

HMS LINCS Center

**Mining LINCS drug-response databases to
identify novel activities of investigational
breast cancer therapeutics**

Caitlin Mills

4/4/2017

U54-HL127365

Disclosure Information
AACR Annual Meeting 2017
Caitlin E. Mills

I have no financial relationships to disclose.

I will not discuss off label use and/or investigational use in my presentation.

Overview

- Overview of current HMS LINCS breast cancer profiling effort
 - Clinically relevant kinase inhibitors
 - Triple negative breast cancer (TNBC)
 - PDX models
- Focused follow-up work on CDK4/6 inhibitors
- Snapshot of available, and future datasets relevant to breast cancer

Dataset collection

- Focus on triple negative breast cancer
 - Unmet clinical need
 - Poor prognosis
 - No targeted therapy options
- Dose response to kinase inhibitors of clinical relevance
- Baseline profiling under matched conditions
 - Transcriptomics
 - Total and phosphoproteomics

Selection of relevant cell lines and drug treatments

Cell Line			Receptor Status	Molecular Subtype	
20 TNBC	BT-20	TNBC	Basal A		
	HCC1143	TNBC	Basal A		
	HCC1806	TNBC	Basal A		
	HCC1937	TNBC	Basal A		
	HCC70	TNBC	Basal A		
	MDA-MB-468	TNBC	Basal A		
	BT-549	TNBC	Basal B		
	CAL-51	TNBC	Basal B		
	HCC1395	TNBC	Basal B		
	HCC38	TNBC	Basal B		
	Hs 578T	TNBC	Basal B		
	MDA-MB-157	TNBC	Basal B		
	MDA-MB-231	TNBC	Basal B		
	MDA-MB-436	TNBC	Basal B		
	SUM1315	TNBC	Basal B		
	SUM149	TNBC	Basal B		
	SUM159	TNBC	Basal B		
	CAL-85-1	TNBC	Basal		
	CAL-120	TNBC	Luminal		
	MDA-MB-453	TNBC	Luminal		
6 HR+	CAMA-1	HR+	Luminal		
	HCC1428	HR+	Luminal		
	HCC1500	HR+	Luminal		
	MCF7	HR+	Luminal		
	MDA-MB-134	HR+	Luminal		
	T47D	HR+	Luminal		
4 Her2amp	HCC1954	HER2amp	Basal A		
	HCC1419	HER2amp	Luminal		
	MDA-MB-361	HER2amp	Luminal		
	SK-BR-3	HER2amp	Luminal		
2 NM	hTERT-hME1	NM	Basal		
	MCF 10A	NM	Basal		
4 from PDX	PDX-DFCI-1206	TNBC	N/A		
	PDX-DFCI-1258	TNBC	N/A		
	PDX-DFCI-1328	TNBC	N/A		
	PDX-HCI-002	TNBC	N/A		
Drug Name			Primary Target	Clinical Status	
Alpelisib/BYL719			PI3Ka	Phase 3	
TGX221			PI3Kb	Preclinical	
Taselisib/GDC0032			PI3Ka, g, d	Phase 1/2	
Pictilisib/GDC0941			pan PI3K	Phase 2	
Buparlisib/NVP-BKM120			pan PI3K	Phase 2	
INK128/MLN0128			mTORC1/2	Phase 2	
Torin2			mTOR/ATM/ATR	Tool	
Everolimus			mTOR1	Approved	
Ipatasertib/GDC0068			AKT	Phase 1/2	
PF-4708671			p70S6K	Phase 1	
Neratinib/HKI272			EGFR/HER2	Phase 3	
Tivantinib/ARQ197			MET	Phase 3	
Cabozantinib			VEGFR2/MET	Approved	
Cediranib/AZD2171			VEGFR/cKIT	Phase 3	
Ceritinib/LDK378			ALK	Phase 2/3	
Saracatinib/AZD0530			SRC	Phase 2/3	
Dasatinib			BCR/ABL	Approved	
Trametinib/GSK1120212			MEK	Phase 2	
Luminespib/NVP-AUY922			HSP90	Phase 2	
Palbociclib/PD0332991			CDK4/6	Phase 3	
Dinaciclib/SCH727965			pan CDK	Phase 1	
Abemaciclib/LY2835219			CDK4/6	Phase 3	
Volasertib/Bi6727			PLK	Phase 2/3	
AZD7762			CHK1/2	Phase 1	
Olaparib/AZD2281			PARP	Phase 3	
ABT-737			Bcl2/XL	Tool	
A-1210477			Mcl-1	Tool	
Vorinostat			HDAC	Phase 2	
Paclitaxel			Chemotherapy	Approved	
Doxorubicin			Chemotherapy	Approved	
Cisplatin			Chemotherapy	Approved	
Etoposide			Topoisomerase II	Approved	
Topotecan			Topoisomerase I	Approved	
Bleomycin			Radiomimetic	Approved	
Ionizing radiation			DNA damage	Approved	

LINCS cell line and compound resources



LINCS

Home Cell Lines Drugs Datasets About

MCF 10A



General Information

Collections: ICBP43, Core 6
Receptor Status: NM
Molecular Subtype: Non malignant, Basal

Media & ATCC Information

Media Base: 1:1 mixture of DMEM/Ham's glutamine, 15 mM Hepes)
Media Additives: 5% horse serum + 20 ng/ml h
100 ng/ml cholera toxin + 10
insulin + 500 ng/ml hydrocort
P/S
ATCC Number: CRL-10317

LINCS

Library of Integrated
Network-based
Cellular Signatures

HMS LINCS
DATABASE

PUBLICATIONS

DATA
EXPLORATION

SOFTWARE

Images

4x



HMS LINCS DB home
Small molecules
Structure search
Salt table
Cell Lines
Primary Cells
Proteins
Antibodies
Other Reagents
Datasets
Libraries
Login

Alpelisib - Small Molecule (ID:10233-101)

[download molfile](#)

HMS LINCS ID:	10233-101
Name:	Alpelisib
Alternative Names:	BYL719
LINCS ID:	LSM-4256
PubChem CID:	56649450
ChEBI ID:	
ChEMBL ID:	
Molecular Mass:	441.14
InChi:	InChI=1S/C19H22F3N5O2S/c 1-10-14(11-6-7-24-13(9-11)18(2,3)19(20,21)22)30-16(25-10)26-17(29)27-9-4-5-12(27)15(23)28/h6-7,9,12H,4-5,8H2,1-3H3,(H2,23,28)(H,25,26,29)/t12-m/s1
InChi Key:	STUWVGJZDJHPWGZ-LBPRGKRZSA-N
SMILES:	CC1=C(SC(=N1)NC(=O)N2CCC(C@H)2C(=O)N)C3=CC(=NC=C3)C(C)(C)F(F)F
Relevant Citations:	
Date Publicly Available:	
Most Recent Update:	2016-07-12

Nominal Targets:

Small Molecule	Protein	Effective Concentration	Key References
Alpelisib	PIK3CA		2012, AACR 103rd Annual Meeting, Abst CT-01.

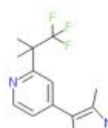
Datasets:

HMS Dataset ID	Dataset Title	HMS Dataset Type
20000	LINCS Compound Protein Targets and Concentrations	Nominal Targets
20237	LINCS Pilot Phase Joint Project: Sensitivity measures of six breast cancer cell lines to a library	Microscopy/Imaging

LINCS

Home Cell Lines Drugs Datasets About

Alpelisib



General Information

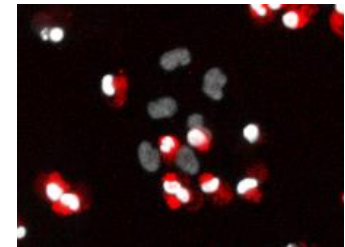
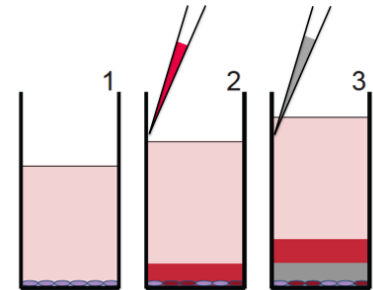
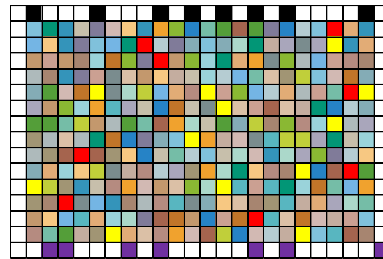
Development Stage: Phase 3
Synonyms: None known
HMS LINCS ID: 10233-101

