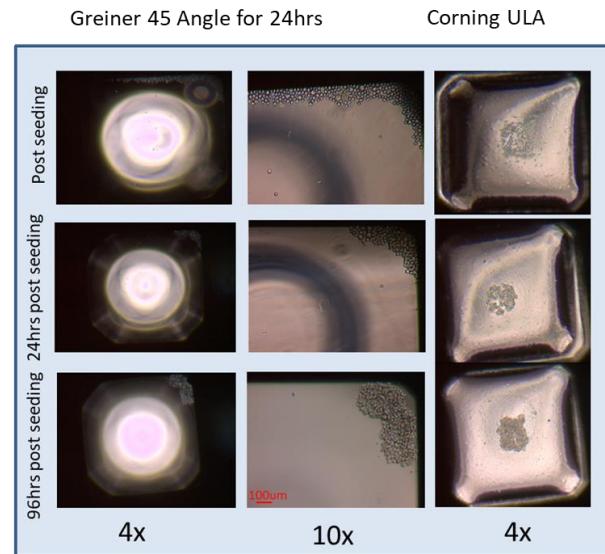


Baillargeon et al *SLAS Technology*. 2019 June. [PMID:31225974](#)

NIH IMAT Technology Grant: 3R33CA206949 PI Spicer, Co-I Scampavia

Advanced Development and Validation of 3 Dimensional Spheroid Culture of Primary Cancer Cells using Nano3D Technology

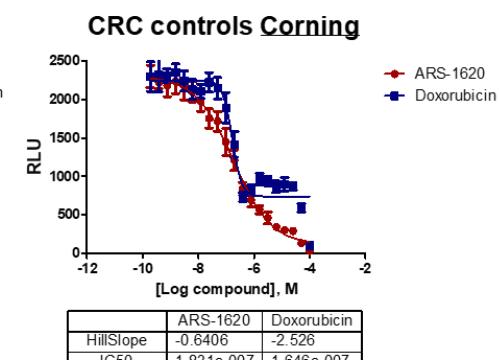
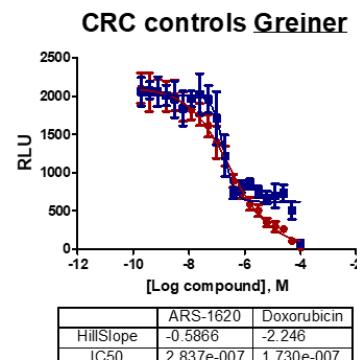
Bigger, Better, Faster, Cheaper



Greiner Angle Method vs. Corning ULA Method

%CV	S/B	Z	Z'
25.38	154.80	0.231	0.688

%CV	S/B	Z	Z'
27.16	144.01	0.044	0.679

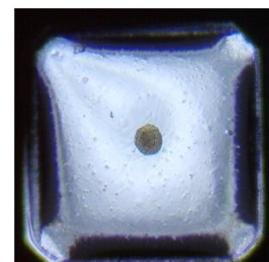


Who Cares? The Patients and Providers

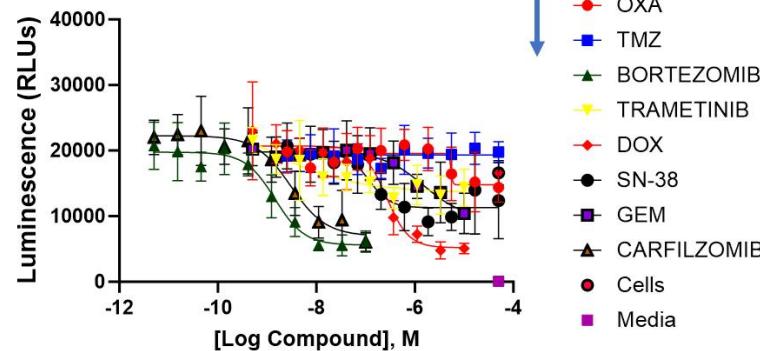


Patient tumors are isolated in Gainesville and sent to Jupiter

Tissue Cell Dissociator



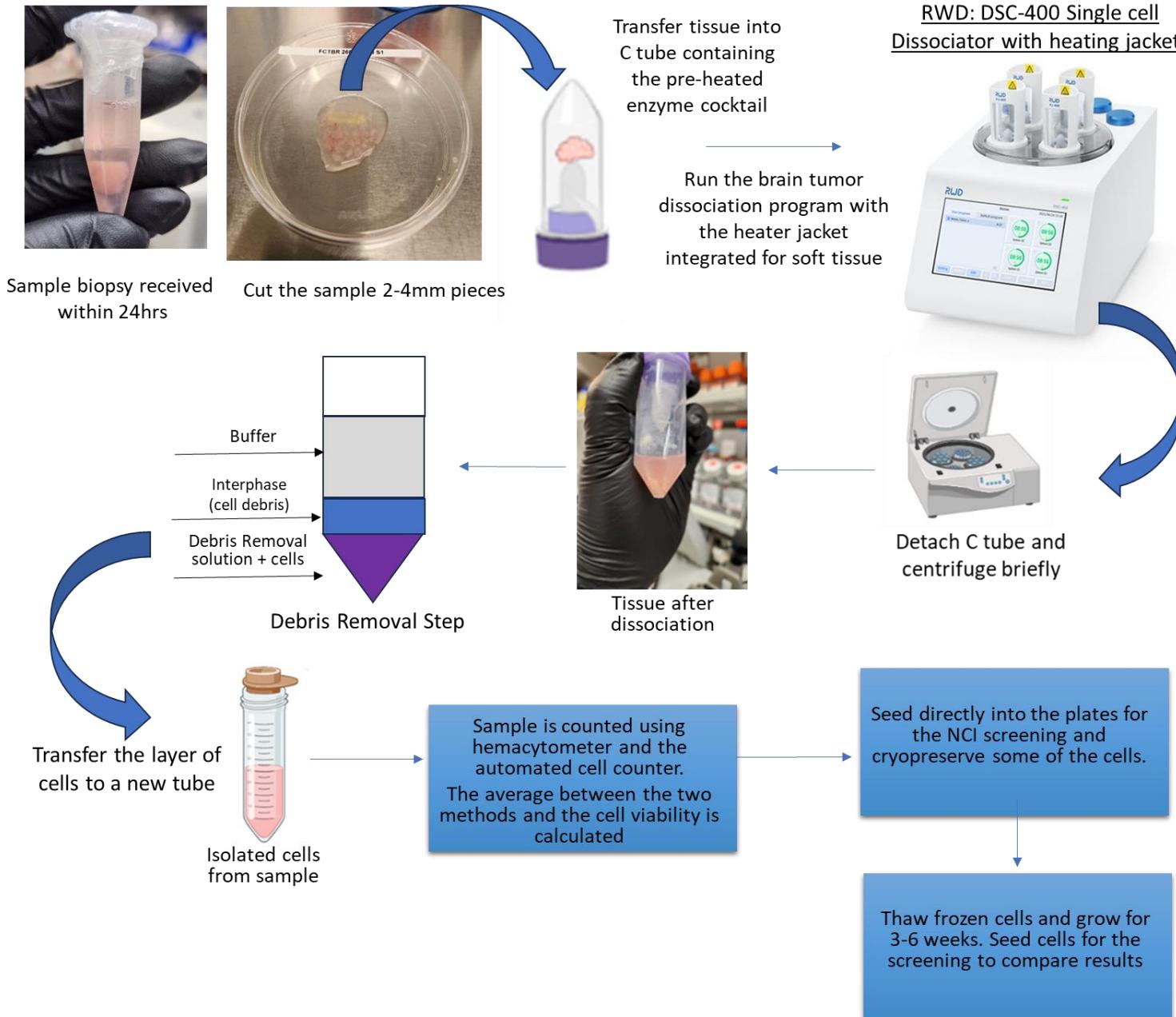
Short list of patient-specific efficacious drugs



