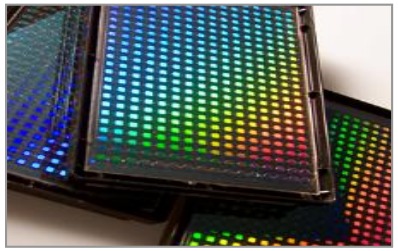
CORNING

Rock the Science of 3D

From Promise to Reality

Corning Life Sciences: Innovative Products for Discovery Research



Business Overview:

- Market leader for over 100 years
- Significant R&D investment for continuous innovation
- Global manufacturing and distribution
- Sales representatives worldwide including Technology and Field Applications Specialists

Laboratory Products and Solutions for:

- Cell Culture and Bioprocess
- Drug Discovery
- ADME/Tox
- Genomics
- Chemistry
- Microbiology
- General Laboratory Products

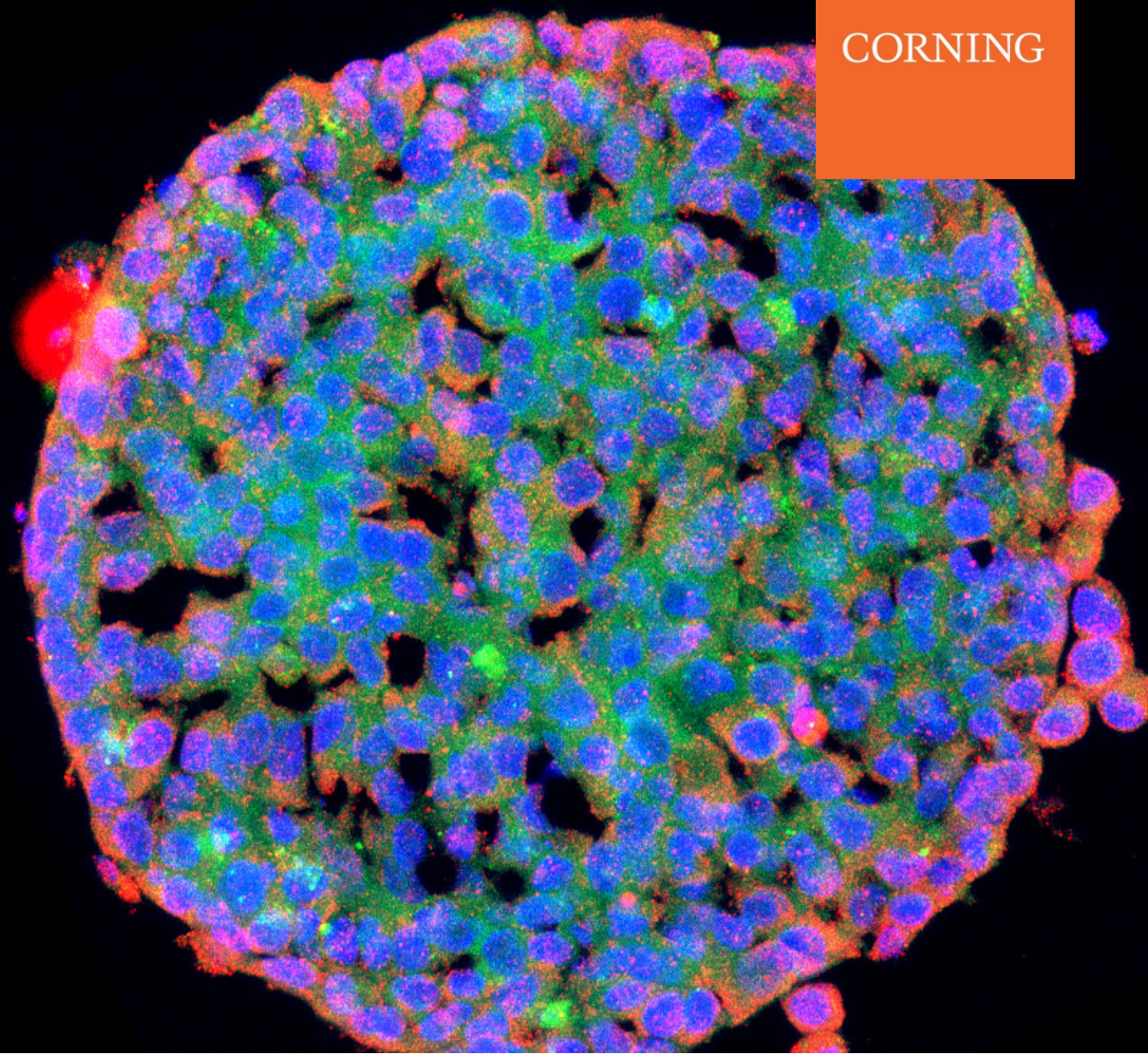
3D Cell Culture:
From Promise to Reality

3D Cell Culture is Exploding

- More *in vivo*-like environments
- More biologically relevant results
- Enables safer, more effective drugs and therapies getting to market in less time, with greater certainty

Applications include:

- Cancer/tumor biology
- Stem cell biology
- ADME/Tox
- Neurobiology and metabolic disease



CORNING

Solutions to cost effectively enable 3D cell culture

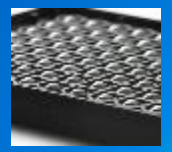
Permeable Supports

Advanced systems, such as **Transwell® permeable supports**, for culturing or co-culturing cells in a 3D system that replicates a tissue

Matrices

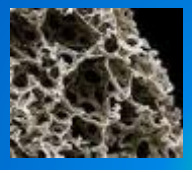
Systems that utilize matrices and gels to grow cells in a semi-solid 3D environment, again mimicking a tissue (e.g. **Corning® Matrigel® matrix**)

Spheroids



Vessels & coatings (e.g. **Corning spheroid microplates**) that force adherent cells to grow together in a 3D structure, similar to how tumors & embryos grow

Scaffolds



Modified materials, such as PS, PLL, Nylon, that form a 3D scaffold for cells to grow on, in and around

KIYATEC®

Changing the Future of Cancer Care by Predicting Patient Response to Oncology Drugs

Corning Exhibitor Spotlight

April 2018

Outline

- About KIYATEC
- Glioblastoma overview
- Predicting patient response *ex vivo*
- What does this mean for cancer patients

About KIYATEC

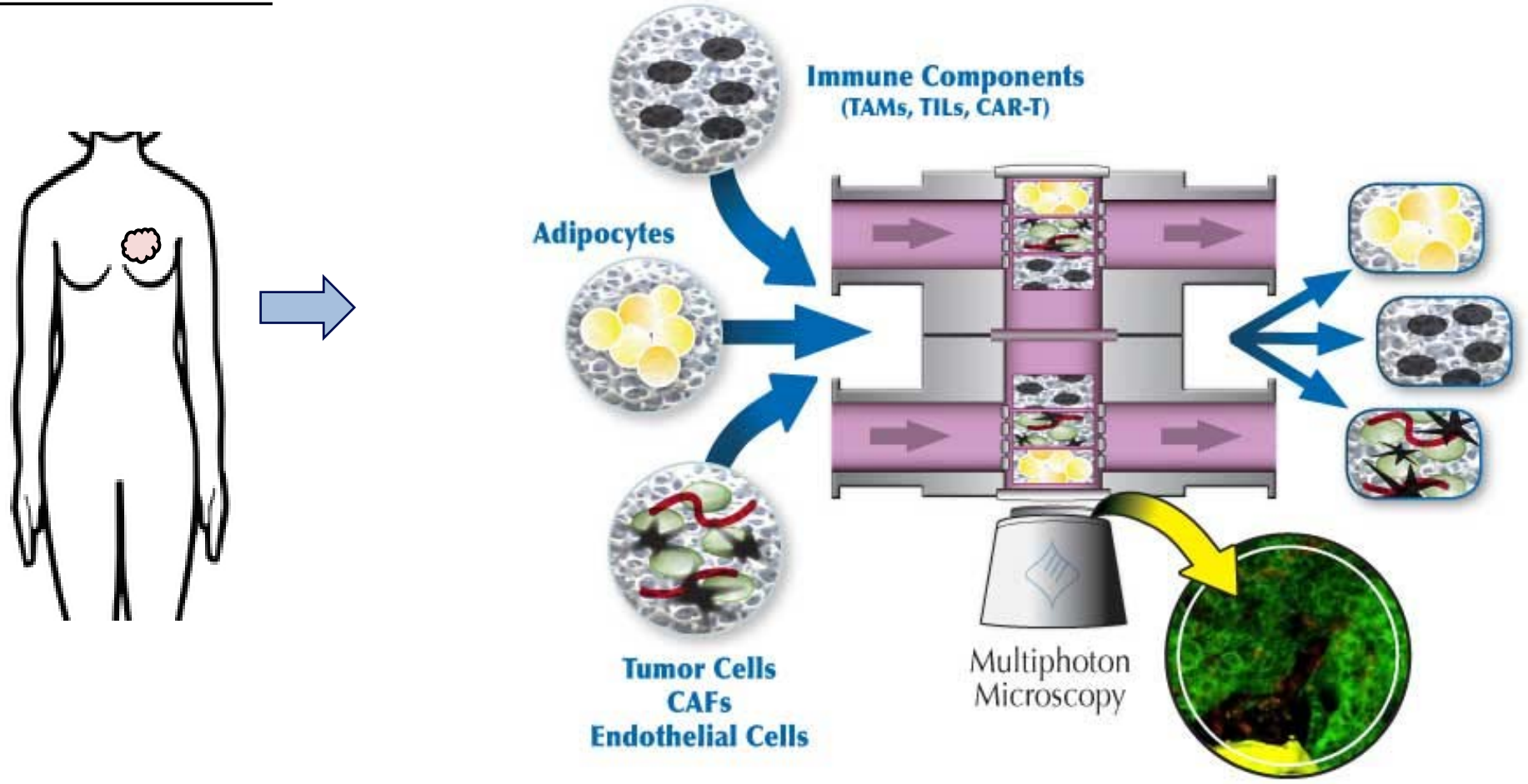
- **Leader in ex vivo 3D cell culture models** with unique, robust tool belt:

		Platform	
		Spheroid	Microtumor
Media Condition	Static	✓	✓
	Perfusion		✓

- Strong focus on **cancer**
- Specialized expertise in **patient derived primary cells, including patient matched immune cells**
- Awarded big projects:
  - $2.3M **Breast Cancer, GBM** 3D Microtumor program (**NCI**)
  - $2.0M **Lung Cancer / Cancer Stem Cell** program (**NCI**)
  - $1.9M **Bone Marrow / Ex Vivo Platelet** program (**NIBIB**)

3D Perfusion Microtumor Platform

Patient-derived 3D Microtumors



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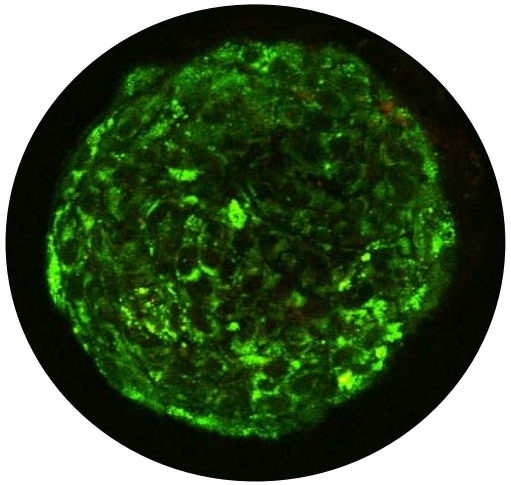
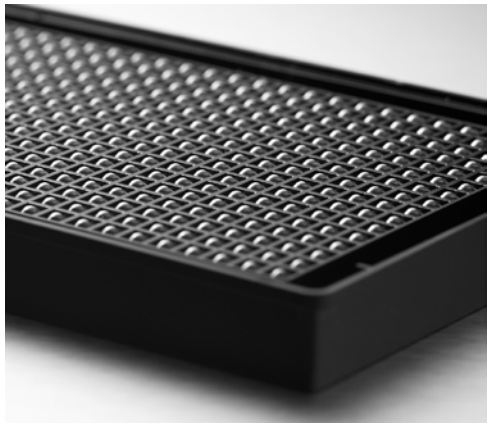
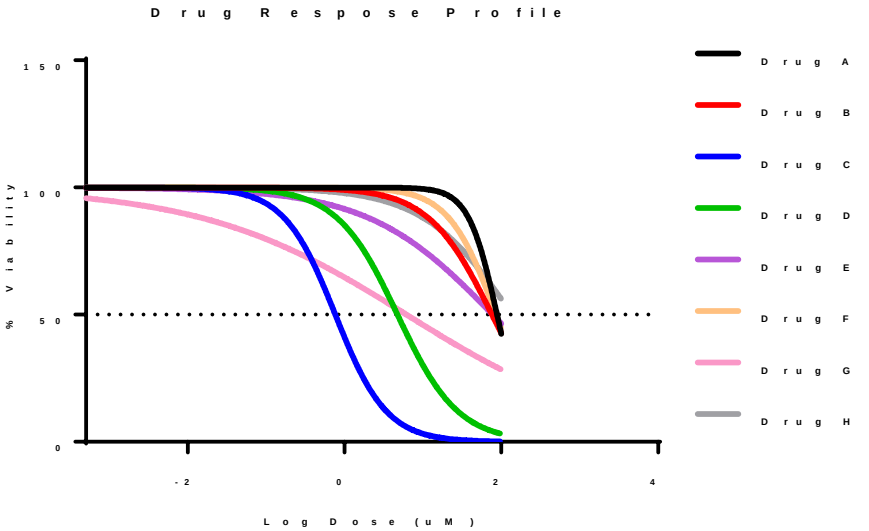
Drug Response Profiling

Cell Sources

- Primary tissue
- Ascites
- Blood
- Cell lines
- PDX







KiYATEC®

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Exceptional Clinical Connectivity