Target Information

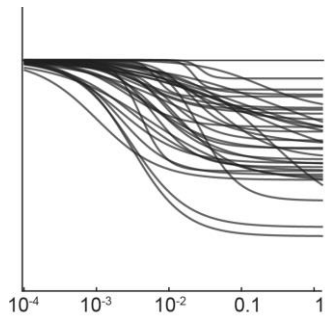
Gene: PIK3CA
Gene class: PI3KA
Pathway: PI3K
Biological function: AKT_Pi3K
Protein: PK3CA_HUMAN
Protein class: KINASE

<http://www.cancerbrowser.org/>
<http://lincs.hms.harvard.edu/db/>

Experimental workflow



t	Treatment	Dose (uM)	Cell count	Dead cell count
72	Staurosporine	1	5091	1833
72	Staurosporine	1	5929	2137
72	Staurosporine	1	5663	2021
72	DMSO	0	8000	297
72	Staurosporine	0.316	6613	1142
72	DMSO	0	7732	329
72	Staurosporine	1	5463	2473
72	DMSO	0	8746	88
72	Staurosporine	0.316	6168	1496
72	Staurosporine	0.1	7941	636
72	DMSO	0	8529	360
72	Staurosporine	0.316	6994	1157
72	DMSO	0	8872	160
72	DMSO	0	9166	73
72	Staurosporine	1	5091	1833
72	Staurosporine	1	5929	2137
72	Staurosporine	1	5663	2021
72	DMSO	0	8000	297
72	Staurosporine	0.316	6613	1142
72	DMSO	0	7732	329
72	Staurosporine	1	5463	2473
72	DMSO	0	8746	88
72	Staurosporine	0.316	6168	1496



Dye-drop assay protocol

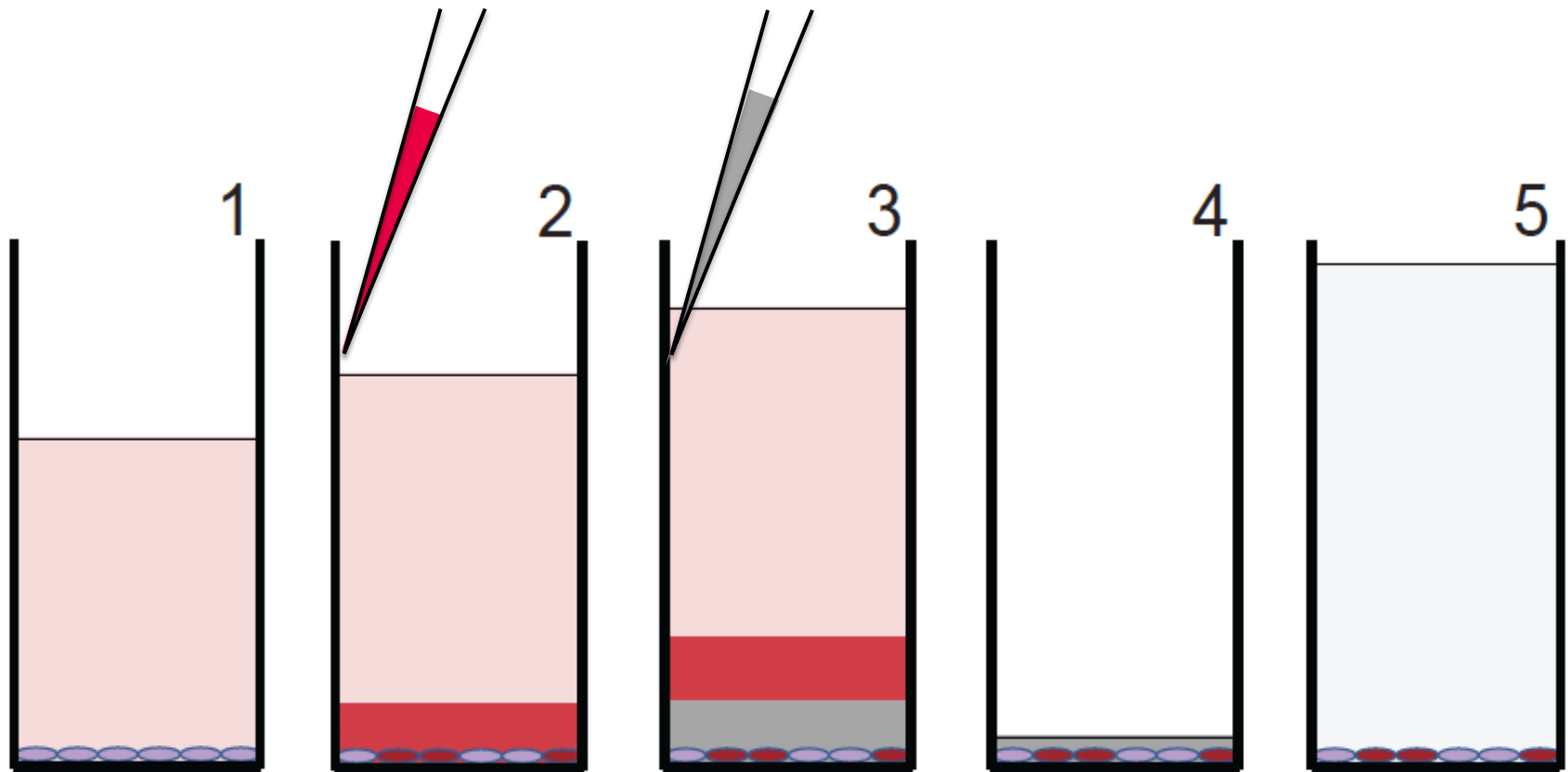
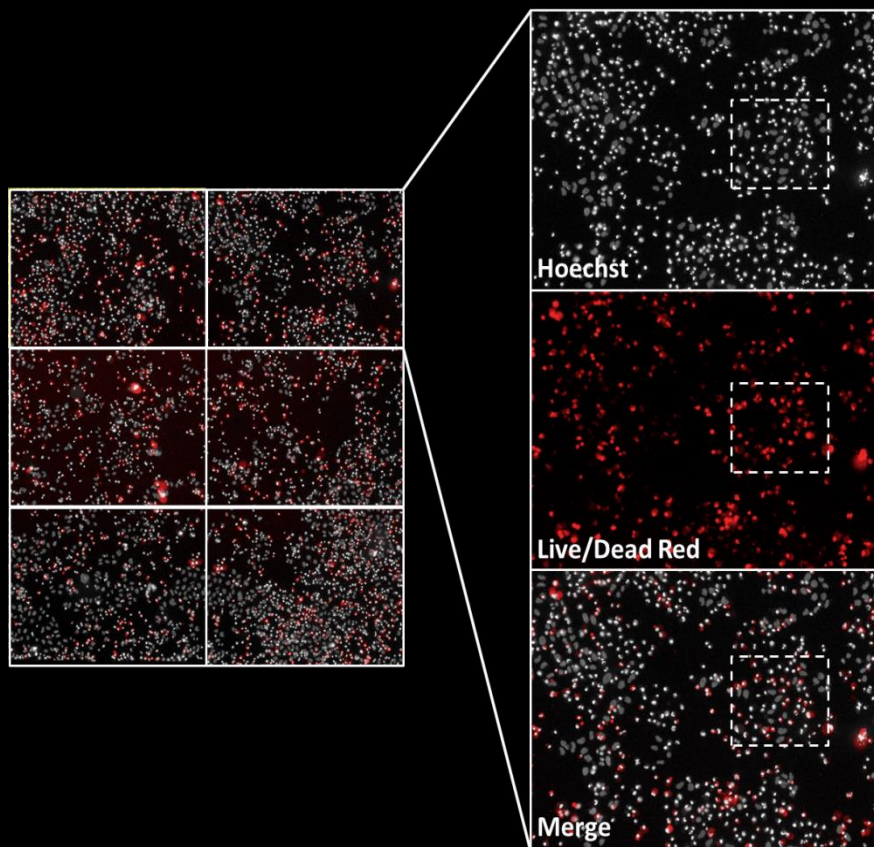
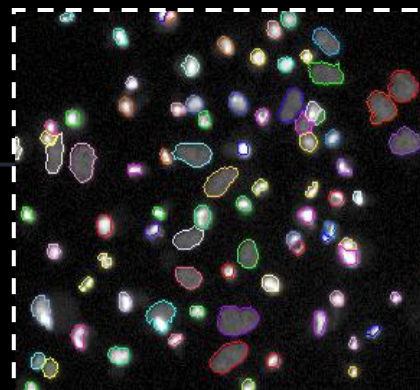