	OXA	TMZ	BORTEZOMIB	TRAMETINIB	DOX	SN-38	GEM	CARFILZOMIB
HillSlope	Unstable	Unstable	-1.450	-0.4355	-1.911	-2.056	-1.051	-1.214
IC50	5.408e-006	Unstable	1.434e-009	3.894e-010	2.645e-007	1.182e-007	1.519e-006	3.013e-009

Slide 8

Single Cell Dissociation-Tumor Proliferation



Single Cell Dissociation-Tumor Proliferation

Greiner Cell Repellent 45 Angle Method

After dissociation, cells were seeded with culture medium: 2500 cells in 25 μ L per well in 384wpf, 500 cells in 5 μ L per well in 1536wpf Greiner Cell Repellent plates 3D plates

Plate with the cells is placed in the 45-angle device

Incubate cells for **48 hrs** | 37°C 5%CO₂ 95% RH

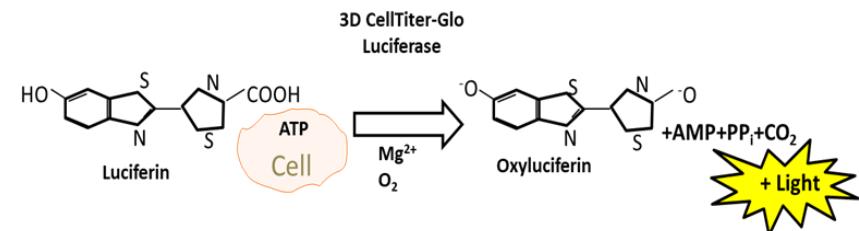
Pintool transfer compounds:
50nL for 384wpf; 10 nL for 1536wpf

Incubate cells for additional **3 days**
| 37°C, 5%CO₂ 95% RH

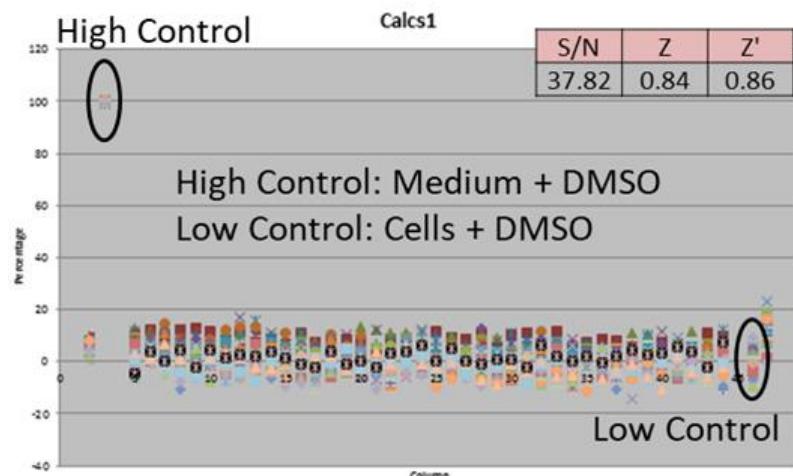
Dispense CellTiter-Glo 3D reagent: 25 μ L per well in 384wpf,
5 μ L per well in 1536wpf.
Incubate for 60min at RT, Read Luminescence



Final volume = 50 μ L in 384wpf; 10 μ L in 1536wpf



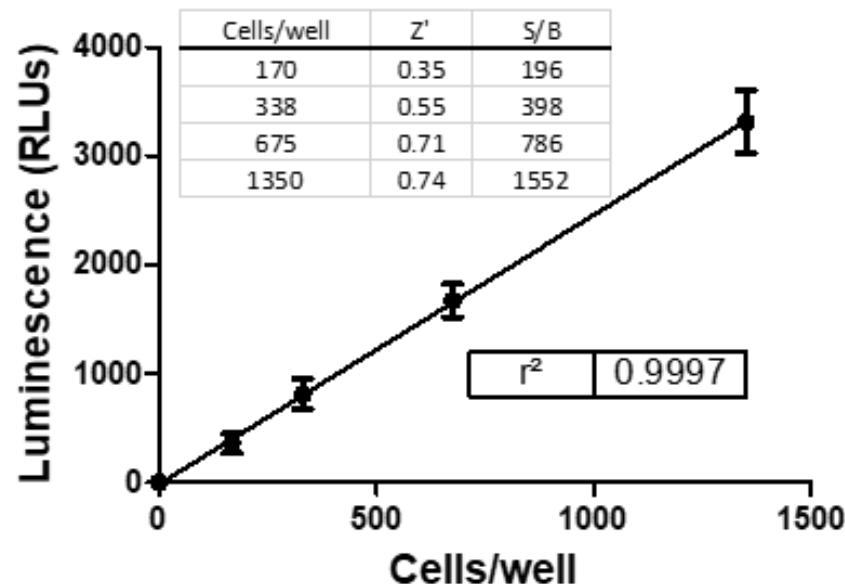
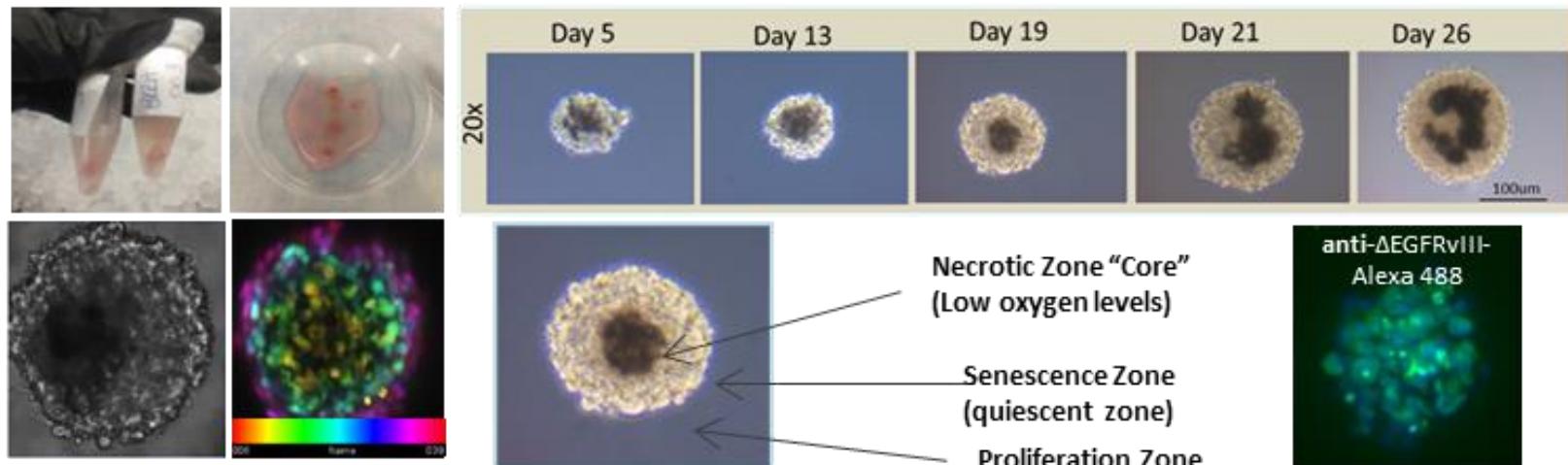
Example of DMSO Plate