- **Co-located with the cancer institute at the Greenville Hospital System** main campus
  - 13th largest US public hospital system
  - Hospital has dedicated Phase I oncology clinical trials unit
  - Ideally positioned for ex vivo integrated adaptive or co-clinical trials
- **5+ years experience sourcing, shipping, processing and culturing primary cancer tissues**
  - IRB approved studies
  - Process involvement of pathologists, surgical oncologists, medical oncologists, gynecologic oncologists, pathologists, and many associated staff
  - Excellent take rates and expansion through proprietary procedures

Existing, Value-add Relationships

- Clinical collaborations

- PDX

- Research collaborations

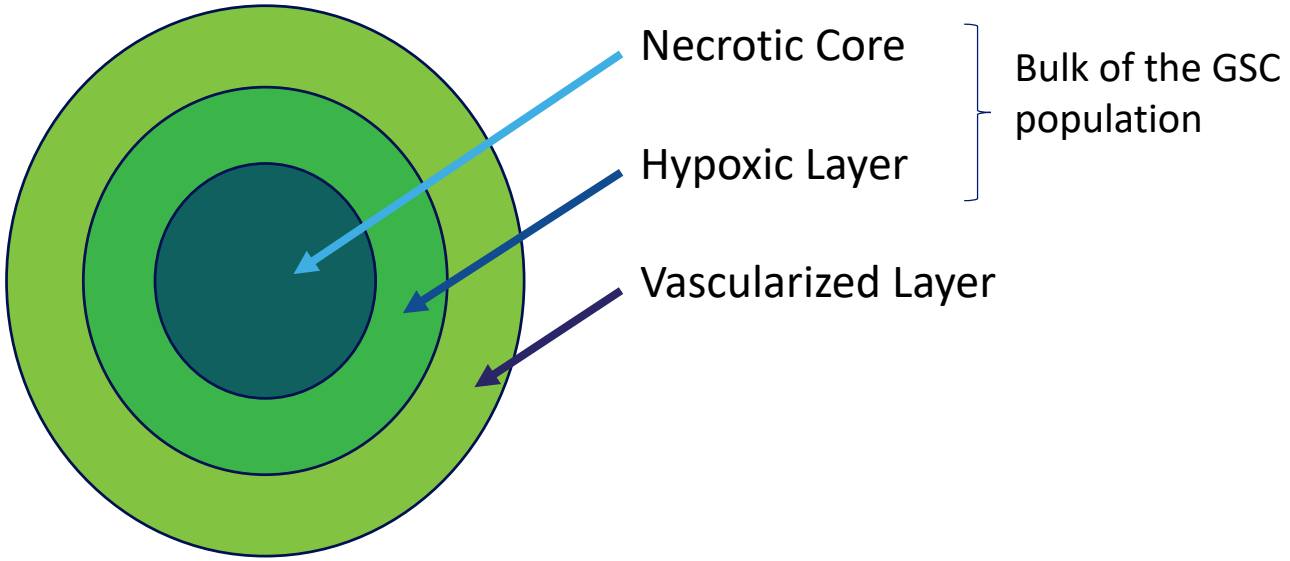
Wasted time is the enemy of cancer patients.

Glioblastoma (GBM)

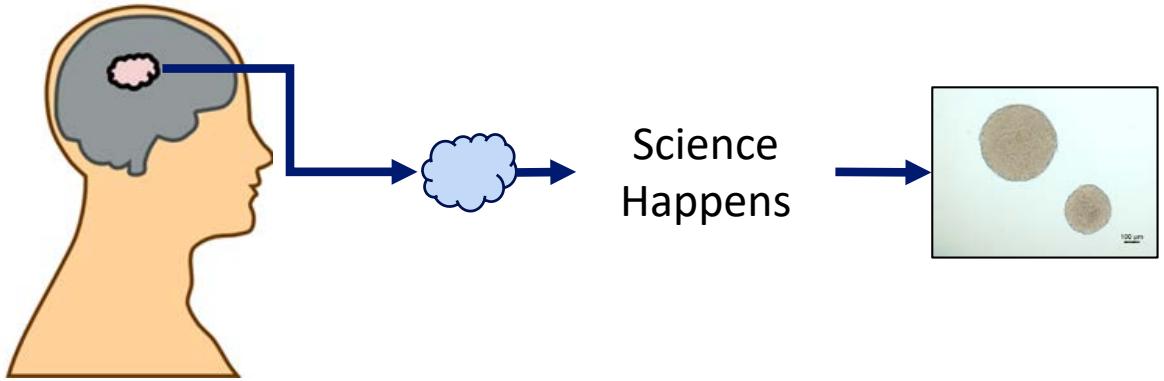
- The most aggressive glioma, marked by high amounts of infiltration into other parts of the brain
- Standard treatment includes surgery, radiation, and temozolomide (TMZ)
- Median survival upon treatment is approximately 14.6 months
- Two-year survival is approximately 30%
- Methylation of MGMT is thought to be a predictor of response to TMZ
  - It is methylated in approximately 45% of GBM
  - Some controversy as to the significance of it for the prognosis of patients PFS and/or OS

Glioma Stem Cells & GBM

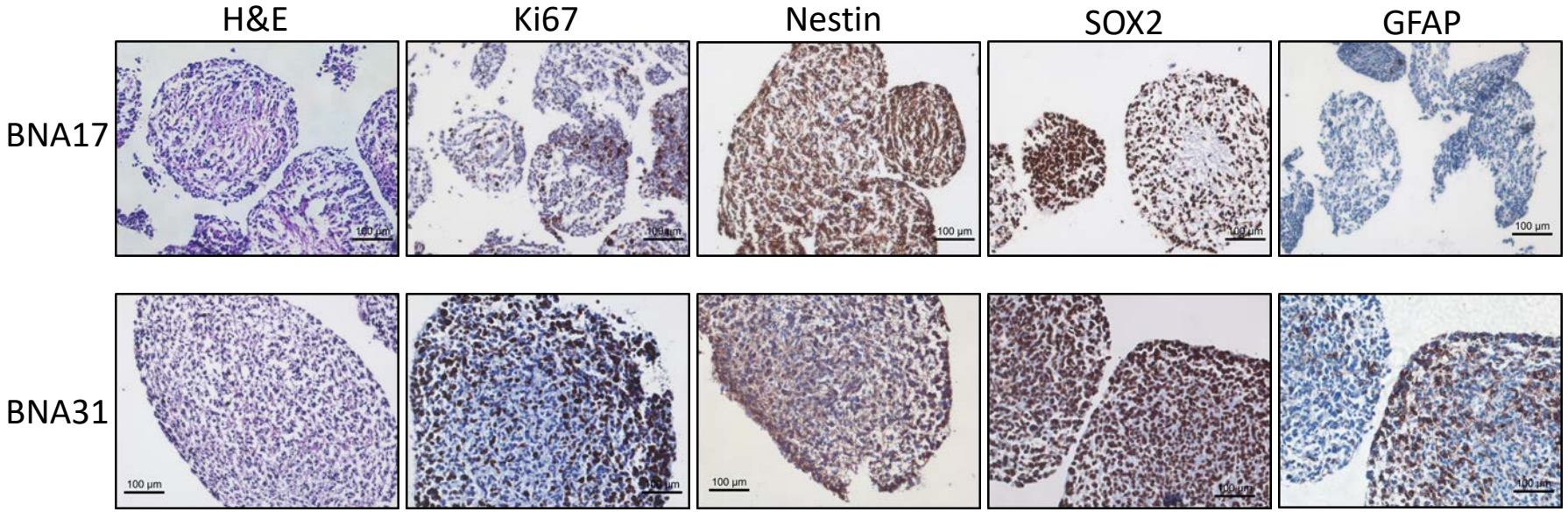
- GSCs
  - Self-renewal & Proliferation
  - Marker Expression
  - Tumor Initiation
- GBM Concentric Layers Model

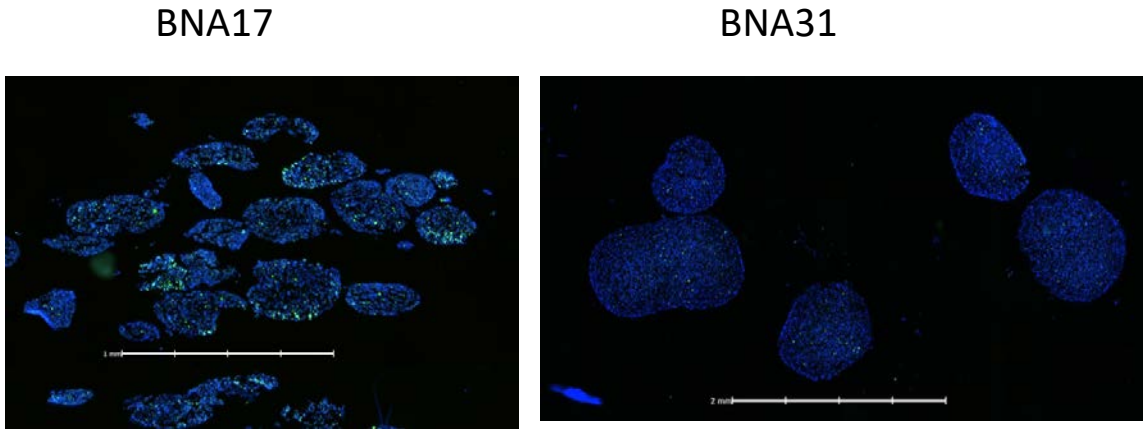


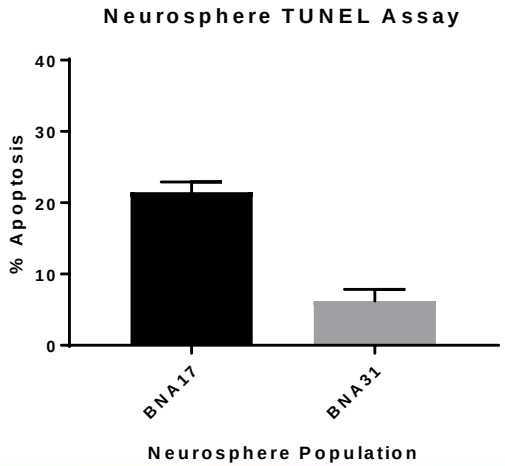
GSC Generation and Drug Response Testing



Neurospheres

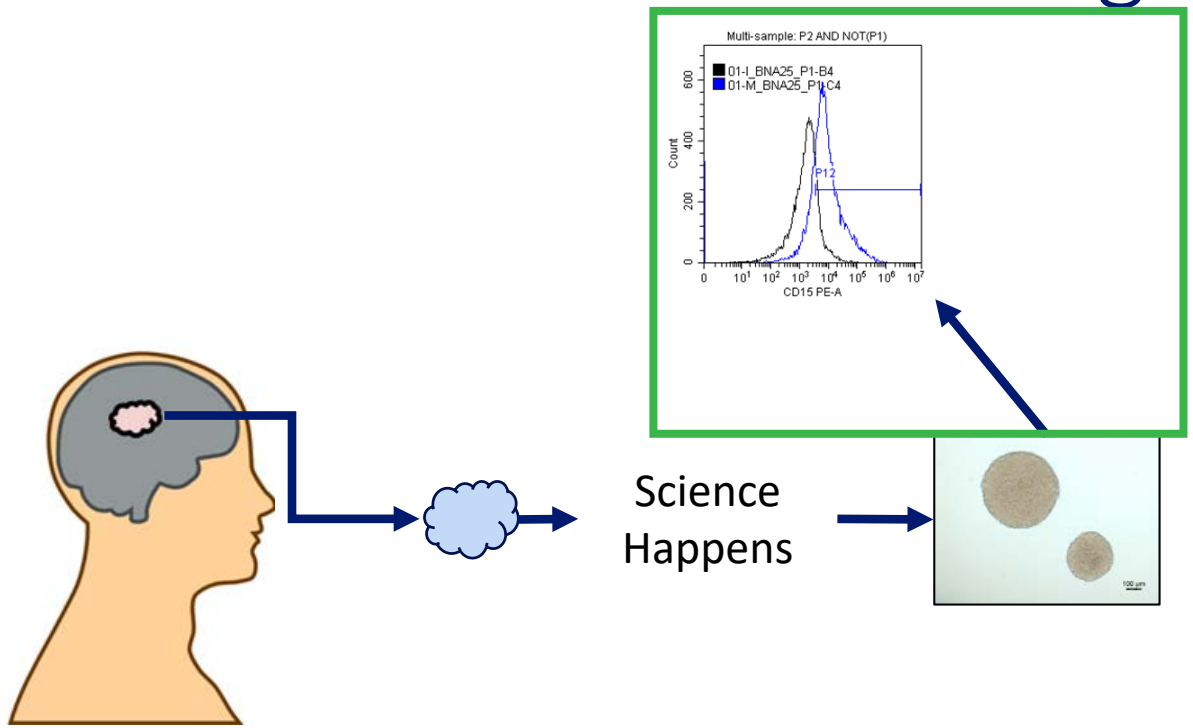




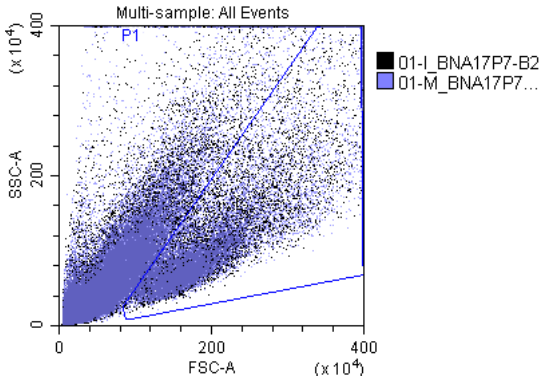


- Ki-67 and TUNEL for proliferation and areas of apoptosis
- Nestin and Sox2 as stem cell markers
- GFAP as a marker of GBM

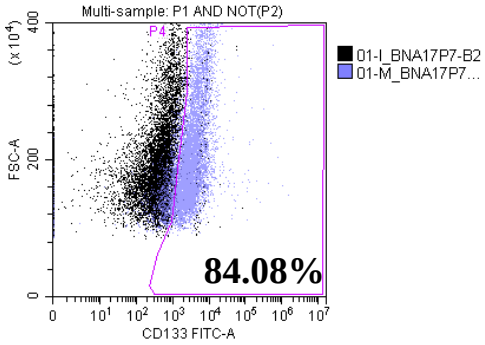
GSC Generation and Drug Response Testing