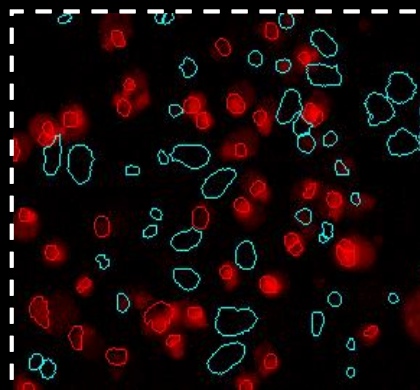
Image analysis



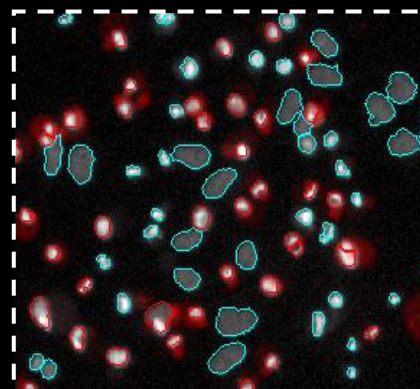
1. Segment nuclei



2. Measure LDR signal

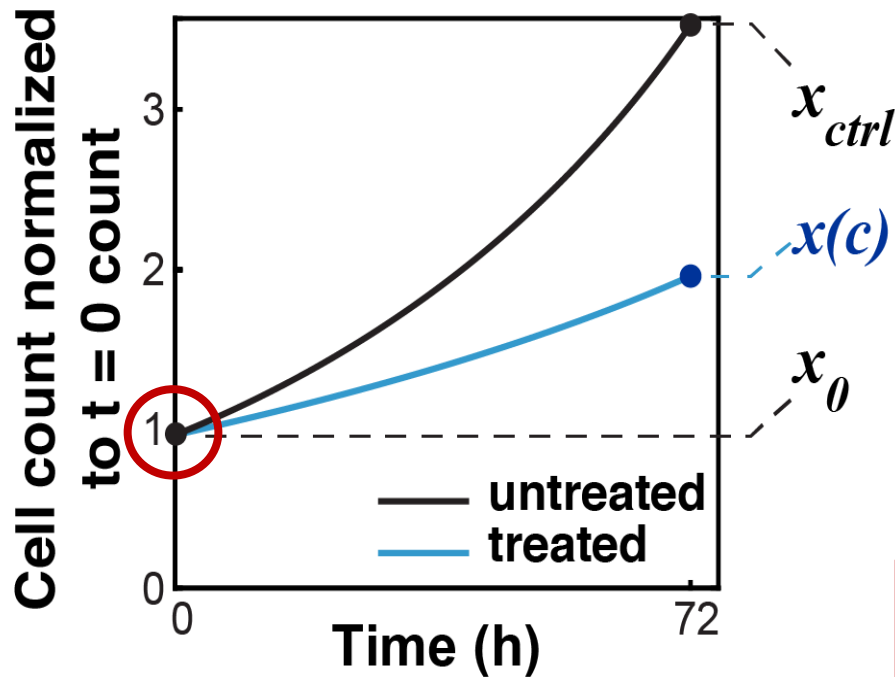


3. Classify live/dead cells



Well	Row	Column	Cell Line	Time point	Treatment	Dose (uM)	Cell count	Dead cell count	Cell count t=0
C2	3	3	2MCF10A	72	Staurosporine	1	5091	1833	1954
C3	3	3	3MCF10A	72	Staurosporine	1	5928	2137	1954
C4	3	3	4MCF10A	72	Staurosporine	1	5663	2021	1954
C5	3	3	5MCF10A	72	Staurosporine	1	5928	2137	1954
C6	3	3	6MCF10A	72	Staurosporine	0.316	6033	1142	1954
C7	3	3	7MCF10A	72	Staurosporine	0	7732	329	1954
C8	3	3	8MCF10A	72	Staurosporine	1	5463	2473	1954
D2	4	4	2MCF10A	72	DMSO	0	8746	88	1954
D3	4	4	3MCF10A	72	Staurosporine	0.316	6168	1496	1954
D4	4	4	4MCF10A	72	Staurosporine	0.1	7941	636	1954
D5	4	4	5MCF10A	72	DMSO	0	8529	360	1954
D6	4	4	6MCF10A	72	Staurosporine	0.316	6994	1157	1954
D7	4	4	7MCF10A	72	DMSO	0	8872	160	1954
D8	4	4	8MCF10A	72	DMSO	0	9166	73	1954
C2	3	3	2MCF10A	72	Staurosporine	1	5091	1833	1954
C3	3	3	3MCF10A	72	Staurosporine	1	5928	2137	1954
C4	3	3	4MCF10A	72	Staurosporine	1	5663	2021	1954
C5	3	3	5MCF10A	72	DMSO	0	8000	297	1954
C6	3	3	6MCF10A	72	Staurosporine	0.316	6613	1142	1954
C7	3	3	7MCF10A	72	DMSO	0	7732	329	1954
C8	3	3	8MCF10A	72	Staurosporine	1	5463	2473	1954
D2	4	4	2MCF10A	72	DMSO	0.316	6168	1496	1954
D3	4	4	3MCF10A	72	Staurosporine	0.1	7941	636	1954
D4	4	4	4MCF10A	72	Staurosporine	0.1	7941	636	1954
D5	4	4	5MCF10A	72	DMSO	0	8529	360	1954
D6	4	4	6MCF10A	72	Staurosporine	0.316	6994	1157	1954
D7	4	4	7MCF10A	72	DMSO	0	8872	160	1954
D8	4	4	8MCF10A	72	DMSO	0	9166	73	1954
C2	3	3	2MCF10A	72	Staurosporine	1	5091	1833	1954
C3	3	3	3MCF10A	72	Staurosporine	1	5928	2137	1954
C4	3	3	4MCF10A	72	Staurosporine	1	5663	2021	1954
C5	3	3	5MCF10A	72	DMSO	0	8000	297	1954
C6	3	3	6MCF10A	72	Staurosporine	0.316	6613	1142	1954
C7	3	3	7MCF10A	72	DMSO	0	7732	329	1954
C8	3	3	8MCF10A	72	Staurosporine	1	5463	2473	1954
D2	4	4	2MCF10A	72	DMSO	0.316	6168	1496	1954
D3	4	4	3MCF10A	72	Staurosporine	0.1	7941	636	1954
D4	4	4	4MCF10A	72	Staurosporine	0.1	7941	636	1954
D5	4	4	5MCF10A	72	DMSO	0	8529	360	1954
D6	4	4	6MCF10A	72	Staurosporine	0.316	6994	1157	1954
D7	4	4	7MCF10A	72	DMSO	0	8872	160	1954
D8	4	4	8MCF10A	72	DMSO	0	9166	73	1954

GR values rely on three measures of cell count



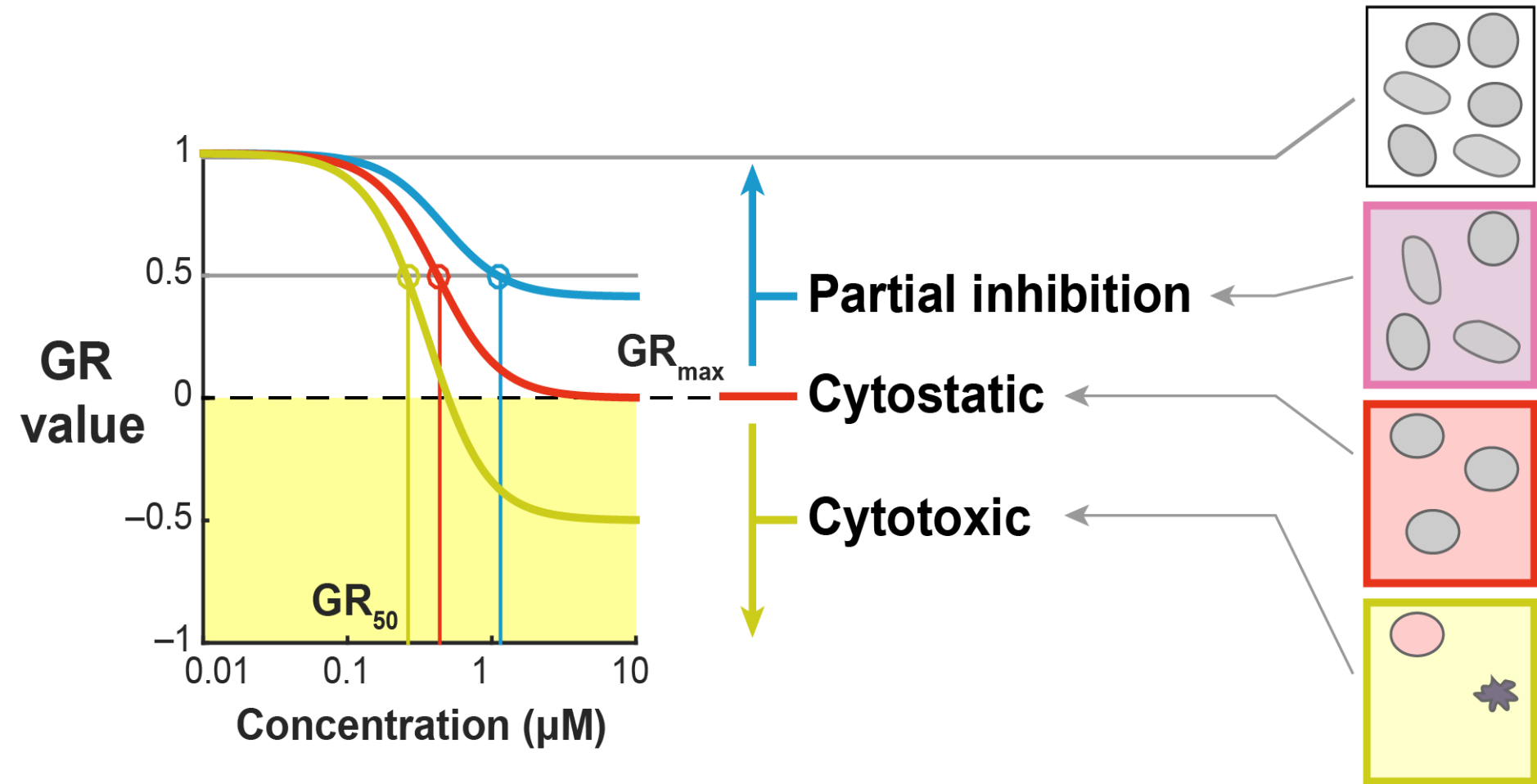
$$GR(c) = 2^{\frac{\log_2(x(c)/x_0)}{\log_2(x_{ctrl}/x_0)}} - 1$$