$$Z' = 1 - \frac{3SD \text{ of Low Control} + 3SD \text{ of High Control}}{(Low Control - High Control)}$$

$$\% \text{ inhibition} = 100 \times \left(1 - \frac{\text{Test Well} - \text{Median High Control}}{\text{Median Low Control} - \text{Median High Control}} \right)$$

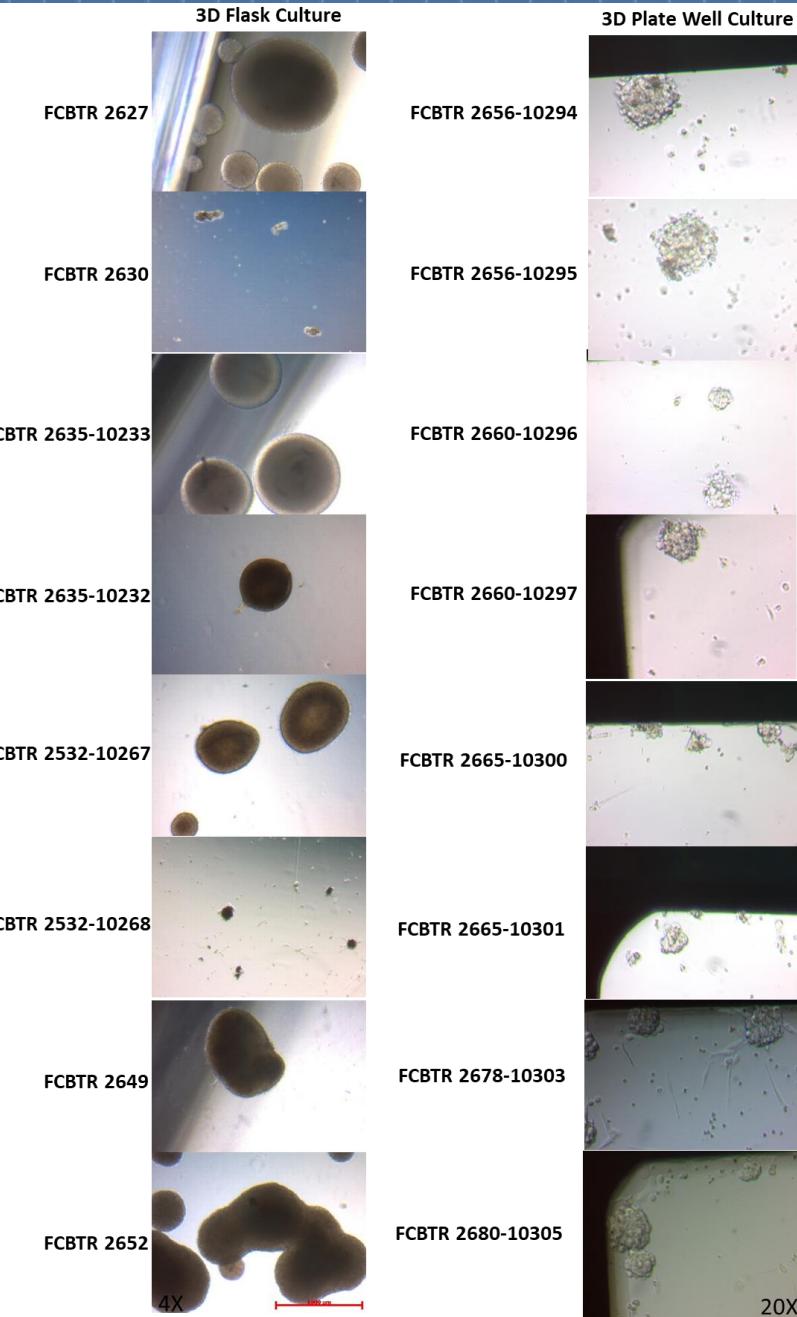
Success in Approach-GBM



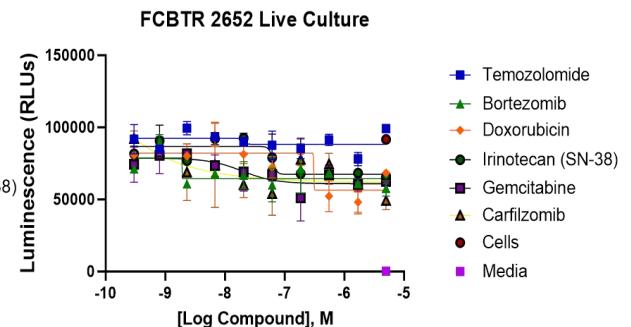
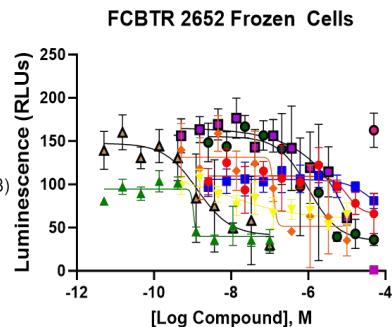
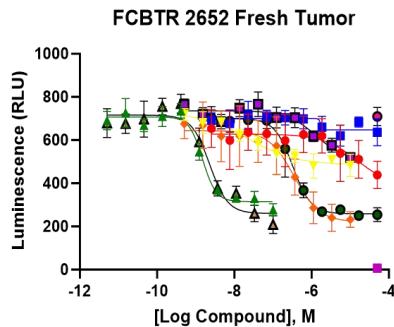
UFL (FCBTR) to UF-Scripps