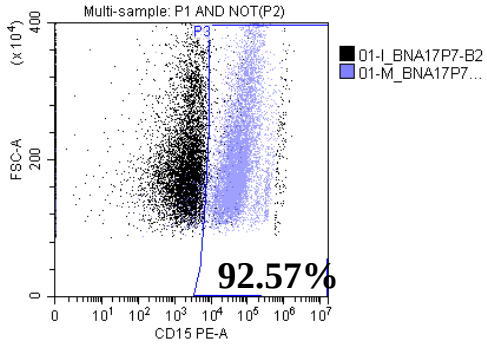
GSCs: Marker Expression - Flow



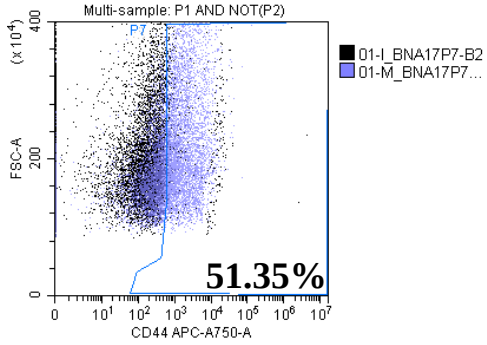
CD133



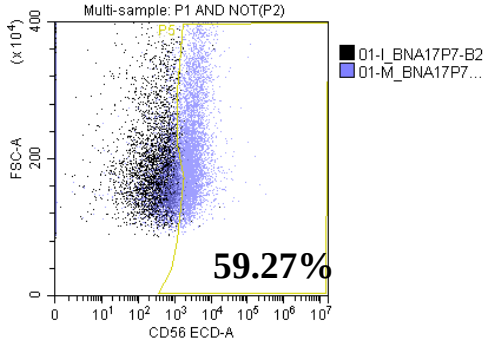
CD15



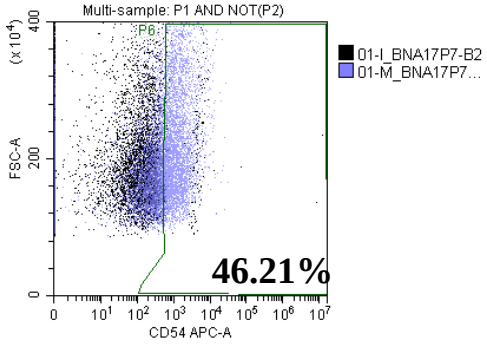
CD44



CD56



CD54



NS	CD133	CD15	CD44	CD54	CD56
BNA17	84	93	51	46	59
BNA20	10	60	91	87	95
BNA21	0	44	16	1	4
BNA24	10	92	42	11	50
BNA25	46	75	75	24	40
BNA31	0	0	49	88	96
BNA36	47	84	93	41	96
BNA40	59	21	96	54	77
BNA42	17	13	90	56	45
BNA43	13	26	98	88	92
BNA46	0	7	80	66	96
BNA47	1	15	88	76	77

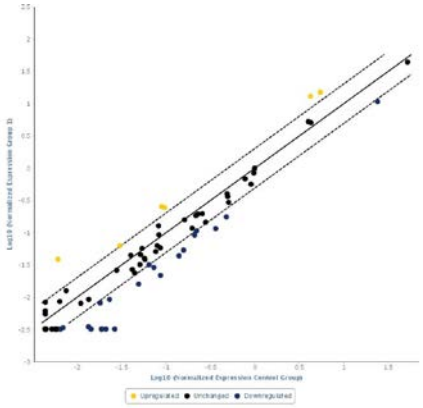
GSC: Marker Expression – MGMT & mRNA

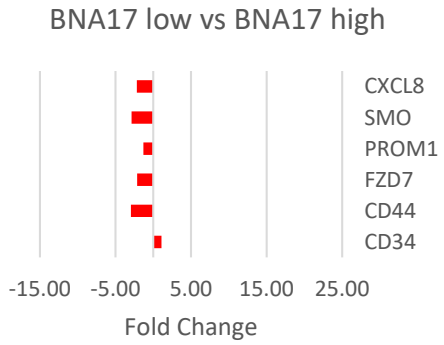
- They maintained their MGMT methylation state over passages

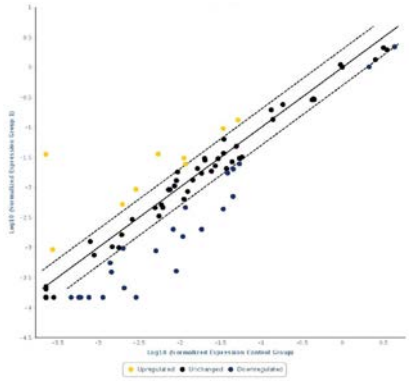
SAMPLE	% UNMETHYLATED	% METHYLATED
BNA17 low	0	100
BNA17 high	0	100

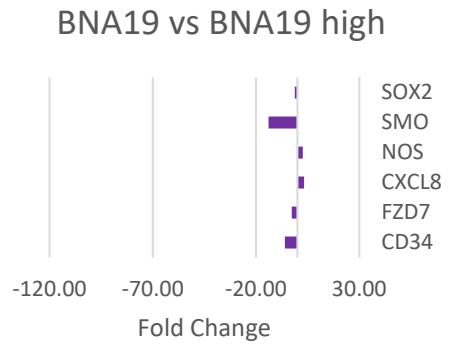
SAMPLE	% UNMETHYLATED	% METHYLATED
BNA19 low	93	7
BNA19 high	100	0

- They maintained their mRNA expression over passages

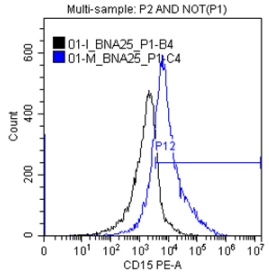


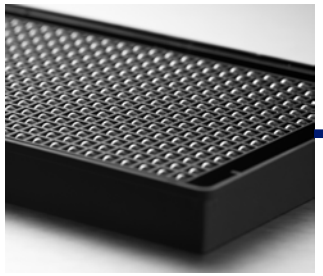




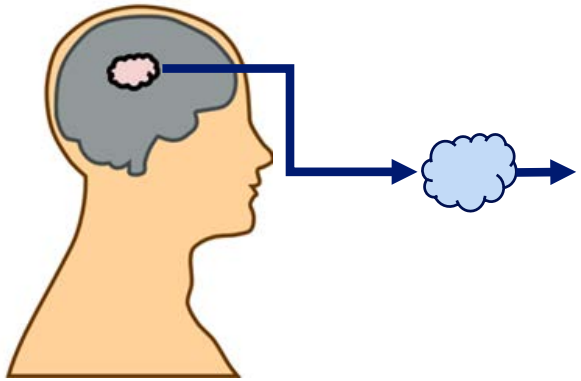


GSC Generation and Drug Response Testing





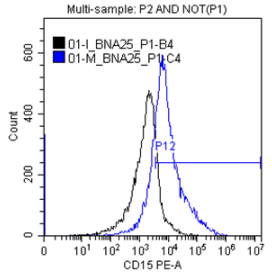
In vitro Limited Dilution Assays

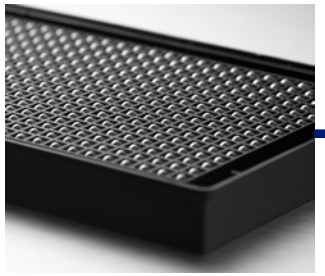


Science Happens

KIYATEC®

GSC Generation and Drug Response Testing





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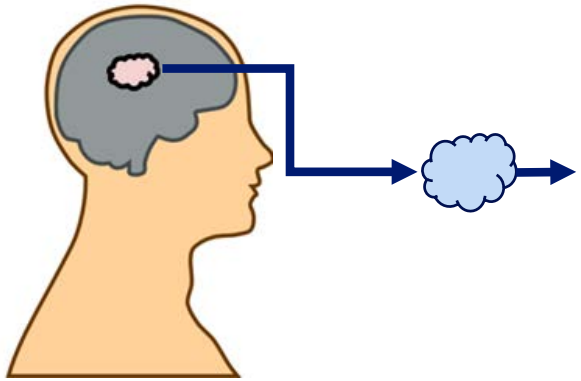

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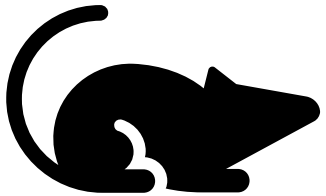

In vivo Limited Dilution Assays

In Vivo Limited Dilution Assay

- Limited dilution in vivo tumor generation was performed with BNA17 to confirm stemness

Cohort Condition	Tumors Produced
100,000 cells	3/3
10,000 cells	5/6
1,000 cells	2/4
100 cells	4/6

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Cohort Condition	Tumors Produced
100,000 cells	3/3
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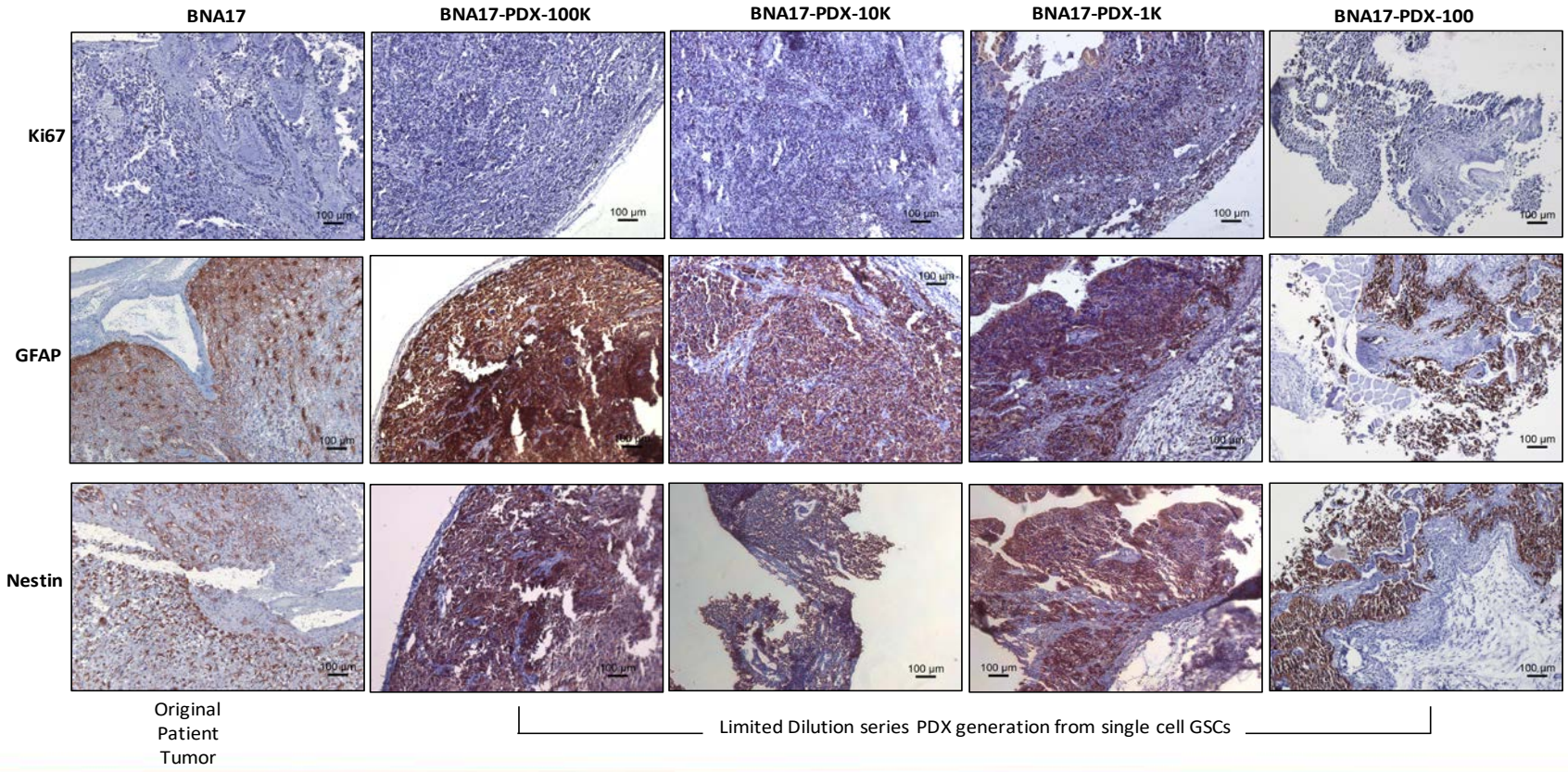
- The resulting tumor histology is similar to the primary tumor histology



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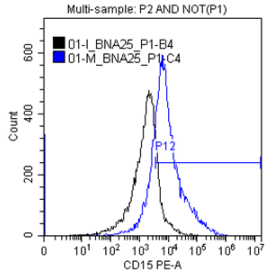

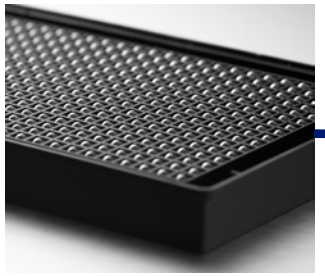

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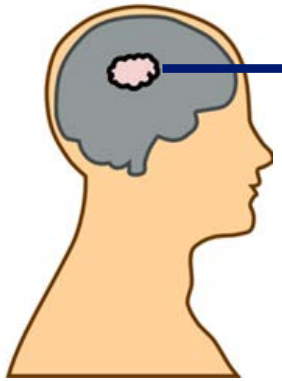