$x(c)$ is the treated cell count

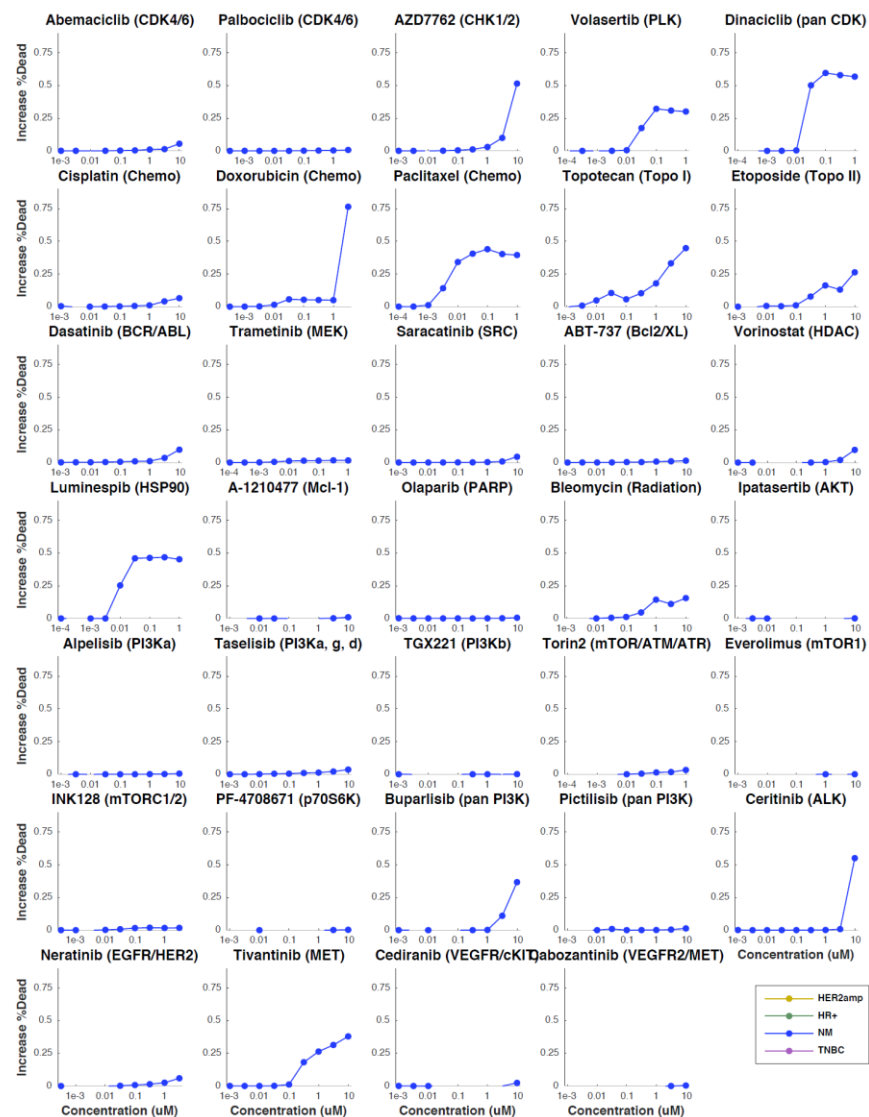
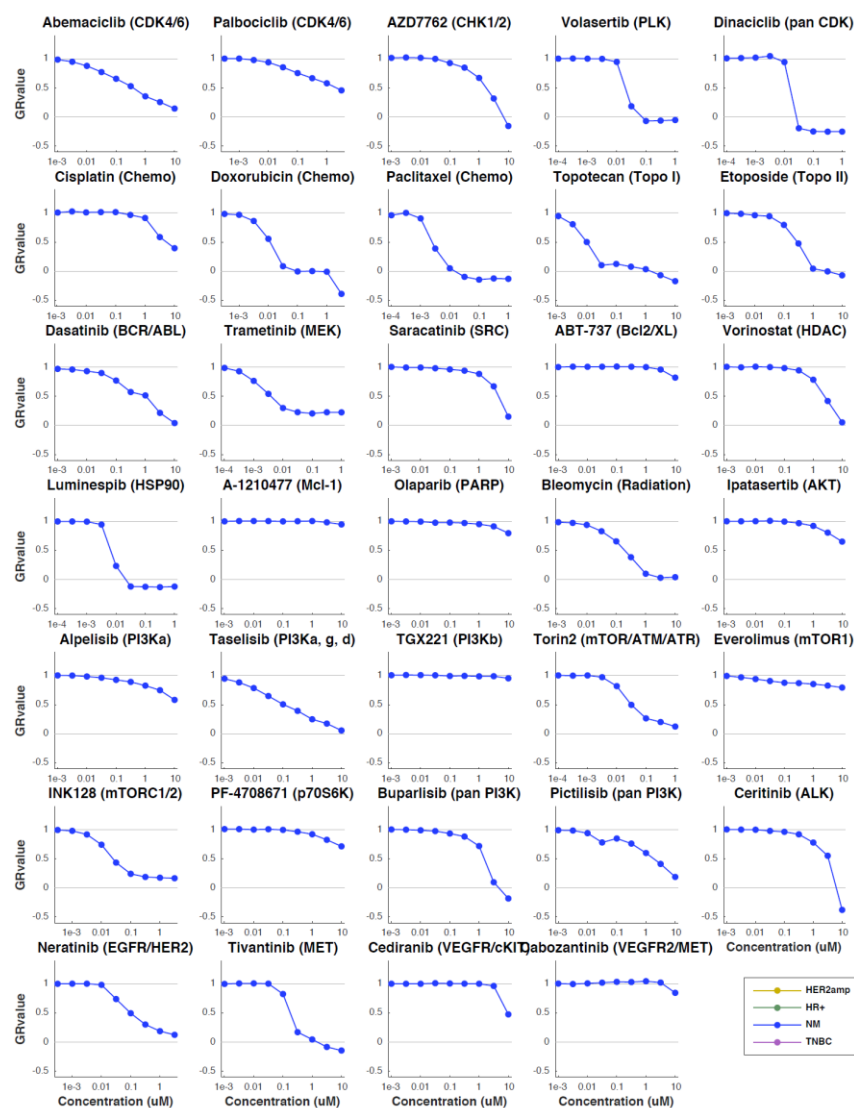
x_{ctrl} is the control cell count