Tumor Samples Tested							
Sample ID	Preliminary Diagnosis	Surgery Date	Date Processed	Weight	Estimated Number of Cells Isolated	Estimated Cell Viability (%)	Assays Performed
FCBTR 2627	Malignant Glioma	7/17/2023	7/19/2023	227mg	3.6M	80%	Cell Density Test in 1536wpf in Greiner Cell Repellent Plates, NCI Screening of 128 compounds
FCBTR 2630	Malignant Glioma	7/20/2023	7/21/2023	315mg	0.27M	60%	Cells grew very slowly, no assays were performed
FCBTR 2635-10233	Malignant Glioma	8/1/2023	8/2/2023	114mg	1.2M	80%	Cells grew very slowly, no assays were performed
FCBTR 2635-10232	Malignant Glioma	8/1/2023	8/2/2023	226mg	0.6M	60%	Cells grew very slowly, no assays were performed
FCBTR 2532-10267	Recurrent GBM	9/7/2023	9/8/2023	121mg	8.4M	30%	CRC Test in Greiner 1536wpf Cell Repellent Plates
FCBTR 2532-10268	Recurrent GBM	9/7/2023	9/8/2023	124mg	6.2M	40%	384wpf 3D spheroid formation with Nanoshuttle test in Greiner Cell Repellent Plates
FCBTR 2649	Malignant Glioma	9/25/2023	9/26/2023	63mg	4.3M	92%	384wpf 3D spheroid formation test in Greiner Cell Repellent Plates
FCBTR 2652	Malignant Glioma	9/26/2023	9/27/2023	261mg	8.4M	91%	CRC Test in 384wpf for: Greiner Cell Repellent 3D, Greiner Nanoshuttle; Corning ULA 384wpf and NCI 128 panel duplicate in 1536wpf.
FCBTR 2656-10294	Malignant Glioma	10/9/2023	10/10/2023	58mg	0.85M	92%	384wpf 3D spheroid CRC test in Greiner Cell Repellent Plates
FCBTR 2656-10295	Malignant Glioma	10/9/2023	10/10/2023	60mg	1.6M	90%	384wpf 3D spheroid CRC test in Greiner Cell Repellent Plates
FCBTR 2660-10296	Malignant Glioma	10/17/2023	10/18/2023	58mg	12.1M	93%	384wpf 3D Spheroid CRC test in Greiner Cell Repellent Plates; CRC Test in Greiner 1536wpf Cell Repellent Plates.
FCBTR 2660-10297	Malignant Glioma	10/17/2023	10/18/2023	47mg	3.5M	93%	384wpf 3D spheroid CRC test in Greiner Cell Repellent Plates; Full NCI Screen in 1536wpf
FCBTR 2665-10300	Malignant Glioma	10/30/2023	11/1/2023	221mg	13.4M	91%	1536wpf Full NCI Screen
FCBTR 2665-10301	Malignant Glioma	10/30/2023	11/1/2023	278mg	17.2M	94%	1536wpf Full NCI Screen
FCBTR 2678-10303	Malignant Glioma	12/13/2023	12/15/2023	298mg	3.86M	76%	1536wpf Full NCI Screen
FCBTR 2680-10305	Malignant Glioma	12/19/2023	12/20/2023	286mg	8.4M	91%	1536wpf Full NCI Screen

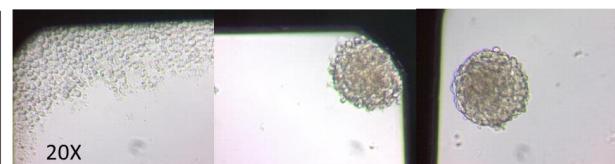
GBM Organoids



Fresh tumor vs. Frozen cells vs. Live culture

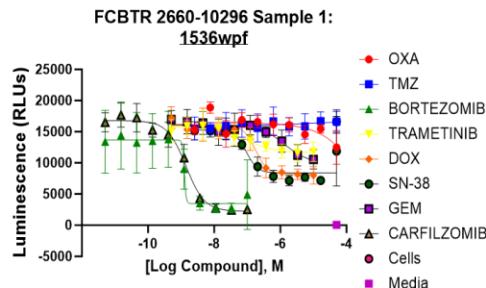
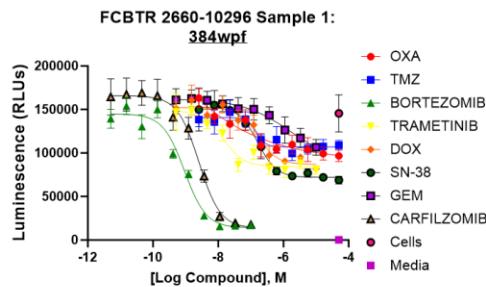


FCBTR 2652 IC50 (M)	Oxaliplatin	Temozolomide	Bortezomib	Trametinib	Doxorubicin	Irinotecan	Gemcitabine	Carfilzomib
Fresh Tumor	1.56E-05	1.73E-06	1.50E-09	2.36E-08	3.53E-07	3.25E-07	1.31E-06	2.29E-09
Frozen Cells	2.05E-08	6.12E-05	1.06E-09	9.06E-09	1.26E-07	1.01E-06	>5.00E-06	1.32E-09
Live Culture	not tested	>5.00E-06	1.21E-09	not tested	3.03E-07	6.36E-08	1.93E-08	2.95E-12



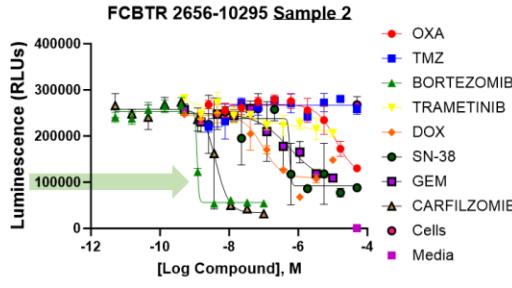
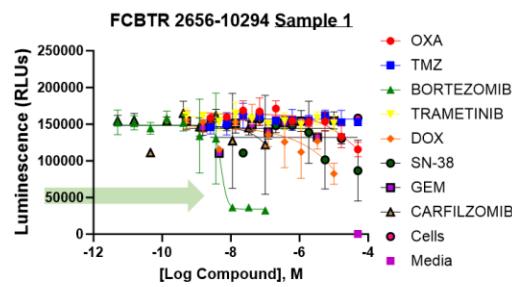
Compatibility Across Technology

FCBTR 2660-10296: 384wpf vs. 1536wpf



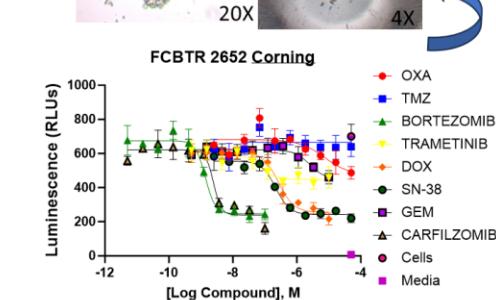
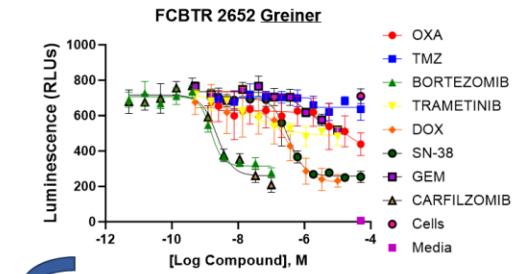
FCBTR 2660 IC50 (M)	384wpf	1536wpf
Oxaliplatin	>5.00E-05	>5.00E-05
Temozolomide	>5.00E-05	1.50E-07
Bortezomib	1.25E-09	9.22E-10
Trametinib	9.17E-08	1.41E-08
Doxorubicin	1.79E-07	1.65E-07
Irinotecan	9.24E-08	1.63E-07
Gemcitabine	1.33E-06	1.74E-06
Carfilzomib	1.40E-09	2.56E-09