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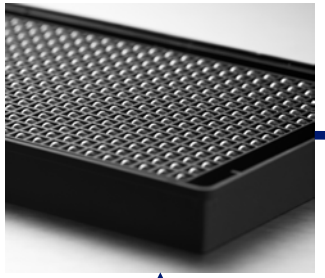
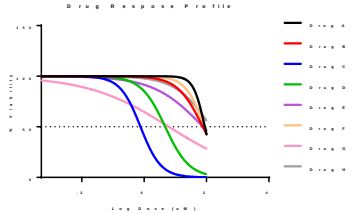
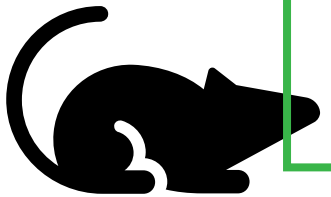
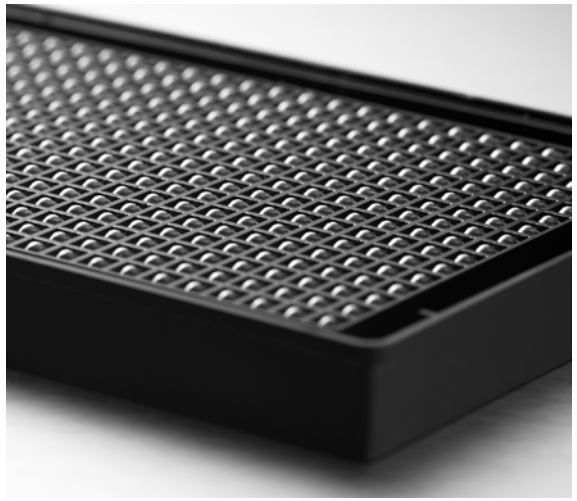


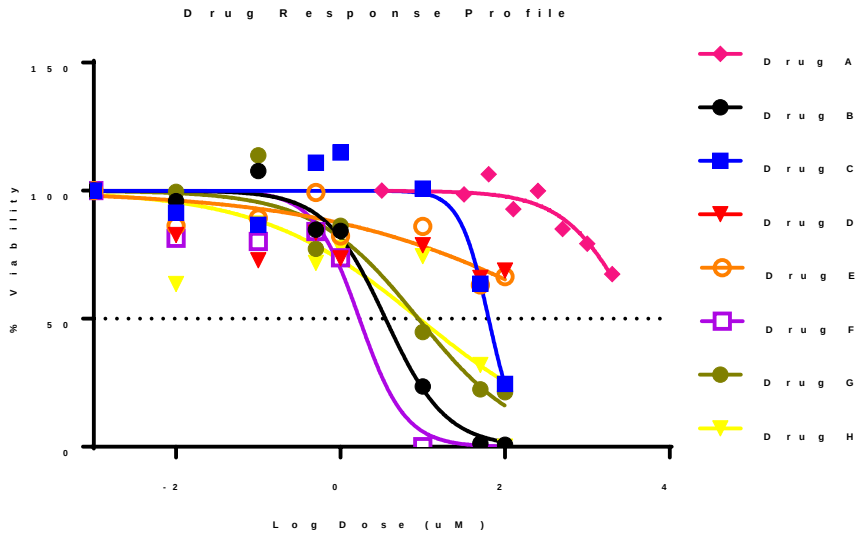




In vivo Limited Dilution Assays

Drug Response Profile



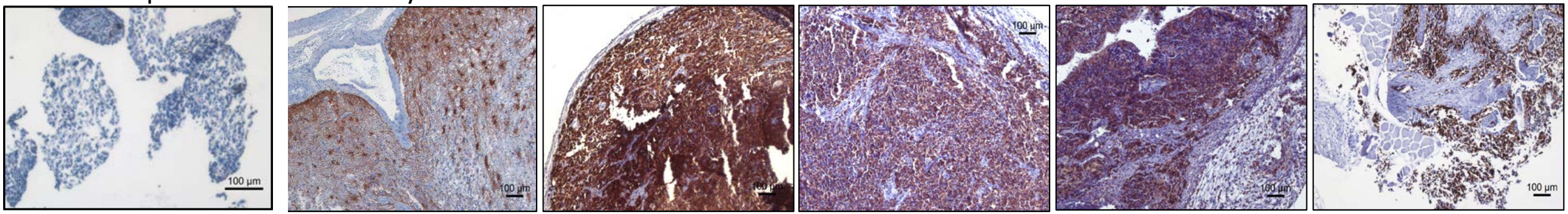


	GSC	LD Mouse
Drug A	2517	2016
Drug B	89	57
Drug C	18	11
Drug D	**86**	**1316**
Drug E	84	156
Drug F	1	2
Drug G	**224**	**5**
Drug H	**55**	**7**

- While the response to some compounds is similar between neurospheres and mice, for others it can vary drastically
  - This may be indicative of the difference between a purer cell population in the neurospheres and a more heterogeneous population in the in vivo tumor in terms of differentiation

For Some Markers, Recapitulation is Patient Specific

Neurosphere | Primary Tumor | Limited Dilution Mice

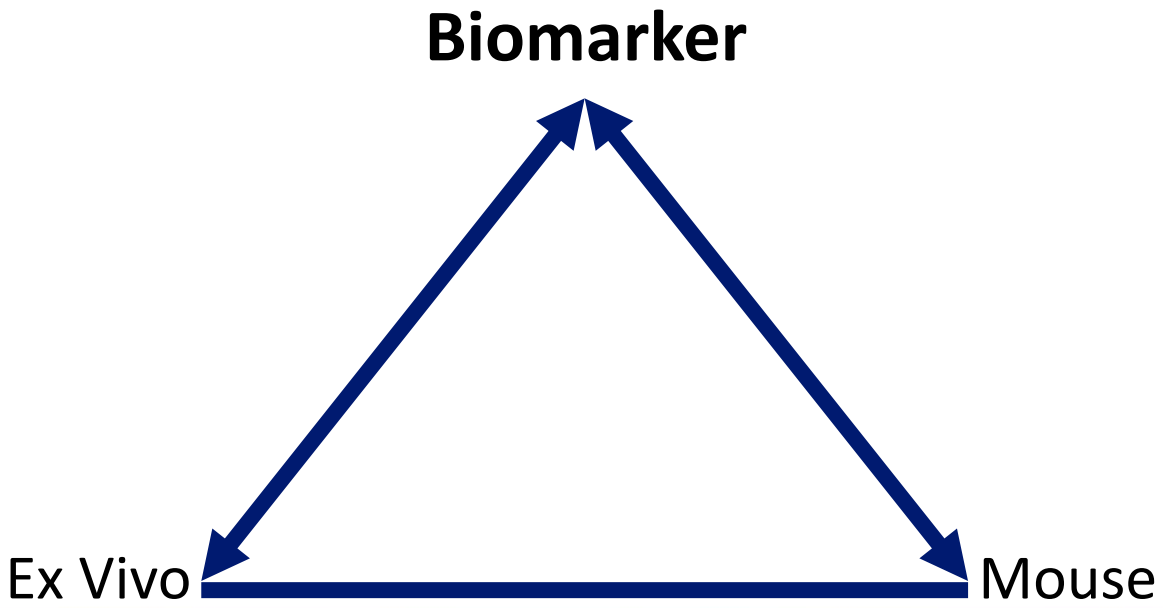


- In this particular case, the neurosphere does not recapitulate the expression of GFAP seen in both the primary tumor and the limited dilution tumors generated in mice
  - But it did for Nestin and Sox2
- Expression was generally recoverable upon implantation in mice
- This reflects the role of the microenvironment in tumor heterogeneity

Implications

Preclinical

- For preclinical studies, correlation to the clinic is not necessary as your test compound and/or combination will not have gone into patients yet.



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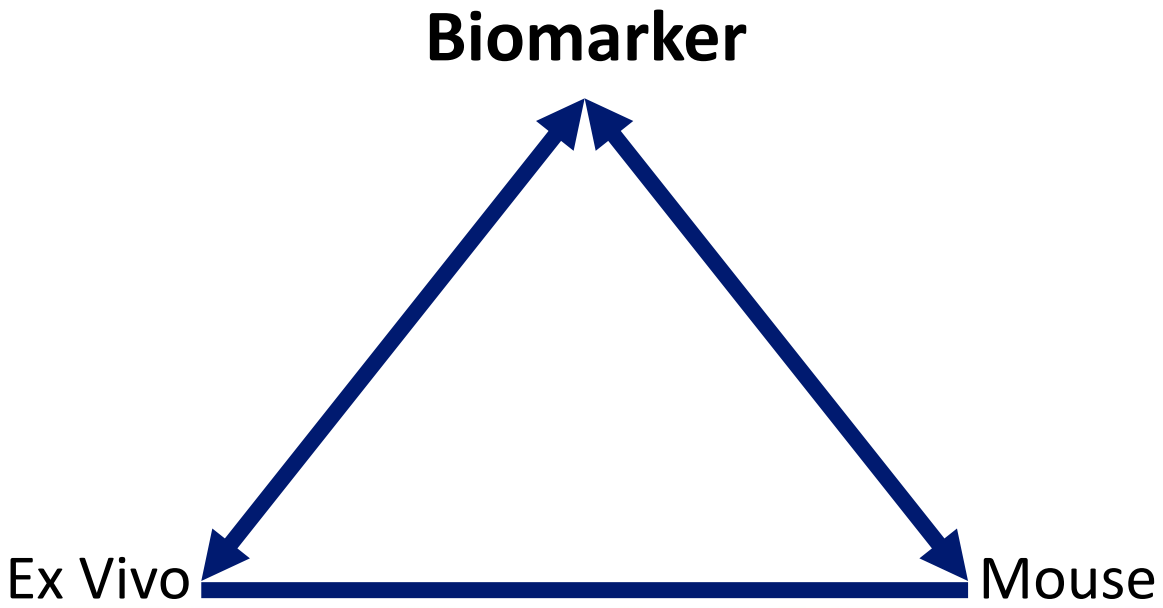
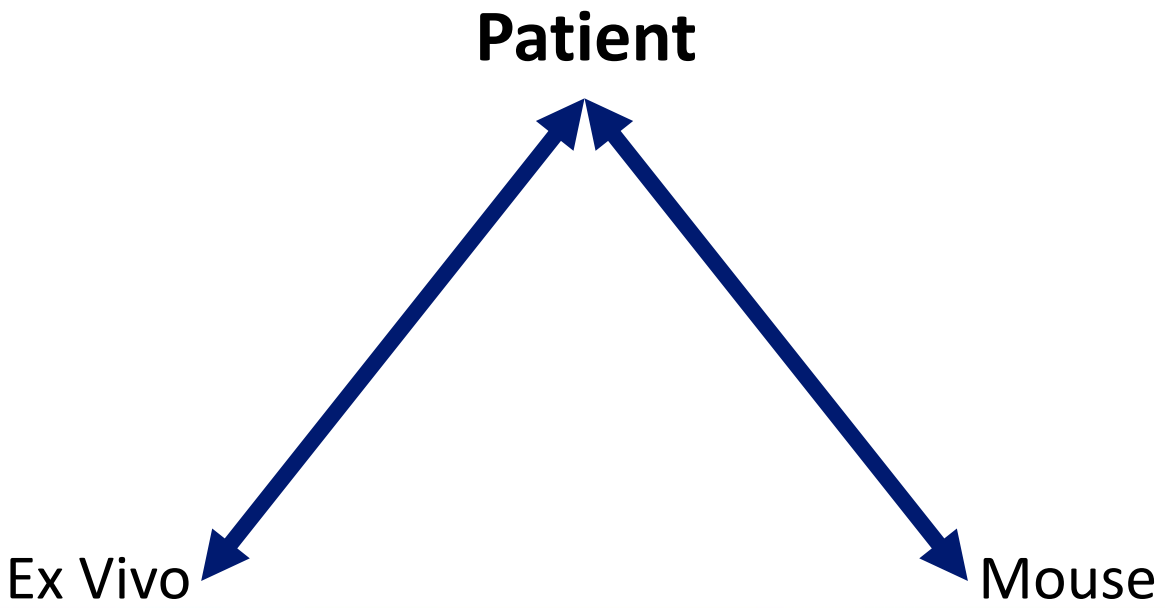


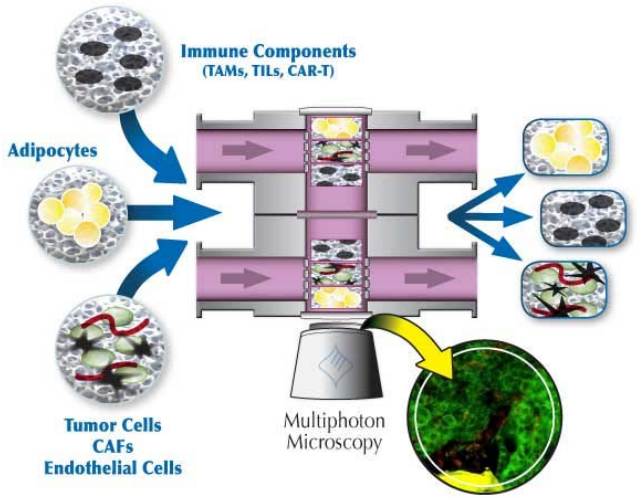
Clinical

- For clinical applications, it is critical that your assay correlate to clinical response.