x_0 is the cell count at the time of treatment

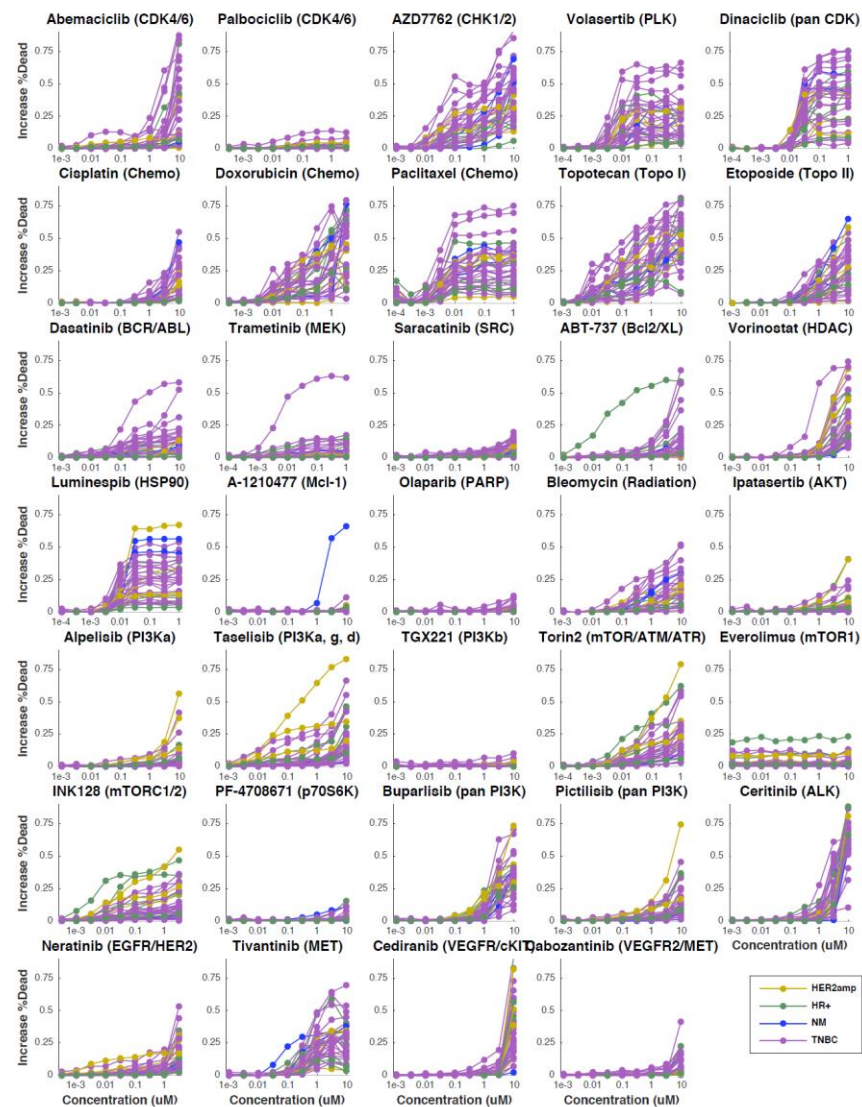
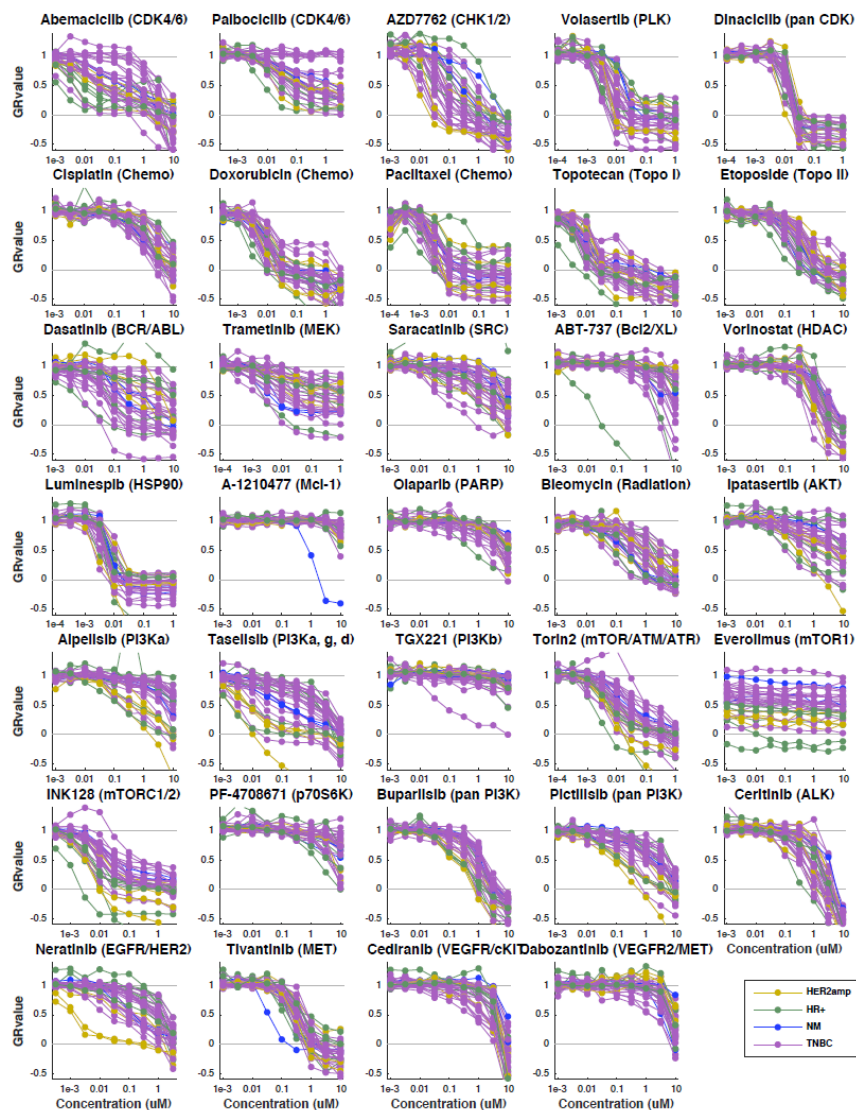
GR values are independent of the division rate and directly relate to the phenotype