FCBTR 2656: Sample Heterogeneity



FCBTR 2656 IC50 (M)	Sample1	Sample2
Oxaliplatin	5.20E-05	1.20E-05
Temozolomide	7.78E-09	8.21E-09
Bortezomib	5.22E-09	1.19E-09
Trametinib	4.19E-08	>1.00E-05
Doxorubicin	>1.00E-05	9.28E-08
Irinotecan	>5.00E-05	5.66E-07
Gemcitabine	>1.00E-05	5.54E-07
Carfilzomib	>1.00E-07	4.10E-09

FCBTR 2652: Greiner vs. Corning

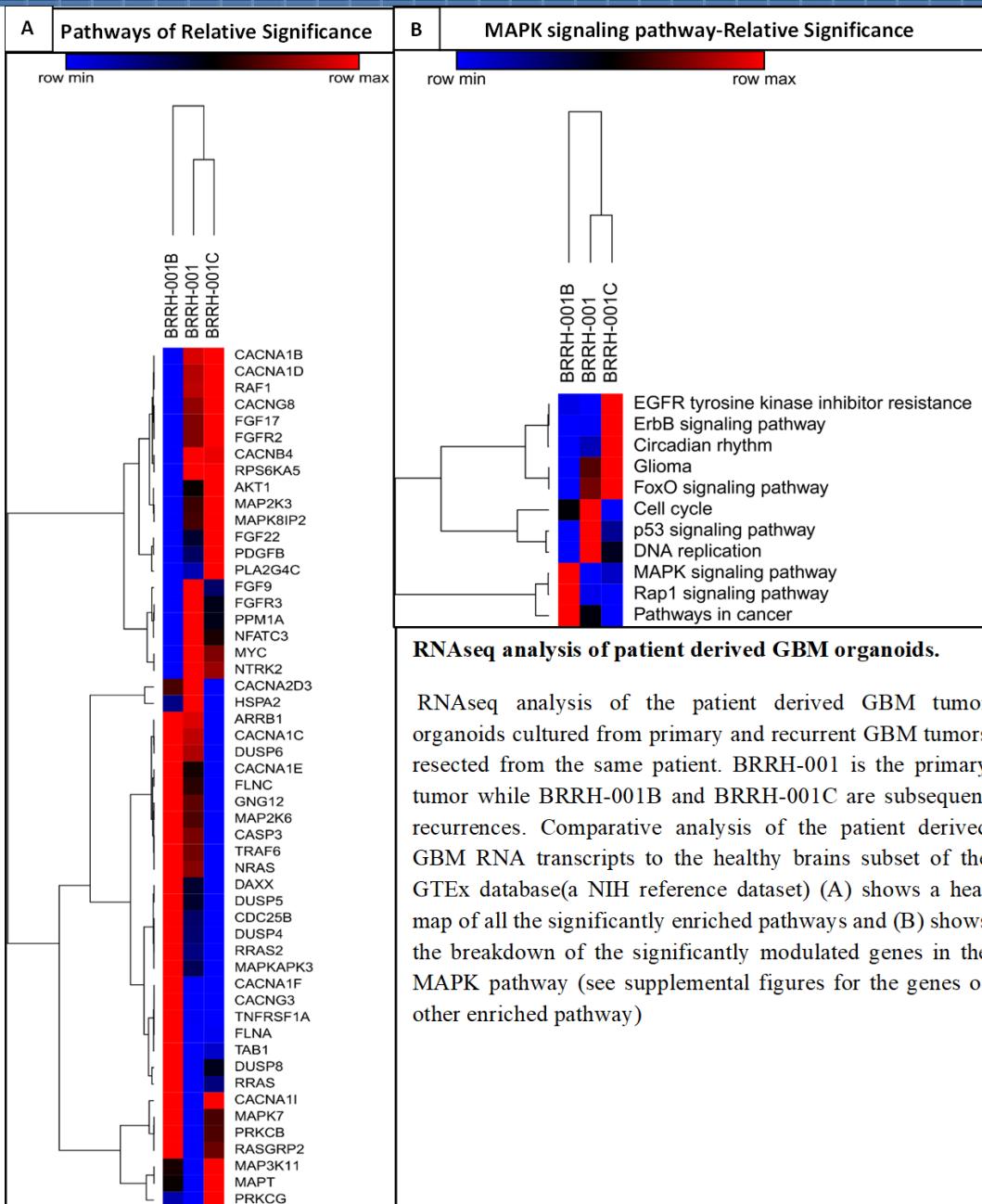


FCBTR 2652 IC50 (M)	Greiner	Corning
Oxaliplatin	1.56E-05	7.39E-06
Temozolomide	1.73E-06	2.35E-08
Bortezomib	1.50E-09	1.38E-09
Trametinib	2.36E-08	6.92E-08
Doxorubicin	3.53E-07	2.67E-07
Irinotecan	3.25E-07	2.19E-07
Gemcitabine	1.31E-06	2.56E-06
Carfilzomib	2.29E-09	2.47E-09

Heterogeneity in Drug Response Across Patients

SAMPLEID	Structure	Vendor Catalog	Molecular Weight	DRUG NAME	Dose Response Curves FCTBR 2652 (pink), FCTBR 2660-10296 (blue), FCTBR 2665-10301 (green)	FCTBR 2652 Final IC50 (N)	FCBTR-2660- 10296 Final IC50 (M)	FCBTR-2665- 10301 Final IC50 (M)			
SR-05000705132-4		NSC758254	517.122			>	6.8E-9	=	10.4E-9	>	6.8E-9
SR-01000000186-17		NSC67574	824.958	Vincristine		=	16.9E-9	>	5.E-6	>	5.E-6
SR-01000000155-14		NSC49842	810.974	Vinblastine		=	60.3E-9	>	5.E-6	>	5.E-6
SR-05003256487-1		NSC826942	679.492			=	833.8E-9	=	1.1E-6	=	4.6E-6
SR-05003256462-1		NSC774769	560.637			=	2.1E-6	>	5.E-6	>	5.E-6
SR-01000939863-7		NSC756655	384.237	Bortezomib		>	5.E-6	=	1.5E-9	=	1.7E-9
SR-01000000033-15		NSC82151	527.520	Daunorubicin		>	5.E-6	=	219.5E-9	=	559.9E-9

Drug gene networking analysis of patient derived GBM spheroids



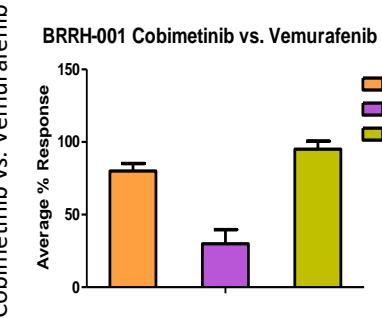
GBM- Patient Study

Drug Gene Network Analyses Proposed Drug Option Treatments

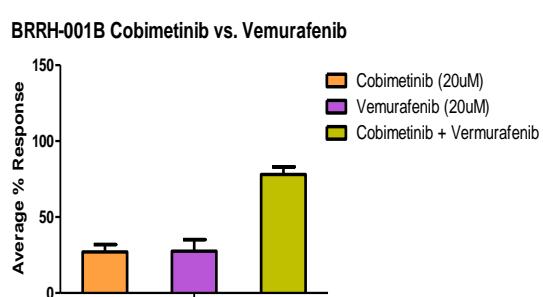
Drug	Gene/protein
Cobimetinib	MAP2K1
Vemurafenib	BRAF
Trametinib	MAP2K1
Dabrafenib	BRAF
Bortezomib	PSMB