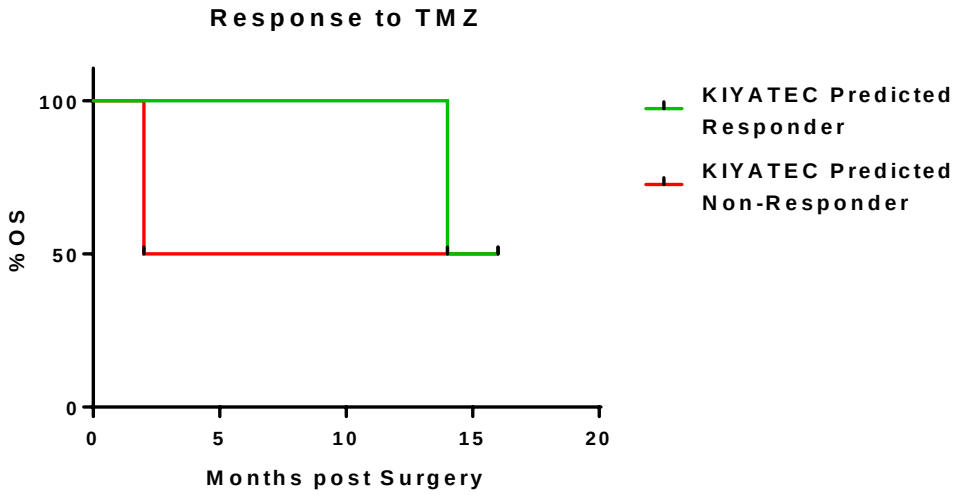


KIYATEC is Solving This Problem

- Multi-cell type 3D microtumors and perfusion bioreactors
- The direct use of patient tumor tissue in a CLIA regulated, clinically validated drug response assay



	Clinical Response	Microtumor Response	GSC	LD Mouse
TMZ	Yes	137.2	2517	2016



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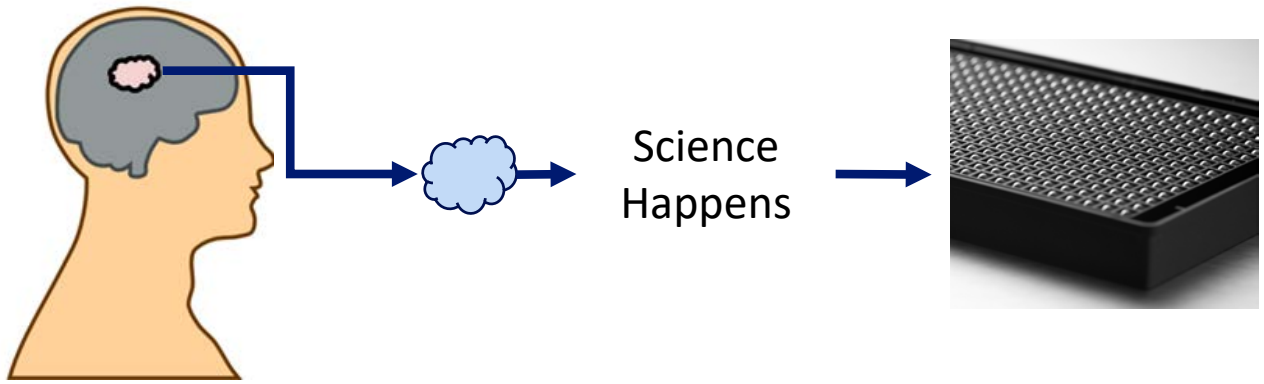

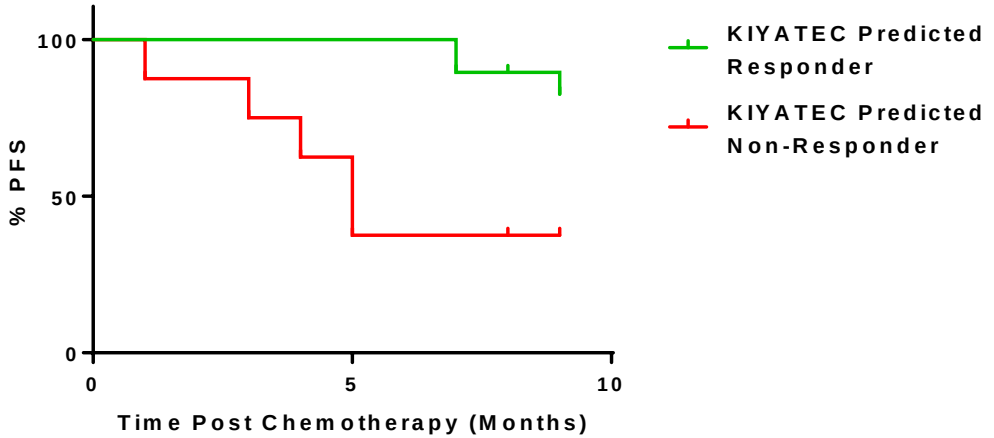

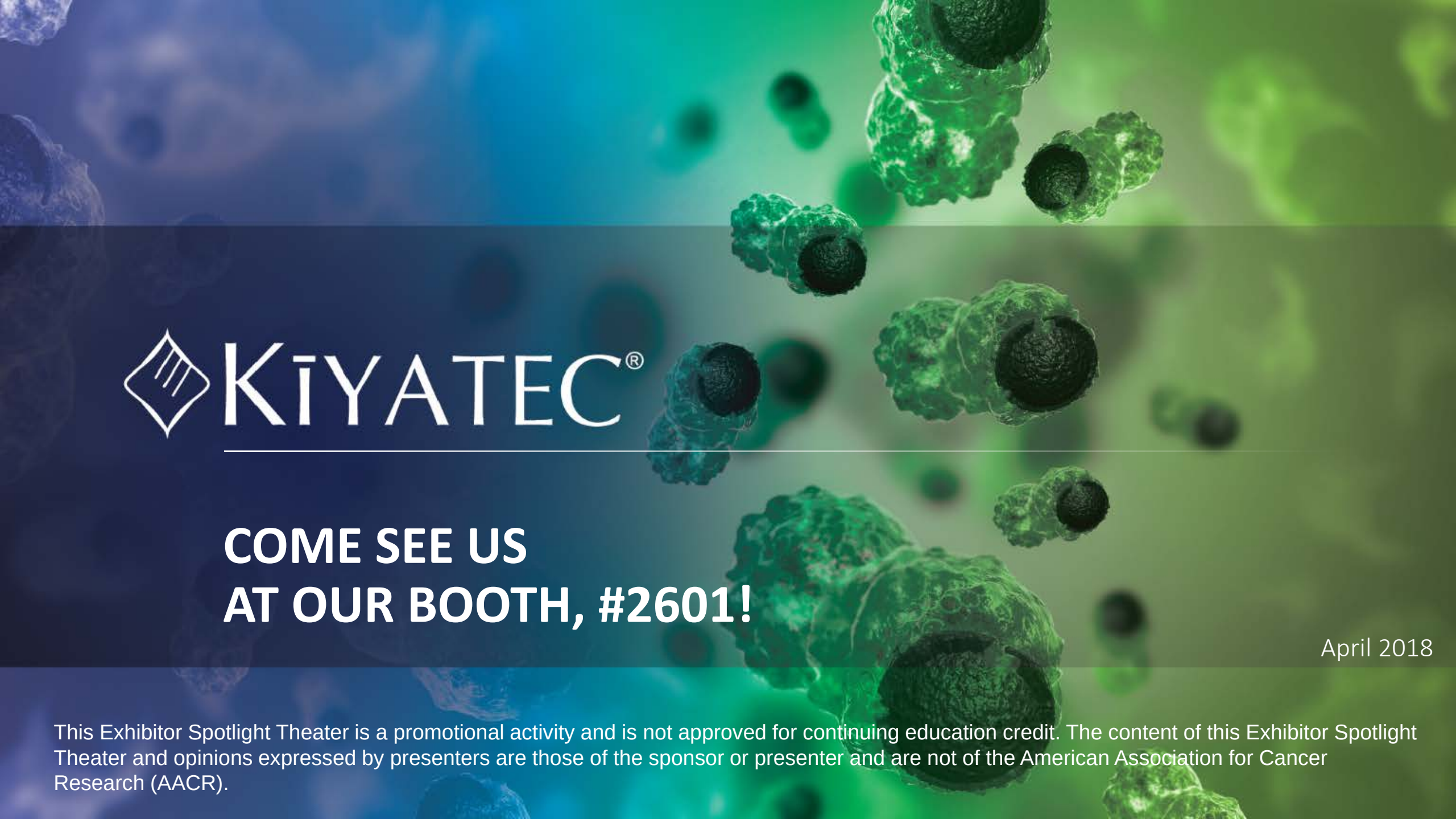

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April 2018

CORNING

A Novel 3D Glioma Blood Brain Barrier Model for High Throughput Testing of Tumoricidal Capability

Hilary Sherman

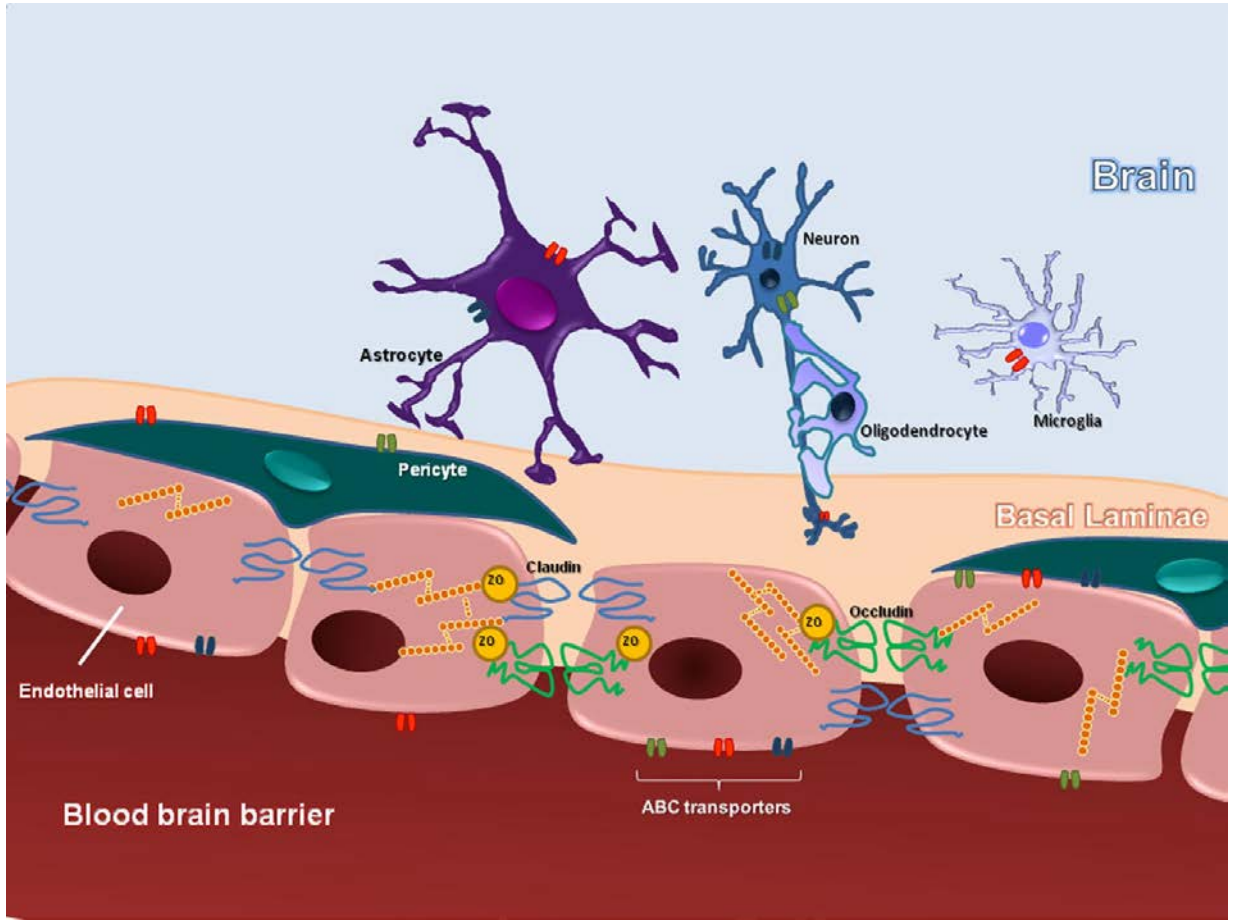
Applications Scientist II, Corning Life Sciences

AACR 2018

Agenda

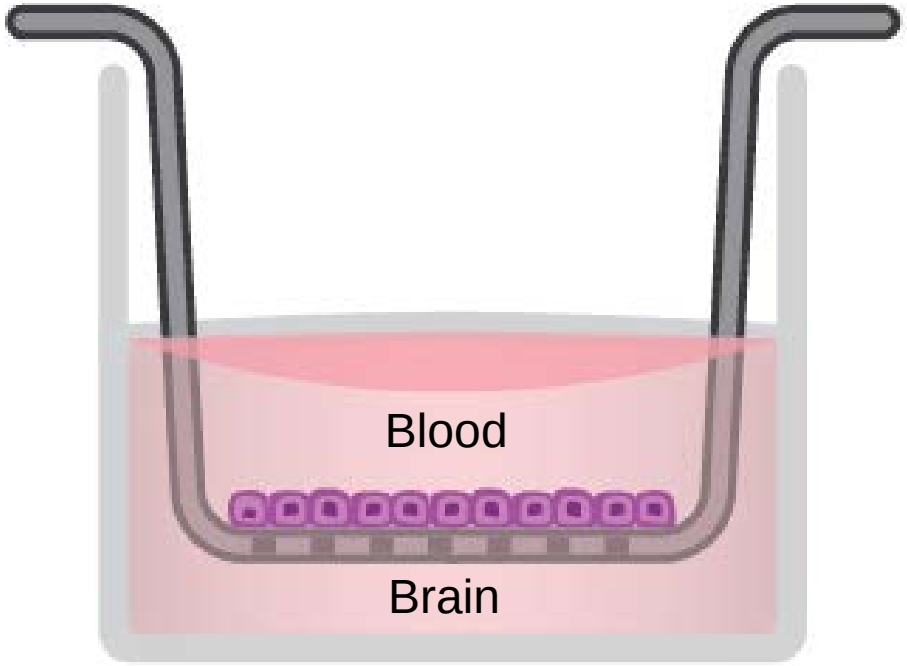
- Blood brain barrier background
- *In vitro* blood brain barrier
- Gliomasphere assay
- 3D glioma blood brain barrier model
- Variations of model

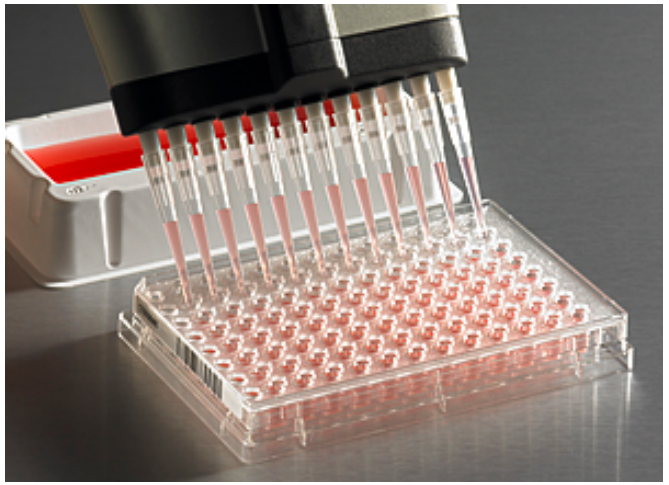
The Blood Brain Barrier



DosSantos, Marcos F., et al. "The role of the blood–brain barrier in the development and treatment of migraine and other pain disorders." Frontiers in cellular neuroscience 8 (2014): 302.