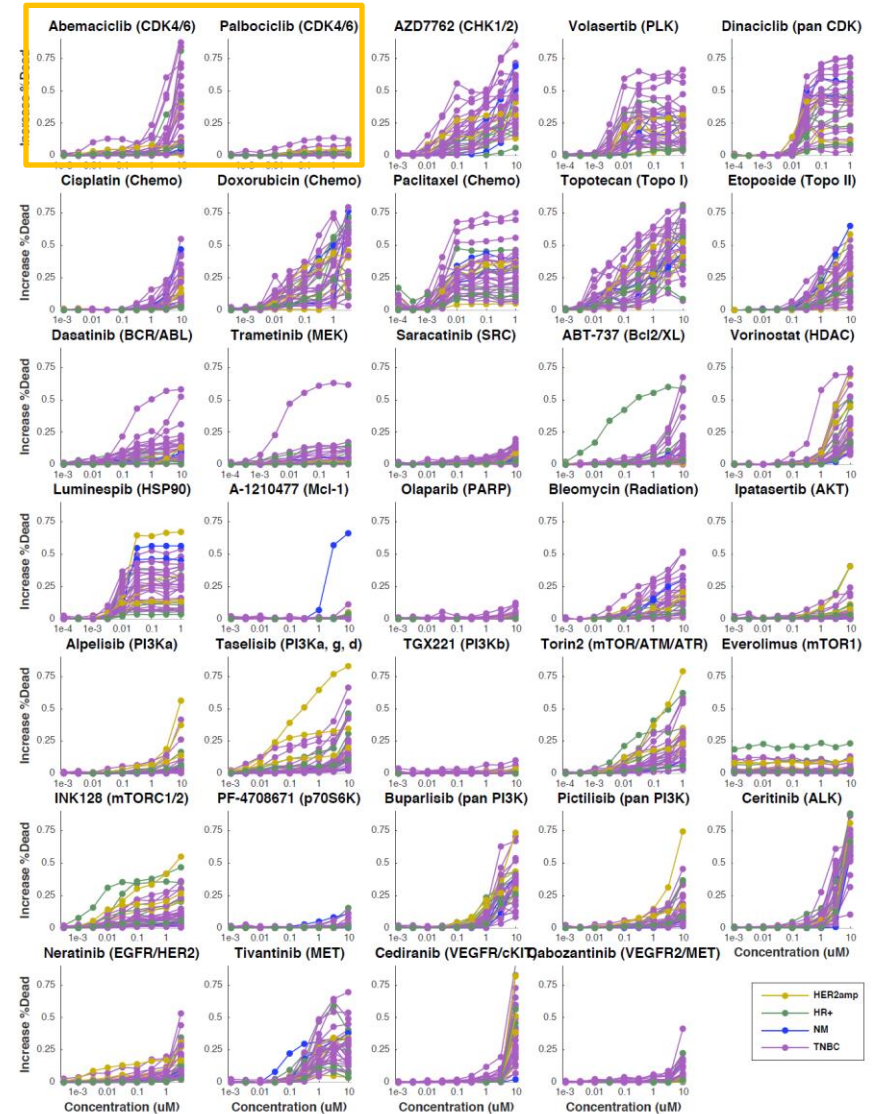
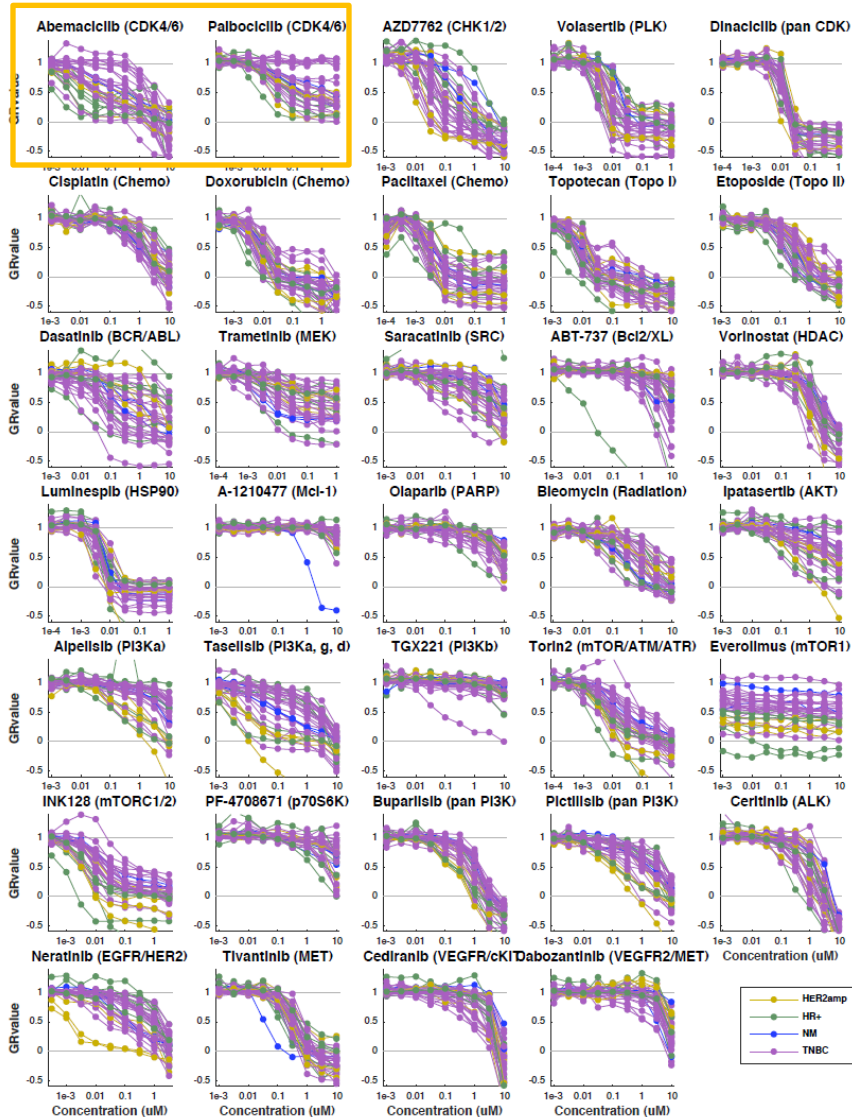
Dose response results – MCF10A cells