Chemoresponse profiling-GBM

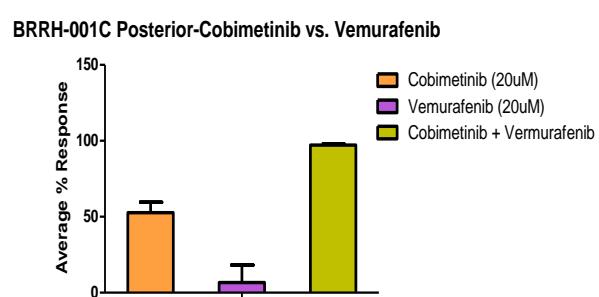
BRRH-001



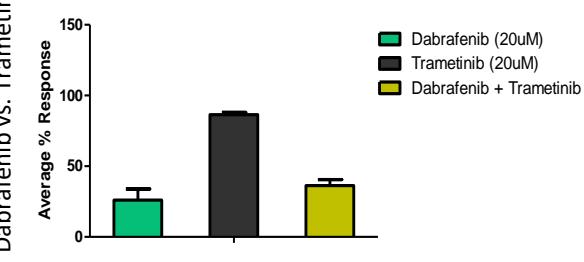
BRRH-001B



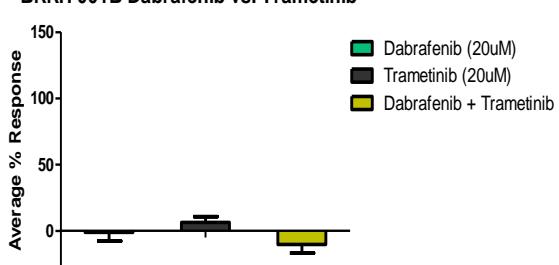
BRRH-001C Posterior



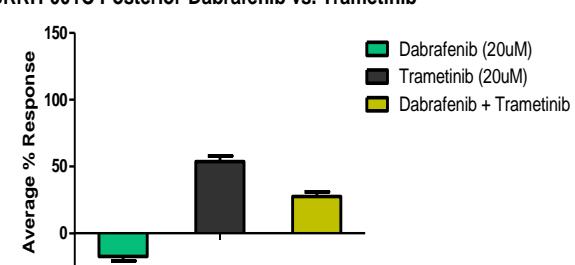
BRRH-001 Dabrafenib vs. Trametinib



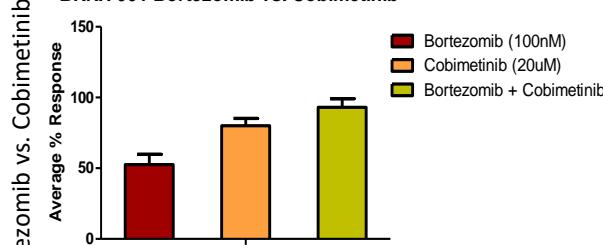
BRRH-001B Dabrafenib vs. Trametinib



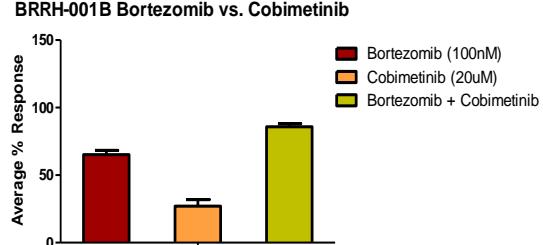
BRRH-001C Posterior-Dabrafenib vs. Trametinib



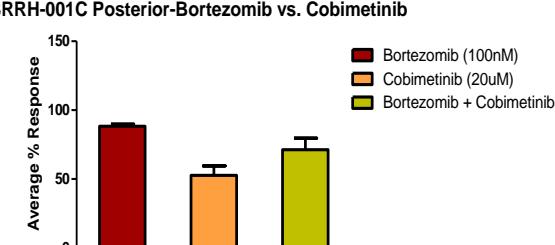
BRRH-001 Bortezomib vs. Cobimetinib



BRRH-001B Bortezomib vs. Cobimetinib

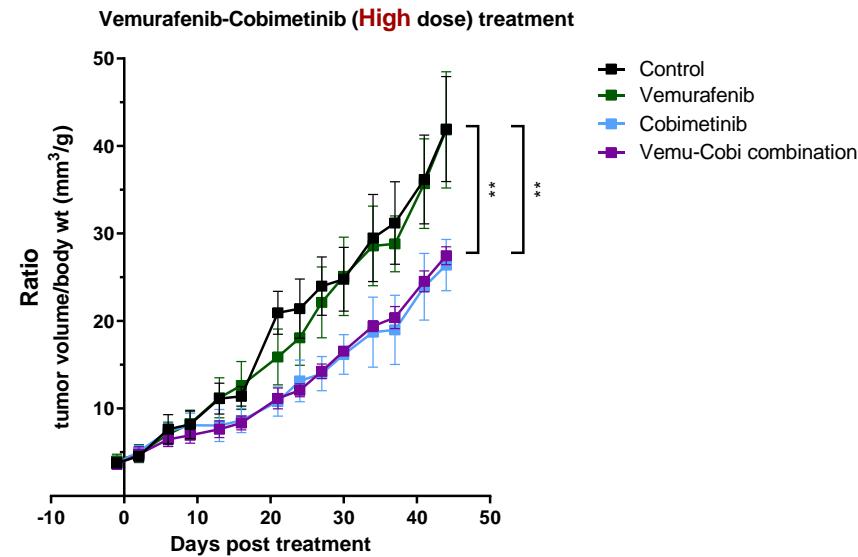
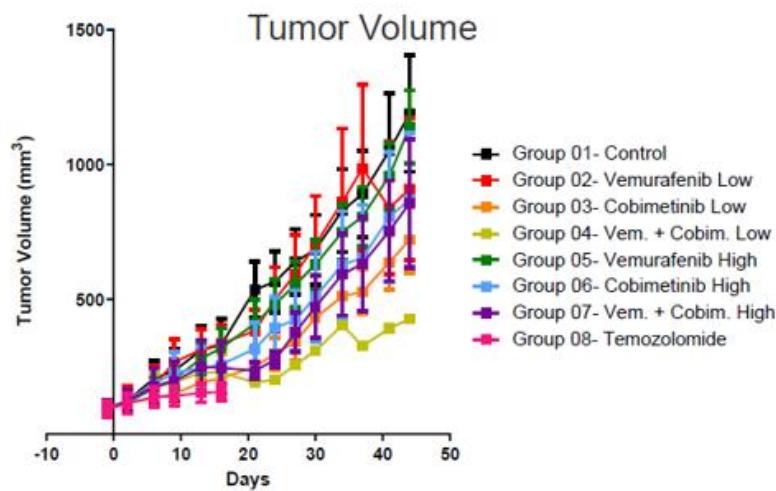