In Vitro Blood Brain Barrier





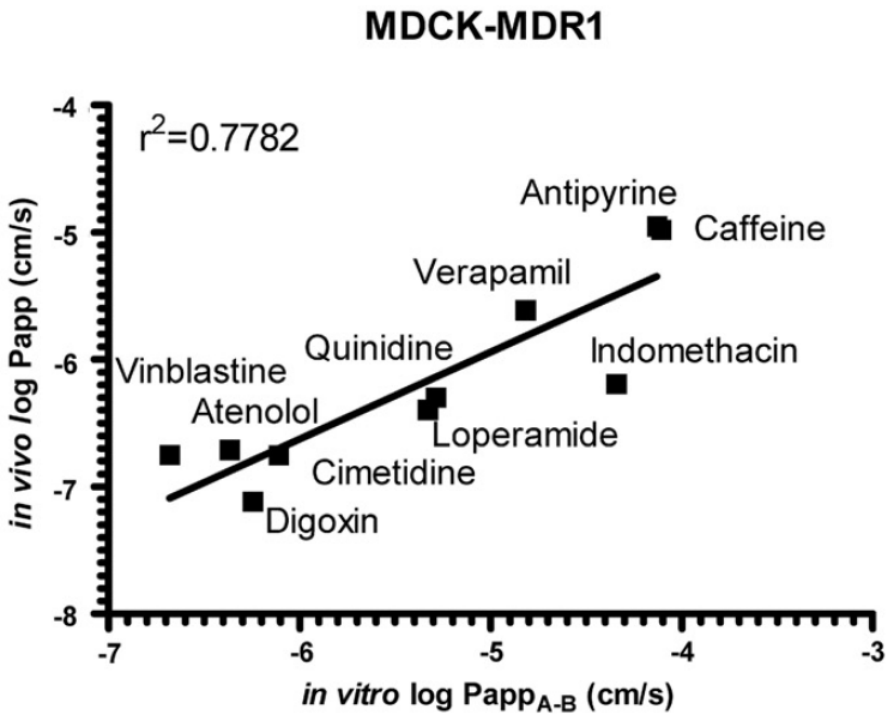
Brain Microvascular Endothelial Cell (BMEC) Models

Table 1 Sources of cells used to replicate BMEC function

Barrier Cell Source	Origin (cell line)	TEER (Ω cm^2)	Advantages	Disadvantages	References
Immortalized	• canine kidney epithelial (MDCK) • human colon adenocarcinoma epithelial (Caco-2) • mouse BMEC (BEnd.3) • rat BMEC (RBE4) • human BMEC (hCMEC/D3 and hBMEC)	40–315 compiled in [96]	• stable over numerous passages • commercially available • can be transfected to express human efflux pumps (MDCK)	• incomplete tight junctions • poor barrier function	[53, 99, 123]
Primary	Mouse, rat, porcine, bovine, human BMECs	130–2200 compiled in [96]	• close initial resemblance to in vivo conditions	• tedious purification with low yields and batch variability • senesce after few passages • difficult to obtain healthy tissue (human)	[98, 124–126]
PSC-derived	Mouse or human iPSC or ESC	250–5350 [19, 101, 102]	• renewable source • patient specific • physiological TEER	• require differentiation and thorough characterization	[20, 101, 102, 112]

Jamieson, John J., Peter C. Searson, and Sharon Gerecht. "Engineering the human blood-brain barrier in vitro." Journal of Biological Engineering 11.1 (2017): 37.

MDCKII/MDR1 cells

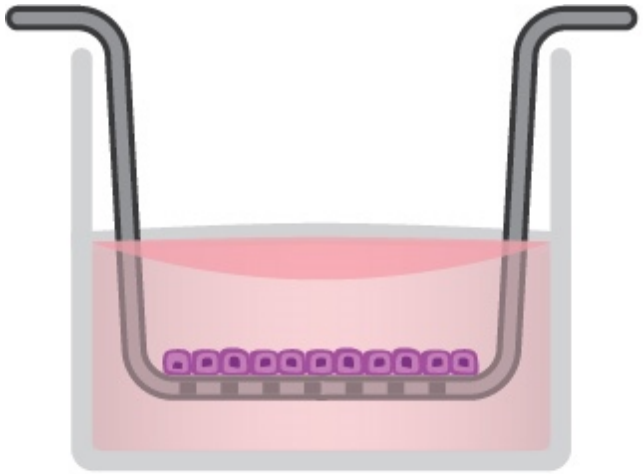


Hellinger, Éva, et al. "Comparison of brain capillary endothelial cell-based and epithelial (MDCK-MDR1, Caco-2, and VB-Caco-2) cell-based surrogate blood–brain barrier penetration models." European Journal of Pharmaceutics and Biopharmaceutics 82.2 (2012): 340-351.

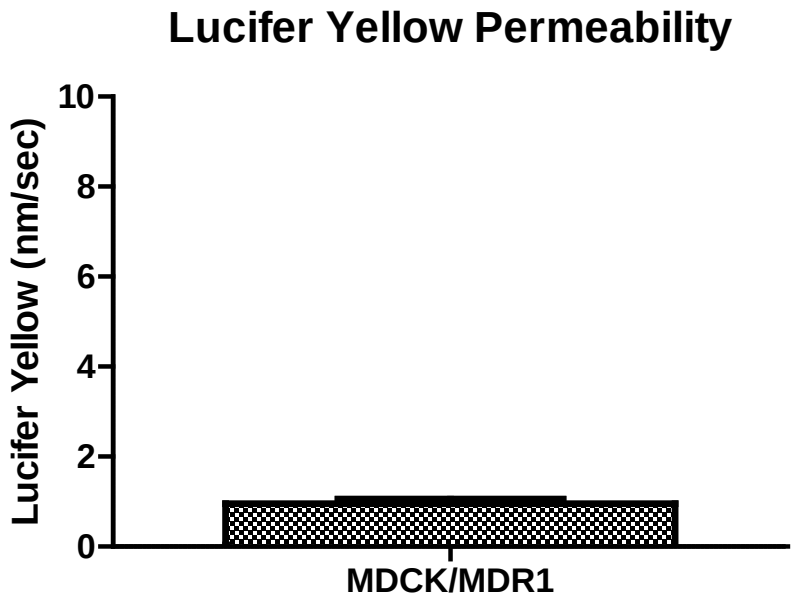
Basic Protocol

1. Seed MDCKII/MDR1 cells at 100,000 cells per cm^2
2. Culture for 5 days with a full medium exchange on day 4
3. Assess effectiveness of BBB model on day 5

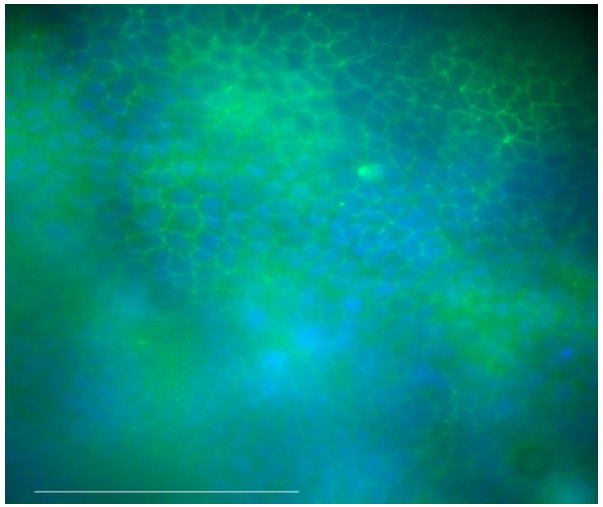
Establishing *In Vitro* BBB



Establishing *In Vitro* BBB

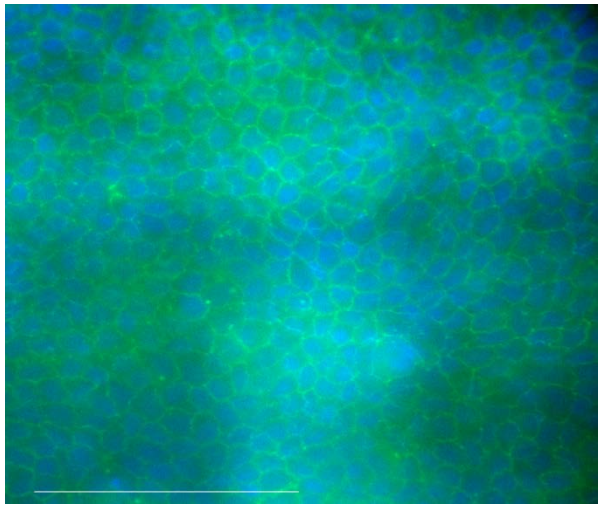


Low Lucifer Yellow (LY) in basolateral chamber demonstrates tight monolayer



ZO-1
(Green)

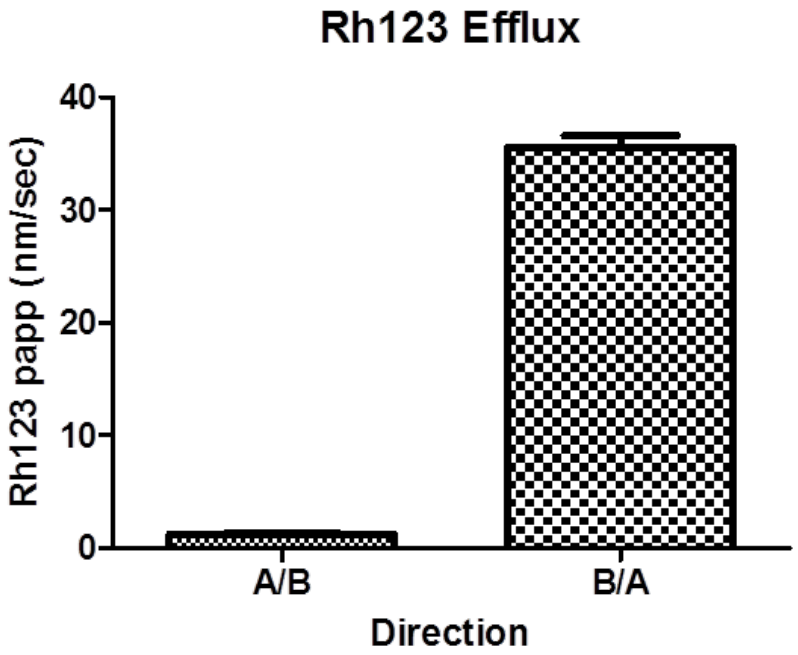
Nuclei
(Blue)



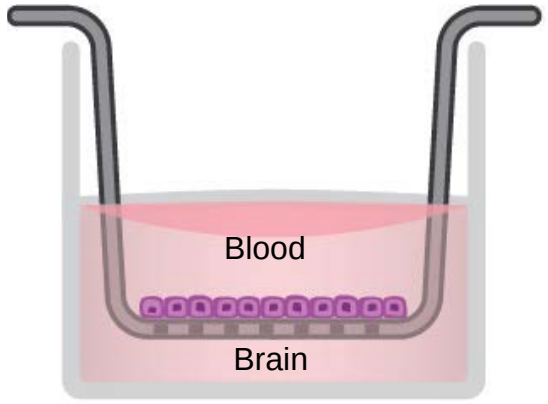
Occludin
(Green)

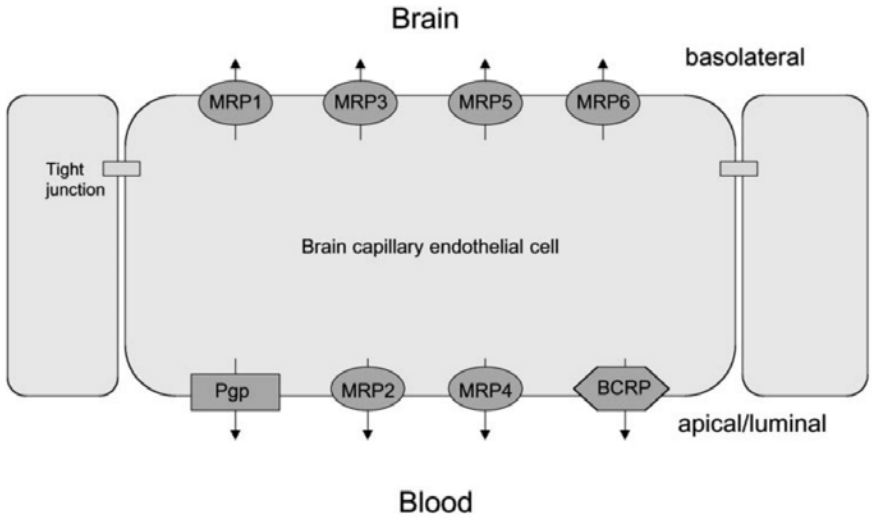
Nuclei
(Blue)

Establishing *In Vitro* BBB



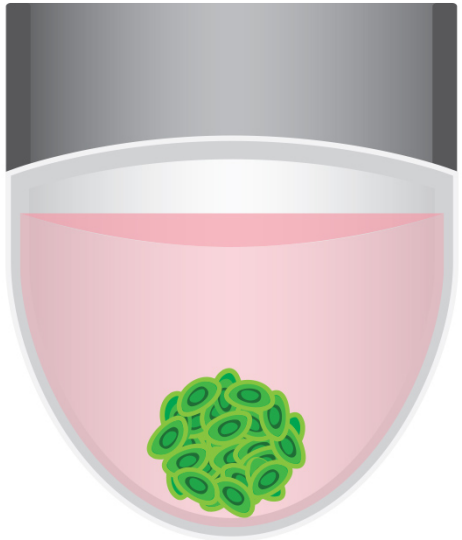
High Rhodamine 123 efflux demonstrates good P-gp functionality.