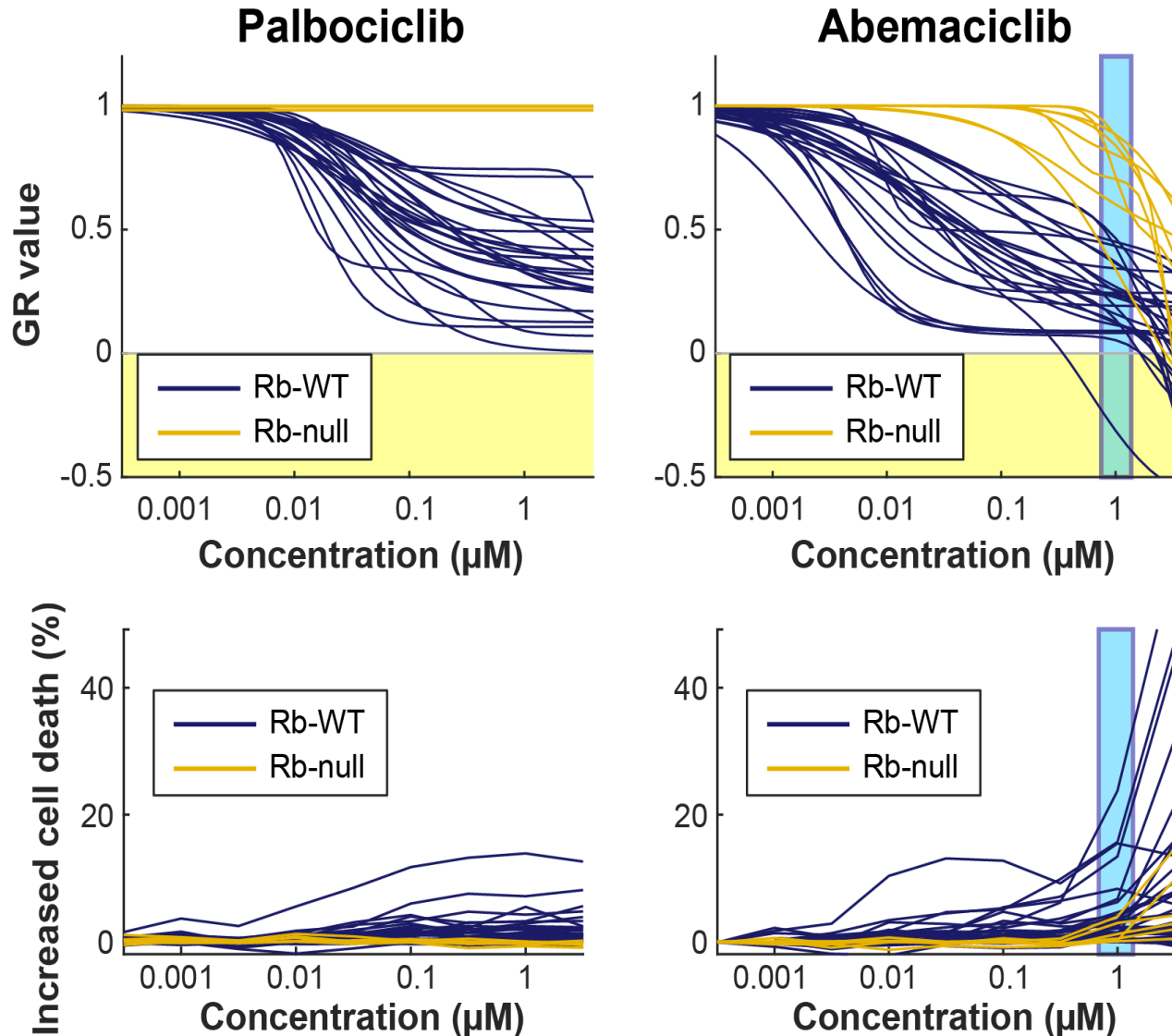
Dose response results – all cell lines



Dose response results – CDK4/6 inhibitors



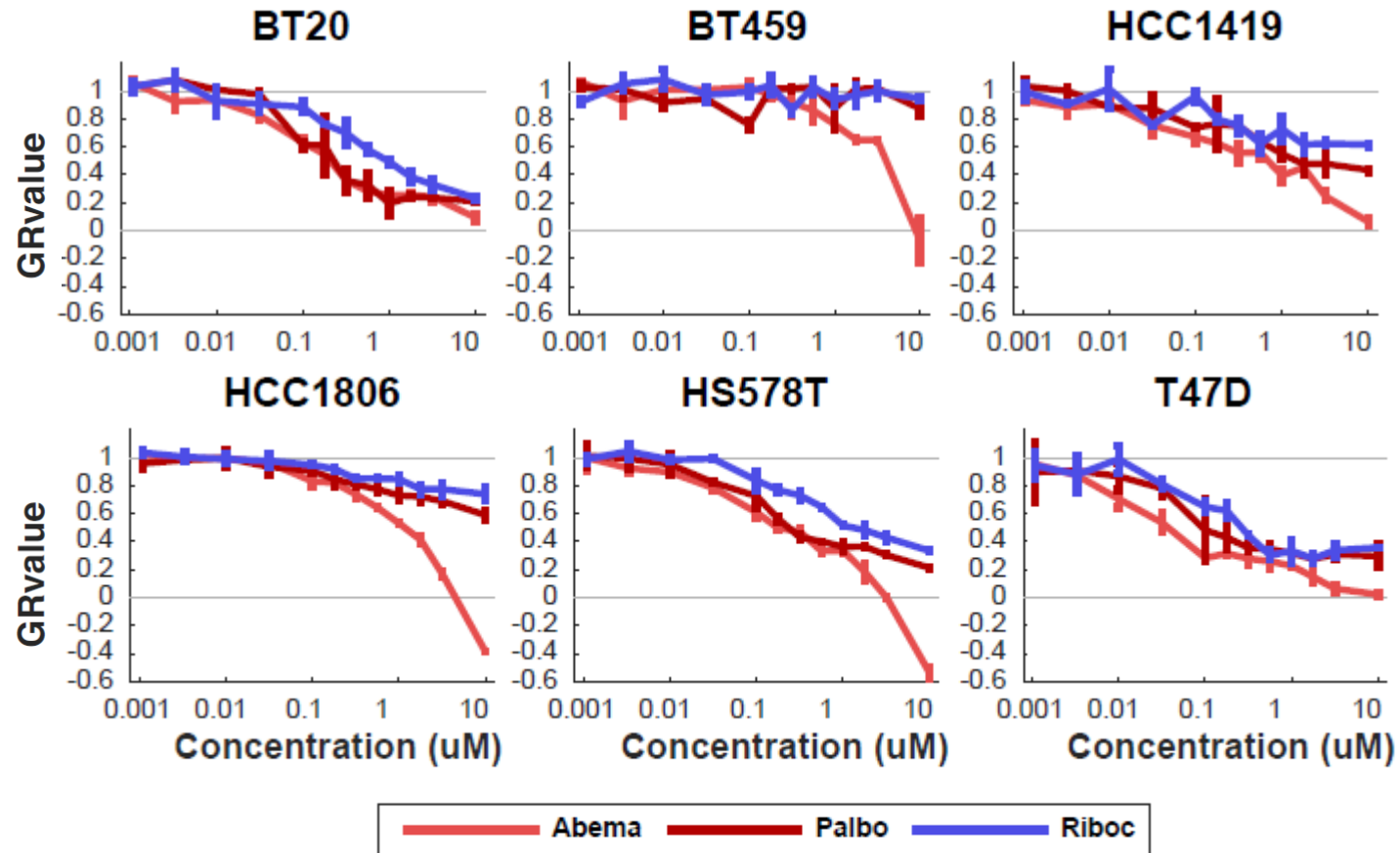
Abemaciclib has a higher efficacy and induces death in all breast cancer cell lines



Clinical characteristics of CDK4/6 inhibitors

Drug	Palbociclib (Pfizer) (PD0332991, Ibrance)	Ribociclib (Novartis) (LEE011)	Abemaciclib (Eli Lilly) (LY2835219)
IC ₅₀ (<i>in vitro</i> kinase assay, recombinant proteins)	CDK4 (D1): 11 nmol/L CDK4 (D3): 9 nmol/L CDK6 (D2): 15 nmol/L CDK1: >10 µmol/L CDK2: >10 µmol/L (66, 67)	CDK4: 10 nmol/L CDK6: 39 nmol/L CDK1: >100 µmol/L CDK2: >50 µmol/L (1, 89)	CDK4 (D1): 0.6–2 nmol/L CDK6 (D1): 2.4–5 nmol/L CDK 9: 57 nmol/L CDK1: >1 µmol/L CDK2: >500 nmol/L (1, 88)
PK	T _{max} 4.2–5.5 hr t _{1/2} 25.9–26.7 hr (69, 70)	T _{max} 4 hr t _{1/2} 24–36 hr (90, 91)	T _{max} 4–6 h t _{1/2} 17–38 h (crosses blood:brain barrier; refs. 92, 93)
PD	Reduced RB phosphorylation in paired tumor biopsies, along with reduced fluorothymidine-PET uptake (75)	Reduced RB phosphorylation and Ki67 expression in paired tumor biopsies (90)	Reduced RB phosphorylation and topoisomerase IIα expression in paired tumor and skin biopsies (92)
Dosing	125 mg daily (3 weeks, 1-week drug holiday) or 200 mg daily (2 weeks, 1-week drug holiday; refs. 69, 70)	600 mg daily (3 weeks, 1-week drug holiday; ref. 90)	200 mg twice daily (continuous dosing; ref. 92)
Major dose-limiting toxicities	Neutropenia, thrombocytopenia	Neutropenia, thrombocytopenia	Fatigue
Other reported adverse events	Anemia, nausea, anorexia, fatigue, diarrhea (69, 70)	Mucositis Prolonged EKG QTc interval Elevated creatinine Nausea (90)	Diarrhea Neutropenia (92)

How does ribociclib compare?

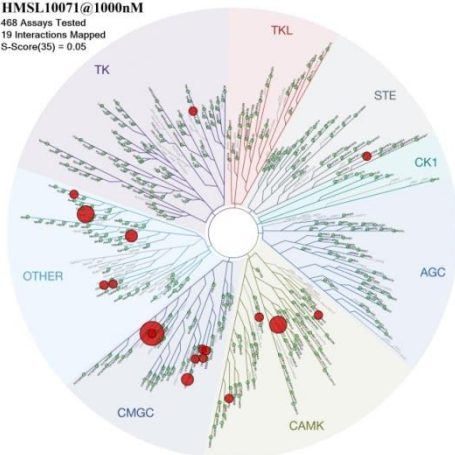


Abemaciclib remains the outlier with dose escalation.

Does polypharmacology play a role?

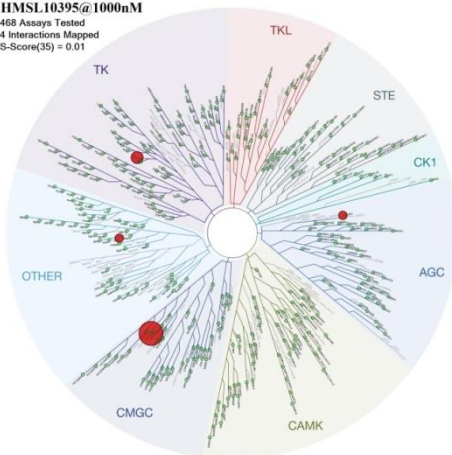
Palbociclib

HMSL10071@1000nM
468 Assays Tested
19 Interactions Mapped
S-Score(35) = 0.05



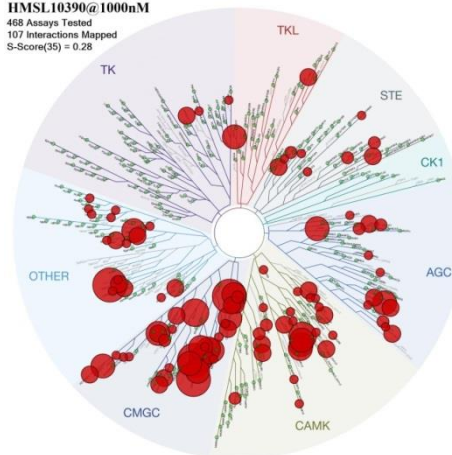
Ribociclib

HMSL10395@1000nM
468 Assays Tested
4 Interactions Mapped
S-Score(35) = 0.01



Abemaciclib

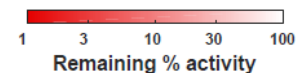
HMSL10390@1000nM
468 Assays Tested
107 Interactions Mapped
S-Score(35) = 0.28