BRRH-001C Posterior-Bortezomib vs. Cobimetinib



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

In-Vivo Pharmacology (Day 44)



In Vivo Study Design (BRRH-001 SubCu): Proof of Concept

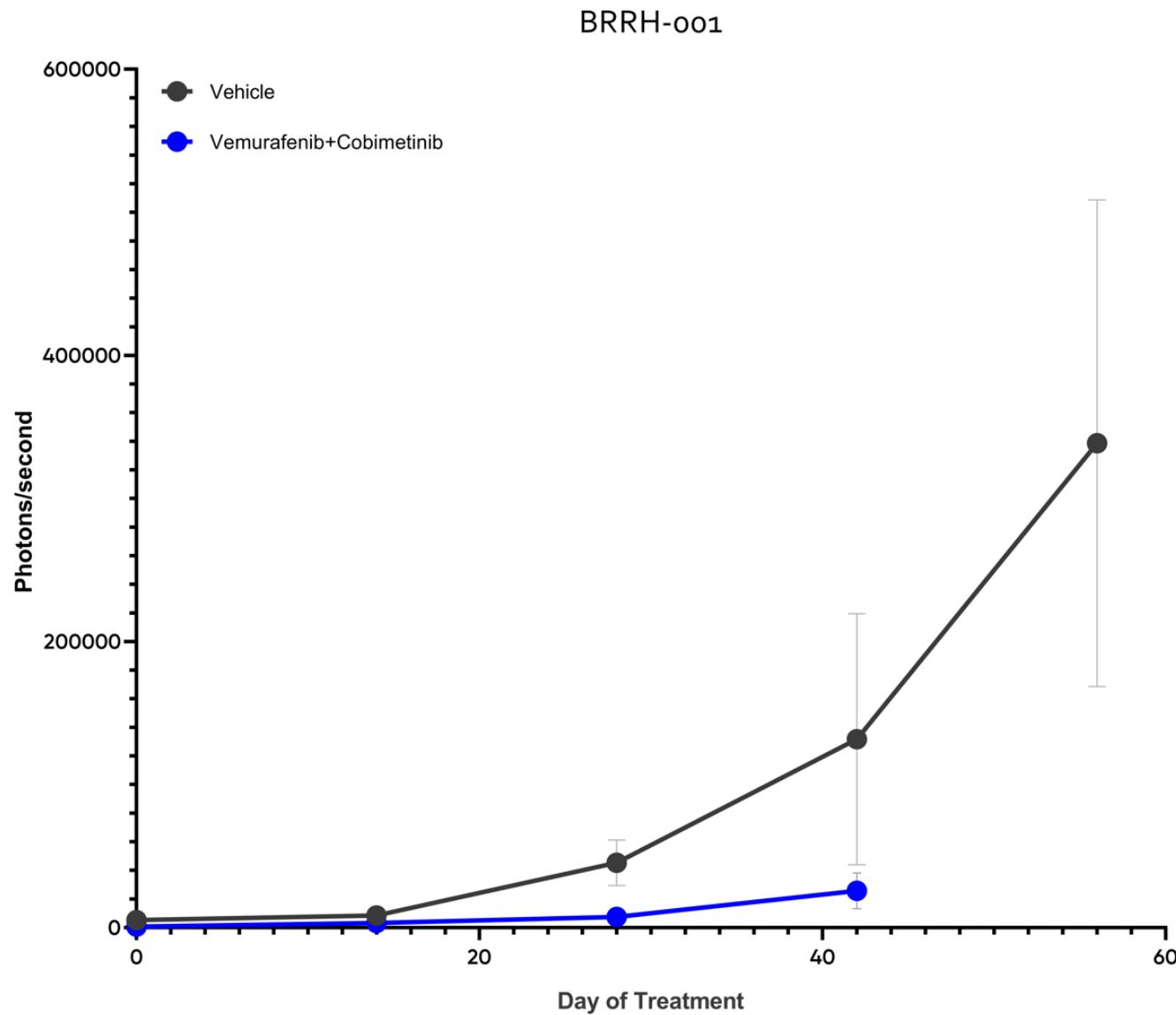
Group	N	Treatment	Dose (mg/kg)	Dose Volume (mL/kg)	Dose ROA	Dose Schedule	Body Weight	Caliper Measurements	MRI
1	6	Vehicle	0	10	PO	QD x 4wks	BiWx4	BiWx4	Pre/Post Tx
2	6	Vemurafenib	10	10	PO	QD x 4wks	BiWx4	BiWx4	Pre/Post Tx
3	6	Cobimetinib	2.5	10	PO	QD x 4wks	BiWx4	BiWx4	Pre/Post Tx
4	6	Vemurafenib + Cobimetinib	10	5	PO	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	PO	QD x 4wks	BiWx4	BiWx4	Pre/Post Tx
8	6	Temozolomide	40	10	IP	QD x 4wks	BiWx4	BiWx4	Pre/Post Tx

*60 total mice implanted April 14th. Randomization to occur around 125-150mm³.

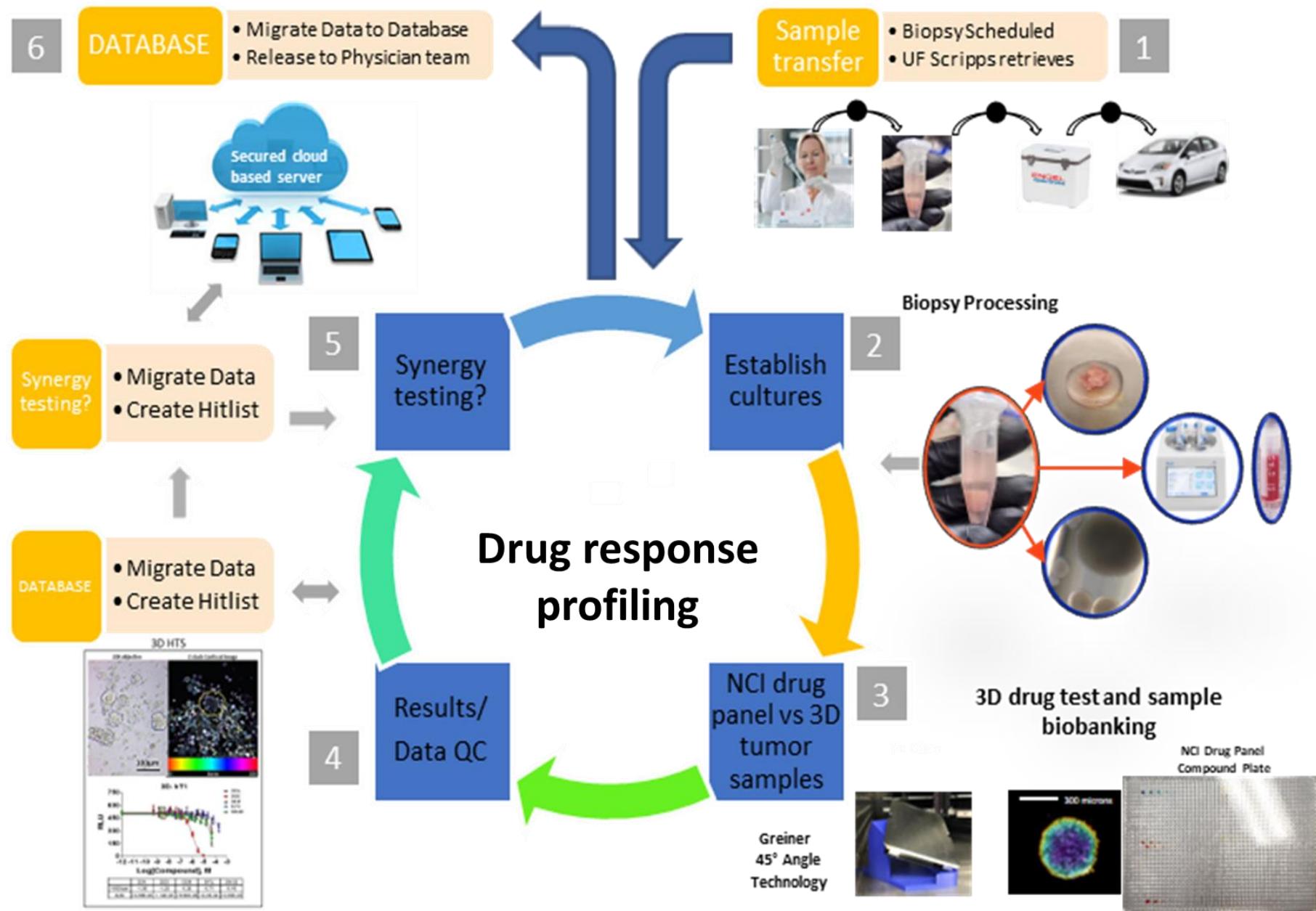
*As of April 29th: 56 out of 60 animals have tumors listed as palpable or growing (13.5 - 58mm³).