Löscher, Wolfgang, and Heidrun Potschka. "Blood-brain barrier active efflux transporters: ATP-binding cassette gene family." NeuroRx 2.1 (2005): 86-98.

Gliomasphere Assay

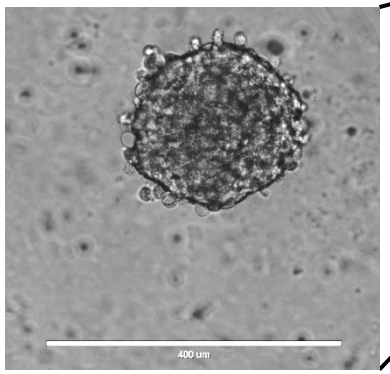


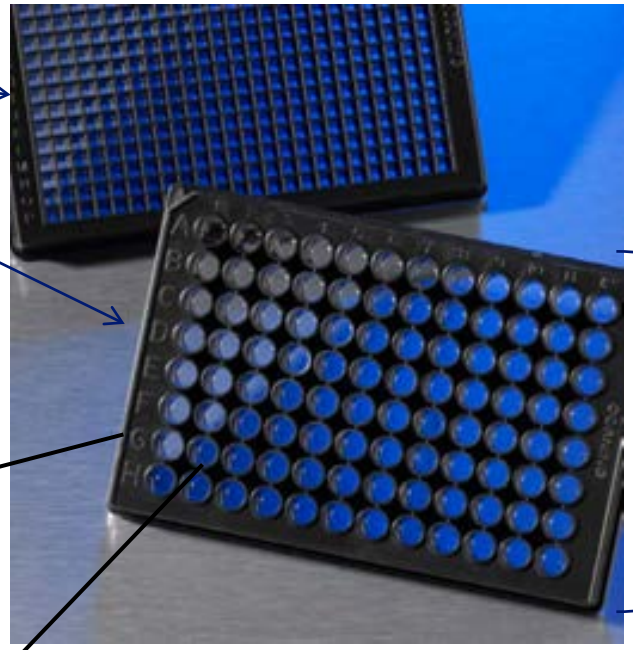
3D Glioblastoma Formation

Corning® Ultra-Low Attachment (ULA) surface on the Corning spheroid microplate features a unique round well-bottom design which enables the formation and growth of a single, uniform spheroid per well with reproducible size.

Standard ANSI/SBS footprint dimensions for Corning spheroid microplate 96-well and 384-well formats

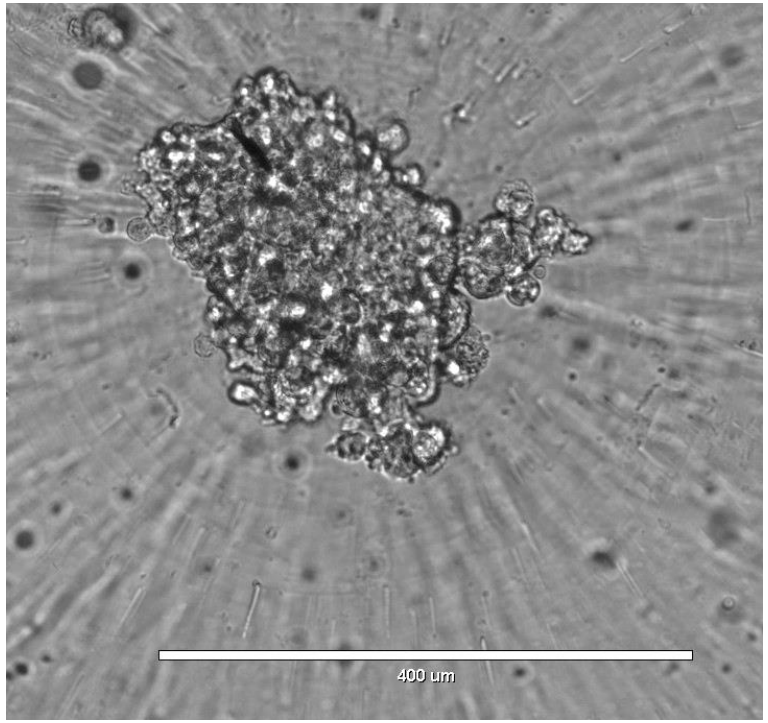
Clear bottom for visualization and imaging





Black sidewalls to reduce cross-talk and background noise in fluorescent- and luminescent-based assays

Spheroid Cytotoxicity

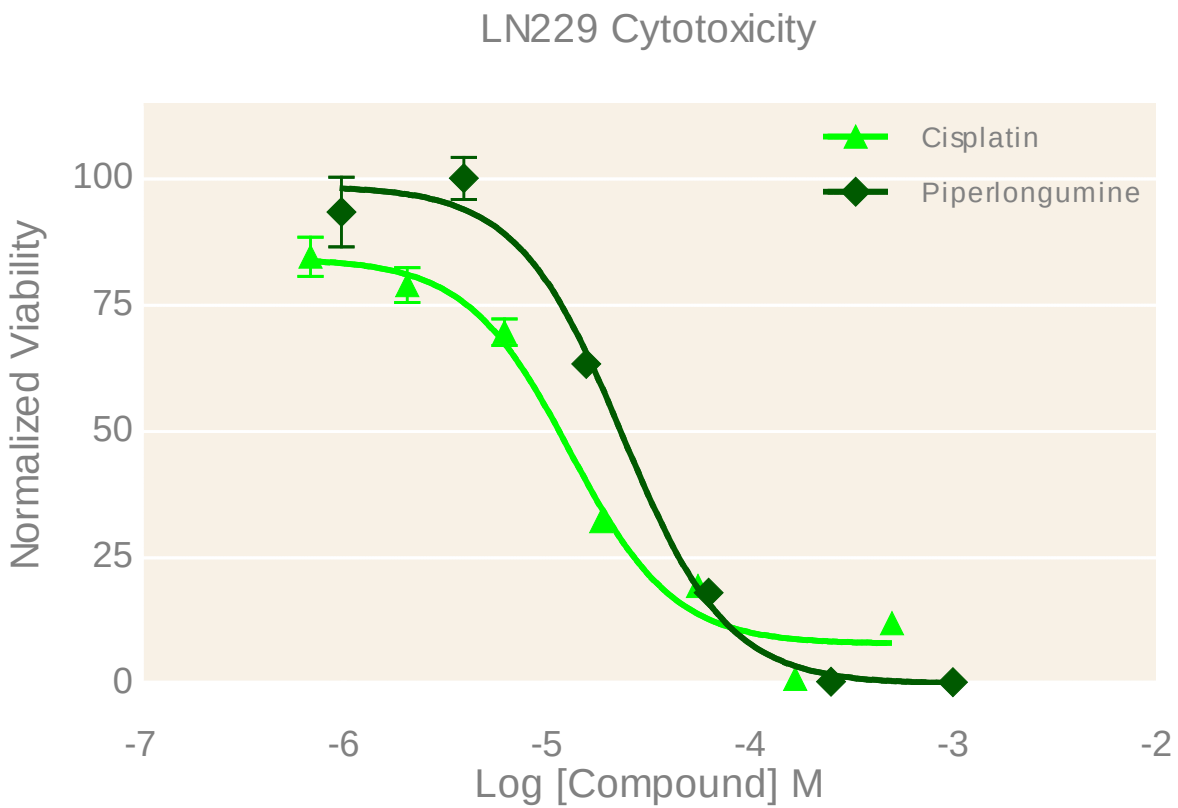


100x Image of LN229 spheroids after 24 hours

Basic Protocol

1. Seed LN229 glioblastoma cells at 1,000 cells/well for 24 hours
2. Remove medium and replace with drugs or negative control
3. Culture additional 48 hours
4. Add equal volume of Promega CellTitier-Glo® 3D reagent to assess cell viability via ATP

Spheroid Cytotoxicity

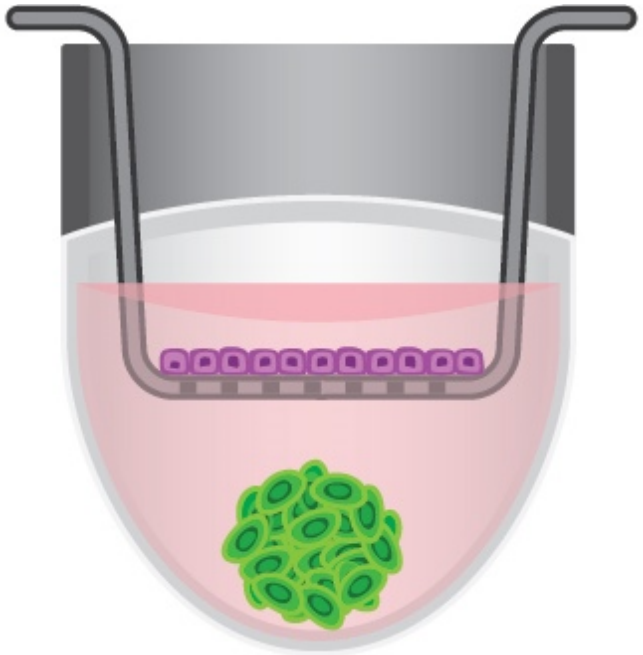


Demonstration of dose dependent cytotoxicity of control compounds

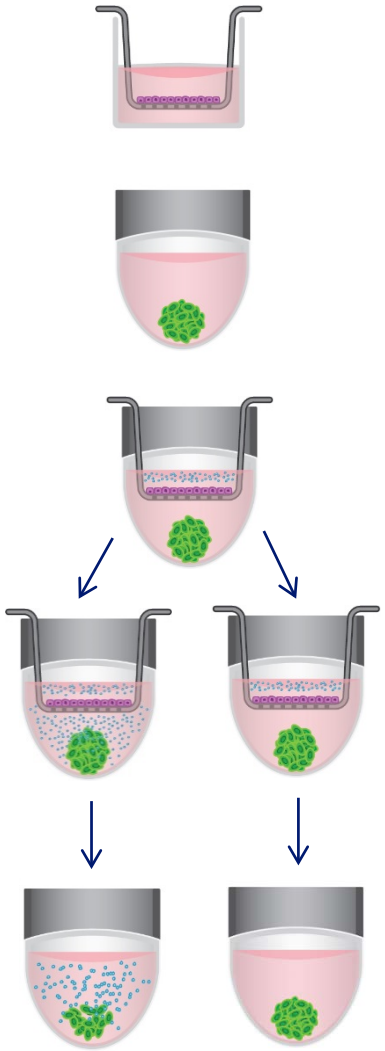
Cisplatin EC_{50} 1.299e-005 M

Piperlongumine EC_{50} 2.356e-005 M

3D Glioma Blood Brain Barrier Model



Assay Schematic



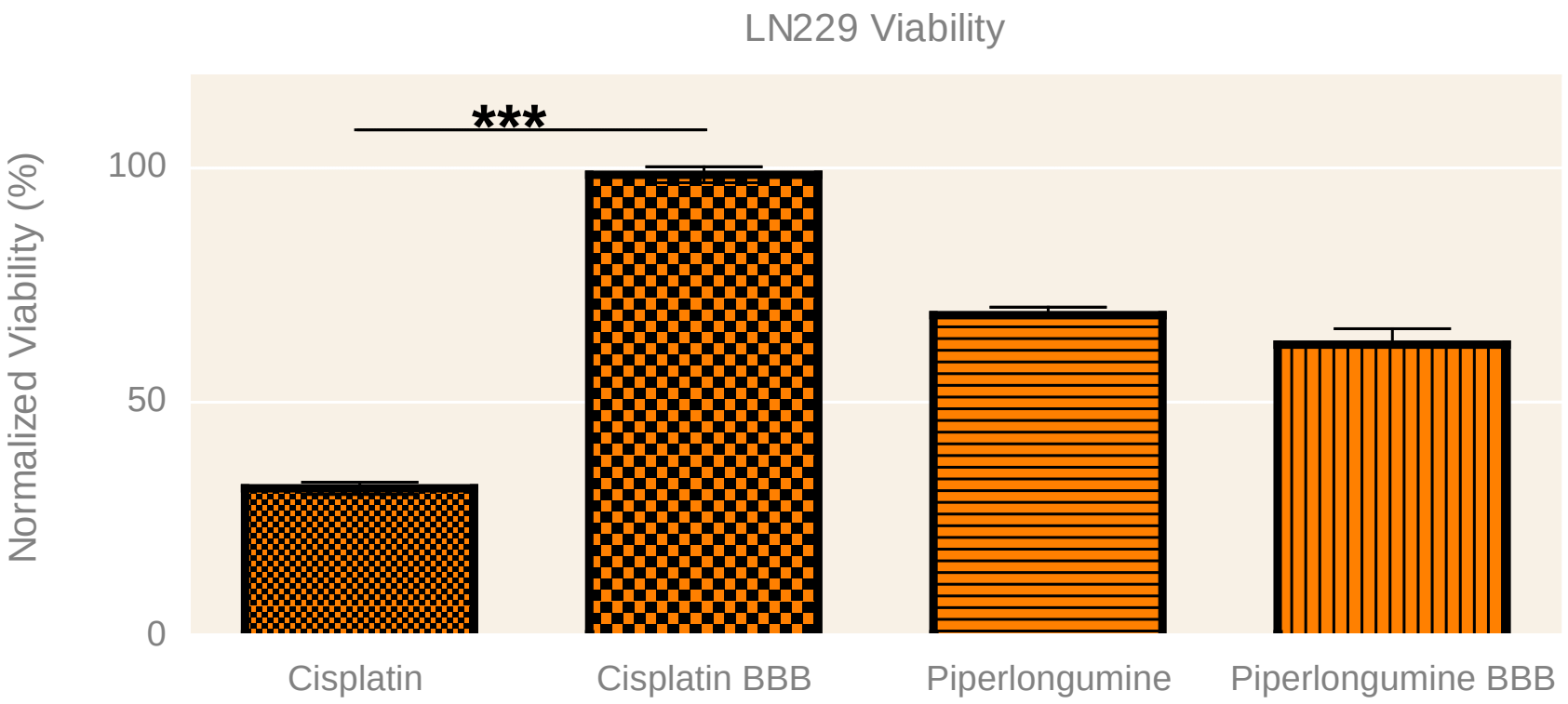
Day 0: Seed MDCKII/MDR1 into 96 HTS Transwells

Day 4: Seed LN229 cells into Spheroid microplate

Day 5: Combine Transwell® permeable supports from Corning with the Corning spheroid microplate and expose apical chamber to drugs for 2 hours. After drug incubation remove Transwell and test for monolayer integrity. Culture spheroids for 2 additional days.