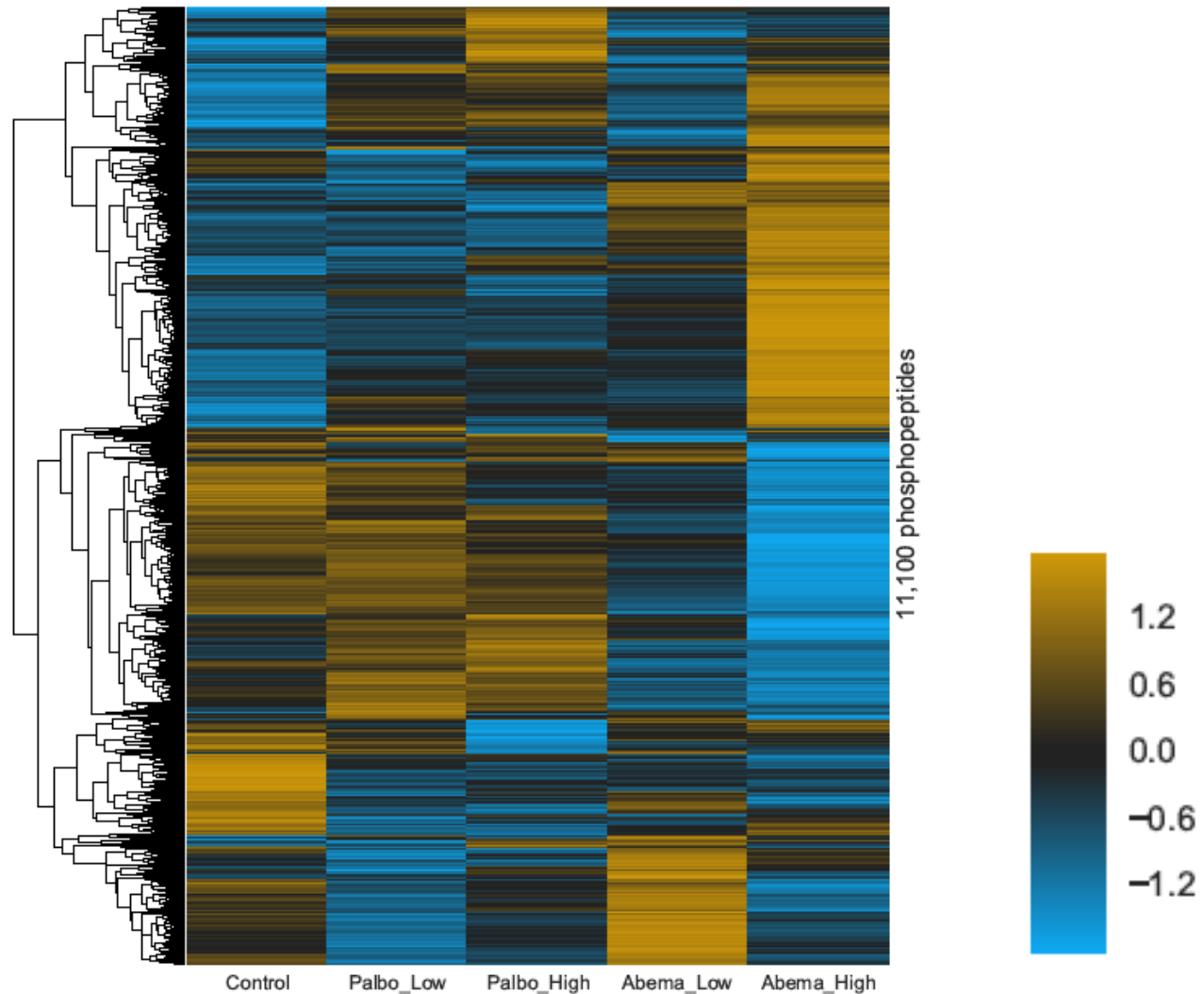
KinomeScan profile @0.10uM



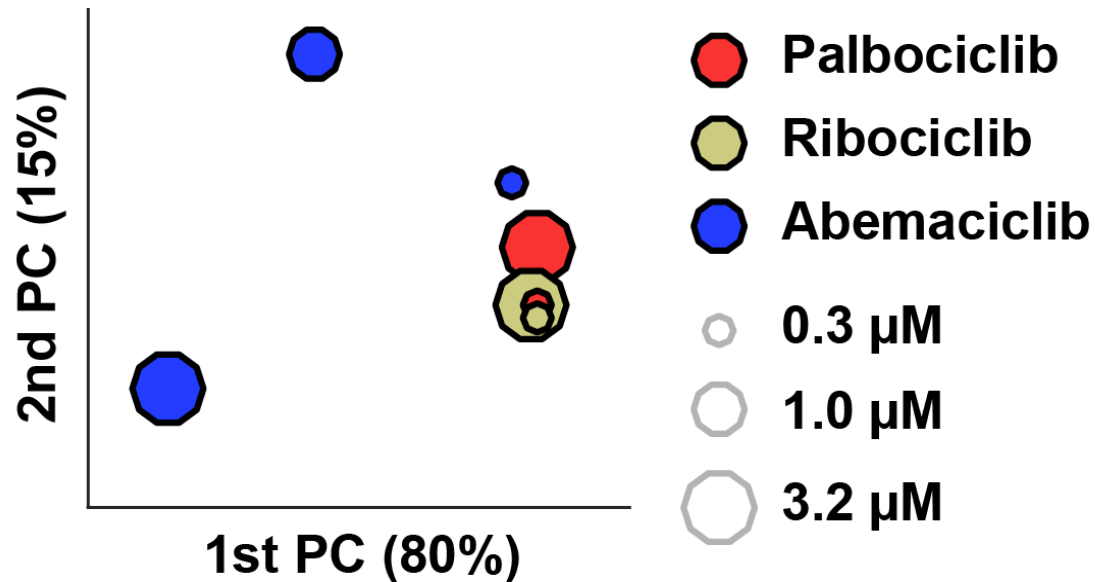
KinomeScan profile @1.00uM



Polypharmacology results in a different phosphoproteomic profile in MCF7 cells



Polypharmacology results in a different transcriptional profile in MCF7 cells at 6h



Summary

- TNBC profiling data collection is complete
 - Expansion is being planned
- First focused follow-up study associated inhibitor polypharmacology with enhanced efficacy
- Additional follow-up projects are underway
- Data will be released as a LINCS resource for future hypothesis testing and generation

Datasets currently available

<https://lincs.hms.harvard.edu>

- **Dose response metrics (20120, 20136)**
 - Dose responses, and immunofluorescence in 4 cell lines treated with 6 compounds
 - Analysis of dose response data for 53 cell lines treated with 62 small molecules
- **LINCS pilot phase joint project (20237-52, 20259-60)**
 - Dose responses, high throughput imaging, and L1000 data for 6 cell lines treated with 107 compounds
- **Density- and context- dependence of dose responses (20256-8)**
 - Dose responses for 6 cell lines at differing plating densities treated with 12 compounds
- **Multiplexed cyclic immunofluorescence imaging (20266-7)**
 - MCF10A cells treated with 9 compounds, probed with 21 antibodies
- **TNBC response to PI3K/Akt/mTor inhibition**
 - 6 cell lines, 28 compounds biased toward the PI3K/Akt/mTor pathway

Datasets currently available

<https://lincs.hms.harvard.edu>

- Dose response metrics (20120, 20136)
 - Dose responses, and immunofluorescence in 4 cell lines treated with 6 compounds
 - Analysis of dose response data for 53 cell lines treated with 62 small molecules
- LINCS pilot phase joint project (20227-52, 20250-60)
 - Dose responses treated with 107
- Density- and cont
 - Dose responses 12 compounds
- Multiplexed cyclic
 - MCF10A cells tr
- TNBC response to
 - 6 cell lines, 28 c

The screenshot shows the LINCS website interface. At the top, the LINCS logo is displayed with the tagline 'Library of Integrated Network-based Cellular Signatures'. Navigation buttons for 'HMS LINCS DATABASE', 'PUBLICATIONS', 'DATA EXPLORATION', and 'SOFTWARE' are visible. A sidebar on the left contains links to various resources: 'HMS LINCS DB home', 'Small molecules' (with sub-links for 'Structure search' and 'Salt table'), 'Cell Lines' (with sub-links for 'Primary Cells' and 'Proteins'), 'Antibodies', 'Other Reagents', 'Datasets' (highlighted), 'Libraries', and 'Login'. The main content area is titled 'Datasets' and includes a search box with the text 'To find datasets from LINCS publications, type the relevant PMID in the datasets search box below.' The search results show 30 datasets, with the first four listed in a table. The first two rows of the table are circled in yellow, corresponding to the datasets mentioned in the bullet points of the slide.

HMS Dataset ID	Dataset Title	HMS Dataset Type
20120	Metrics other than potency reveal systematic variation in responses to cancer drugs	Analysis
20136	Breast cell line dose response to target inhibition measured by high throughput microscopy	Microscopy/Imaging
20137	Basal profile of receptor tyrosine kinase signaling network measured by ELISA	ELISA
20138	Cell signaling response to growth factors measured by high throughput microscopy	Microscopy/Imaging

Acknowledgments

Sorger lab & LSP

- Peter Sorger
- Liz Williams
- Marc Hafner
- Mario Niepel
- Mirra Chung
- Robert Everley
- Alison Erikson
- Sarah Boswell
- Artem Sokolov
- Kartik Subramanian
- Chris Chen
- Stephanie Davis

MGH

- Dejan Juric

ICCB-L

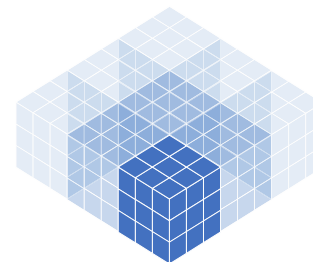
- Caroline Shamu
- Jen Smith
- Stuart Rudnicki
- Richard Siu
- Rachel Warden

Zhao lab

- Thanh Von
- Haluk Yuzugullu

Brugge lab

- Dan Stover



NIH LINCS
PROGRAM