Collaboration work: Certis Oncology

In-Vivo Pharmacology Orthotopic Transplant



Chemoresponse profiling



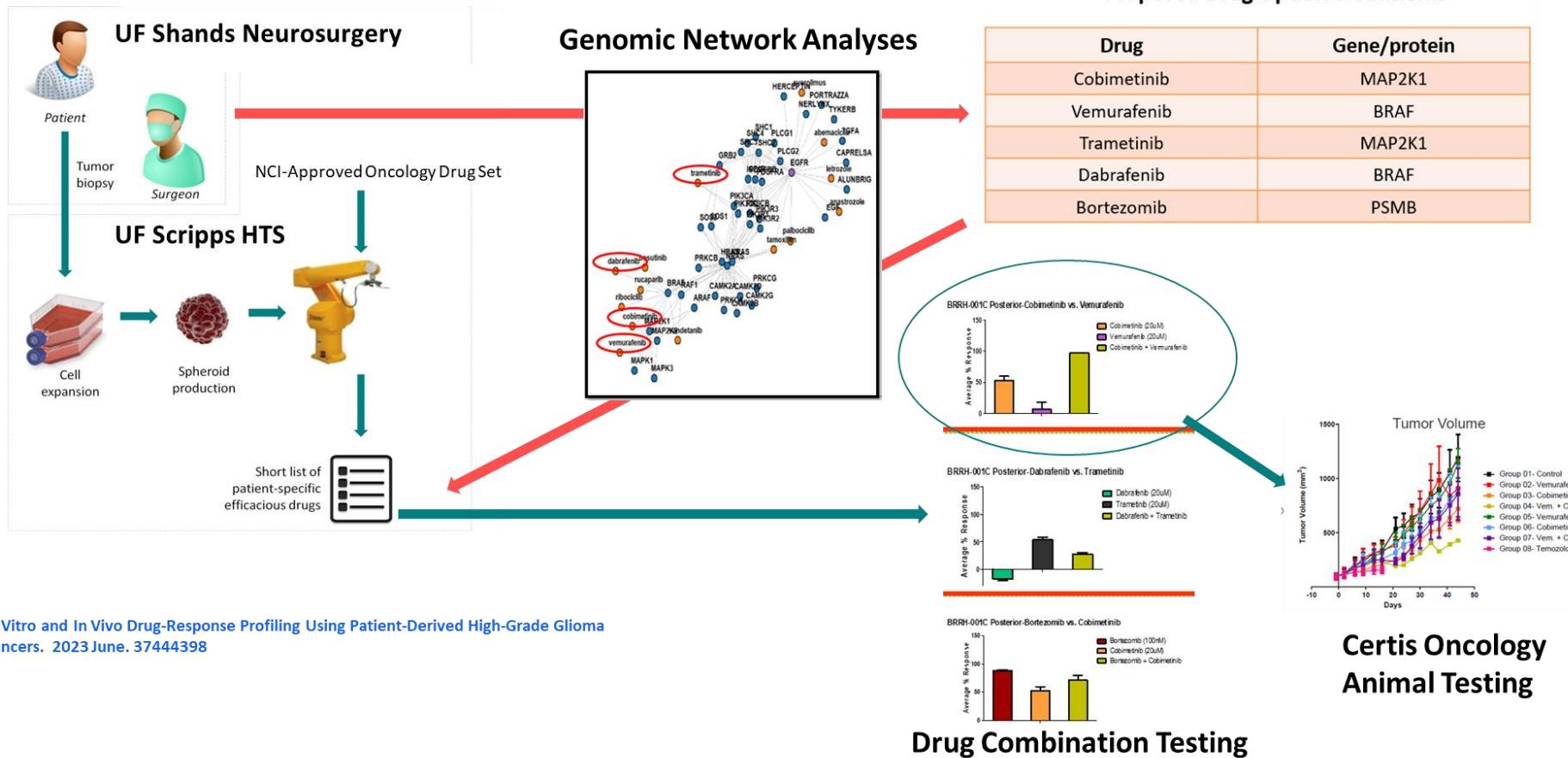
Success?

A turnkey, fully automated, CLIA certified/CAP accredited precision oncology platform placed worldwide into clinical test centers!



- We average 72 hours from tumor sample delivery
 - This is tumor biased and sample biased
- Delivery of CLIA certified test center: months-1 year
- Delivery of a fully automated test platform: 2-4 years

Accelerating AI-Powered HTS Drug Discovery



In Vitro and In Vivo Drug-Response Profiling Using Patient-Derived High-Grade Glioma Cancers. 2023 June. 37444398

Drug Combination Testing

**Certis Oncology
Animal Testing**

Summary

Demonstrated:

- 3D biology is cost effective and practical for physiologically relevant HTS Drug Discovery
- Patient derived 3D spheroids can be rapidly generated for oncology drug profile testing
- Miniaturization allows for cost effect application of precision medicine
- DNA/RNA profiling analysis has shown clear differences in the expression levels of certain genes in the tumor samples that provide strategic guidance to formulating drug combination studies

Ongoing Investigations:

- In-vitro 3D synergy drug profiling based on predicted points of intervention from genomic analyses
- In-vivo PDX drug combination profiling based on insights from in-vitro 3D spheroid drug profiling

Clinical Studies: FDA Expanded Access for “compassionate use”

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