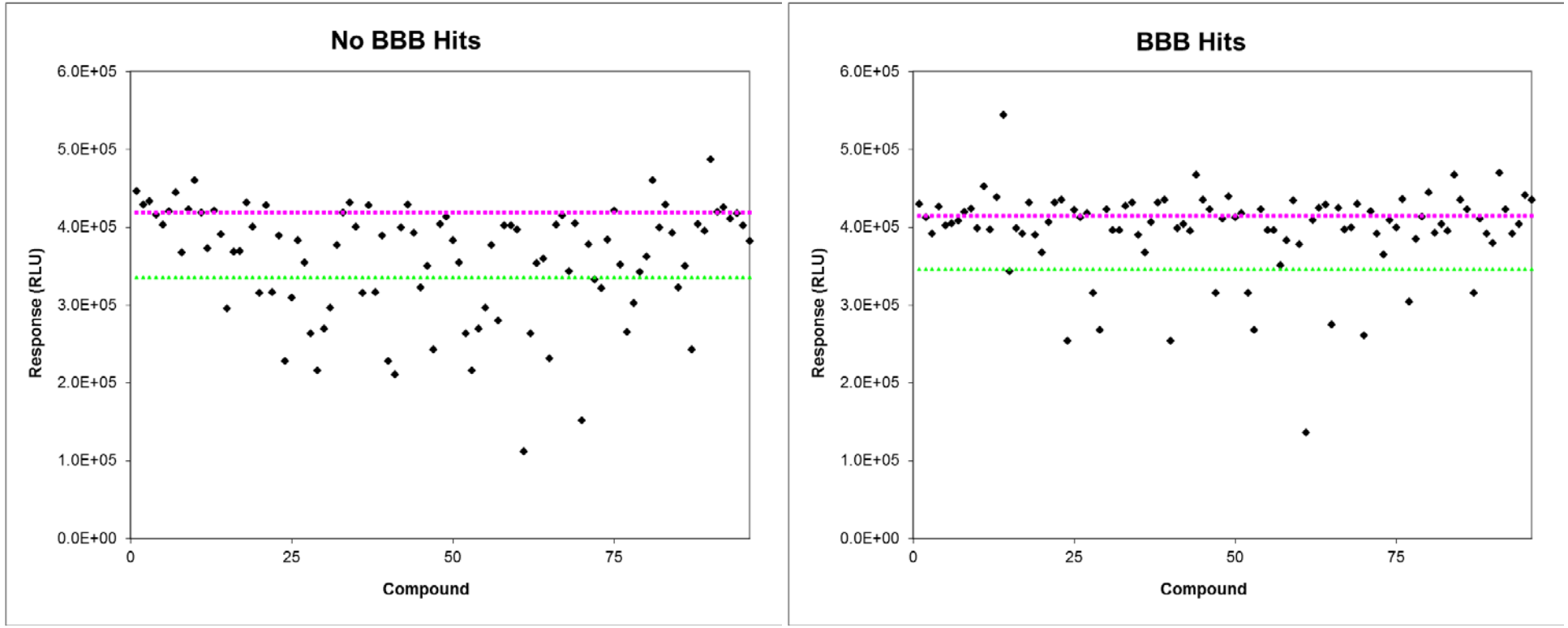
Day 7: Assay spheroids for cytotoxicity

Combined Model



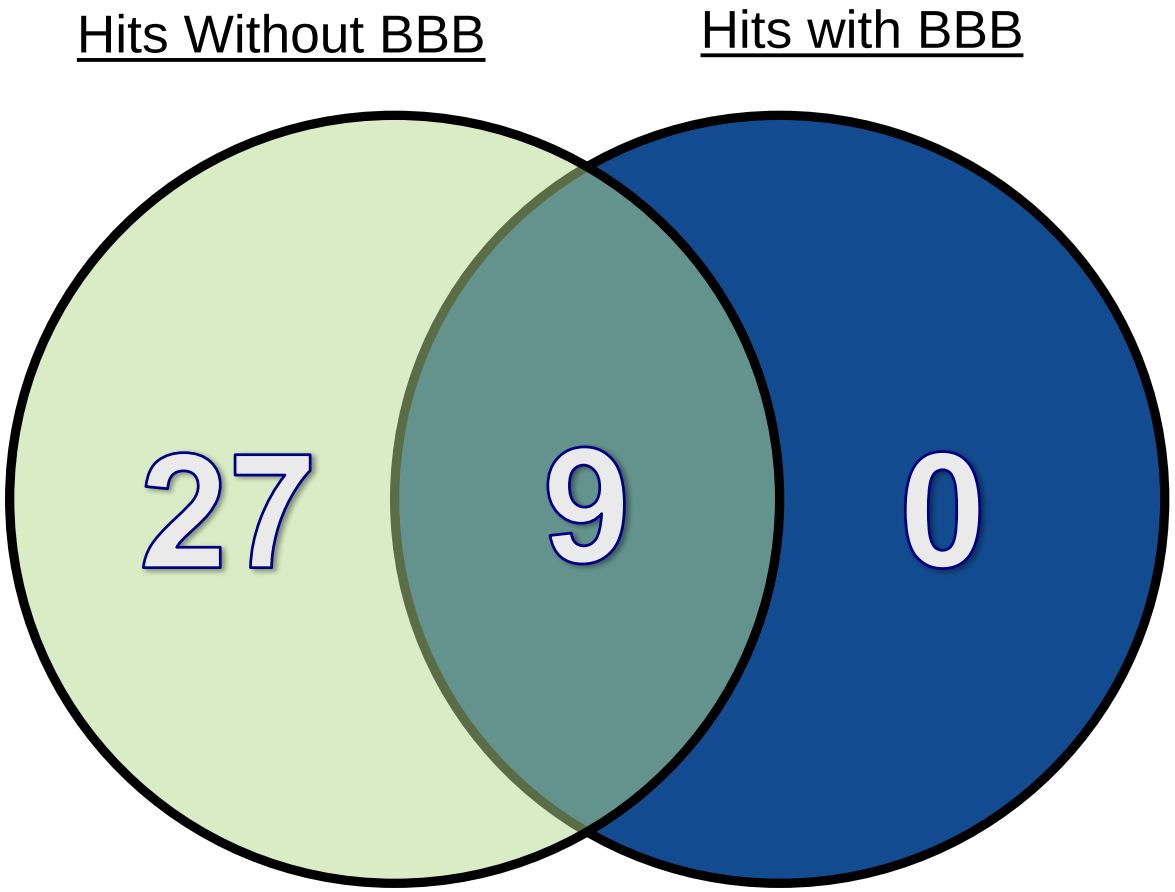
LN229 Cytotoxicity with or without MDCKII/MDR1 cells after 2 hours of exposure to 250 µM of drug as compared to buffer control.

Drug Screen



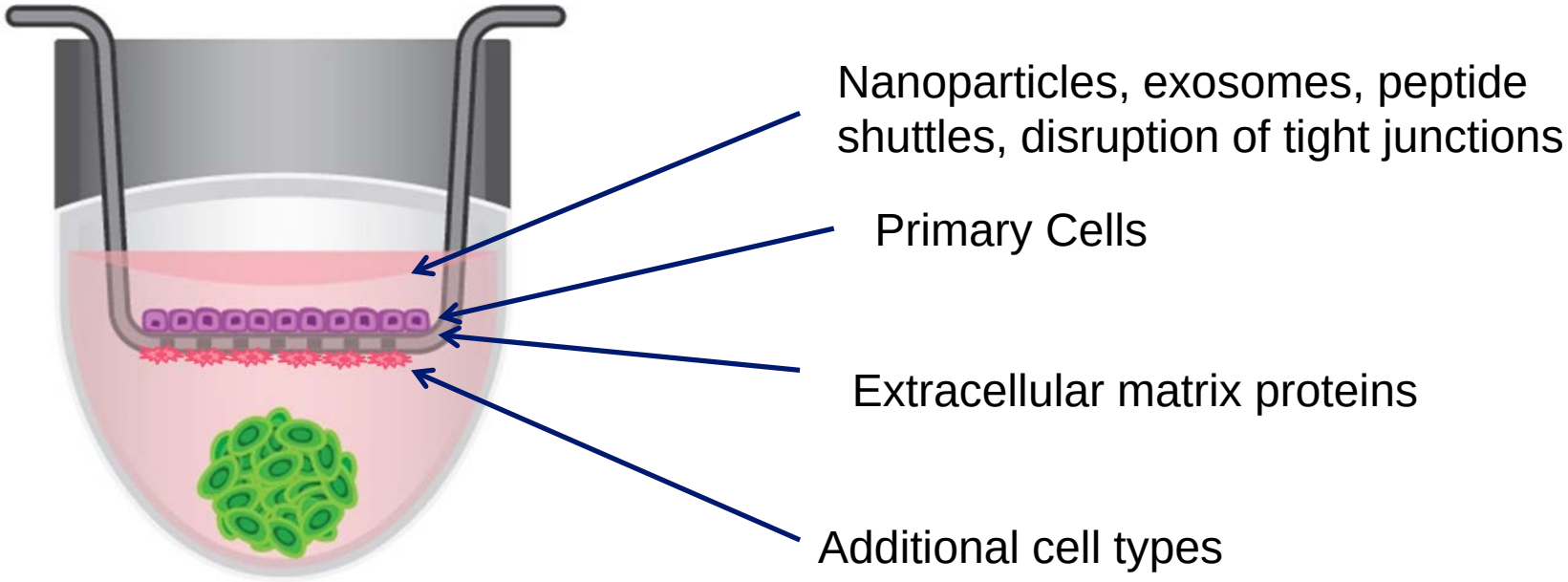
Representative screen from drug library containing 80 compounds showing hits found with and without BBB. Pink line is average buffer control and green line represents 3 sigma below buffer response (Hit).

Drug Screen Summary

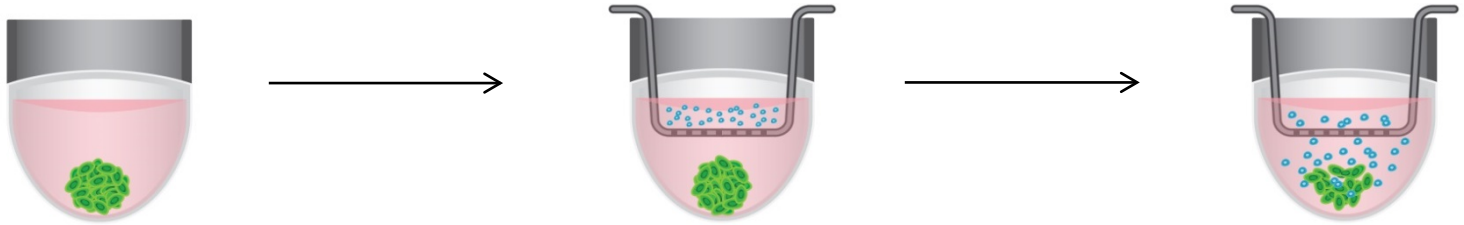


Compilation of hits discovered with and without BBB. Hits were considered if they were 3 sigma below buffer response in at least 2 of 3 independent screens.

Variations


Nanoparticles, exosomes, peptide shuttles, disruption of tight junctions
Primary Cells
Extracellular matrix proteins
Additional cell types

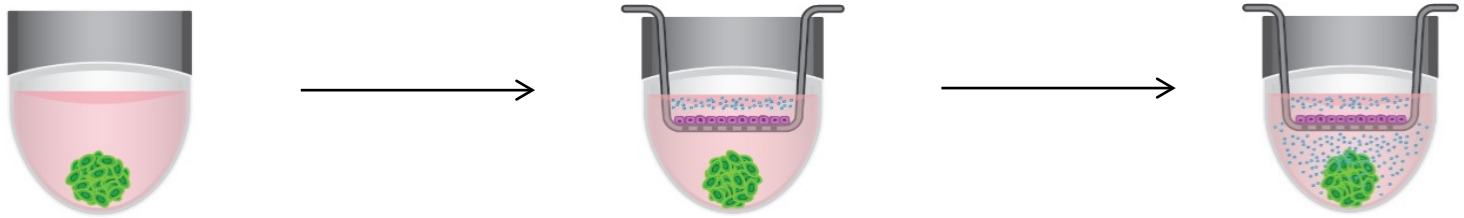
Other Applications



Cancer cells added to spheroid microplate

Immune cells added to insert

Plates incubated together to allow for migration, infiltration, and cytotoxicity



Neural stem cells added to spheroid microplate

Placental barrier is inserted into spheroid microplate and virus added to insert

Plates incubated together to look for viral infection

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Appendix

Hits Without BBB

NH125
GF 109203X
LY 294002 hydrochloride
U0126
SB 239063
SL 327
Ro 31-8220 mesylate
API-2
Ro 08-2750
BIBX 1382 dihydrochloride
Arcyriaflavin A
ZM 447439
ER 27319 maleate
ZM 306416 hydrochloride
IKK 16
10-DEBC hydrochloride
TPCA-1
SB 218078
PD 198306
Ryuvidine
IMD 0354
CGK 733
CGP 57380
PI 828
NU 7026
H 89 dihydrochloride
Iressa

27

Hits with BBB

U0126
SL 327
BIBX 1382 dihydrochloride
ER 27319 maleate
ZM 306416 hydrochloride
PD 198306
IMD 0354
PI 828
Iressa

9

Compilation of hits discovered with and without BBB. Hits were considered if they were 3 sigma below buffer response in at least 2 of 3 independent screens.