NIH LINCS PROGRAM

HMS LINCS Center

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

Caitlin Mills

4/4/2017

U54-HL127365

HiHiS
Harvard Program in Therapeutic Science

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
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
CAMA-1	HR+	Luminal
HCC1428	HR+	Luminal
HCC1500	HR+	Luminal
MCF7	HR+	Luminal
MDA-MB-134	HR+	Luminal
T47D	HR+	Luminal
HCC1954	HER2amp	Basal A
HCC1419	HER2amp	Luminal
MDA-MB-361	HER2amp	Luminal
SK-BR-3	HER2amp	Luminal
hTERT-hME1	NM	Basal
MCF 10A	NM	Basal
PDX-DFCI-1206	TNBC	N/A
PDX-DFCI-1258	TNBC	N/A
PDX-DFCI-1328	TNBC	N/A
PDX-HCI-002	TNBC	N/A

20 TNBC

6 HR+

4 Her2amp

2 NM

4 from PDX

X

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

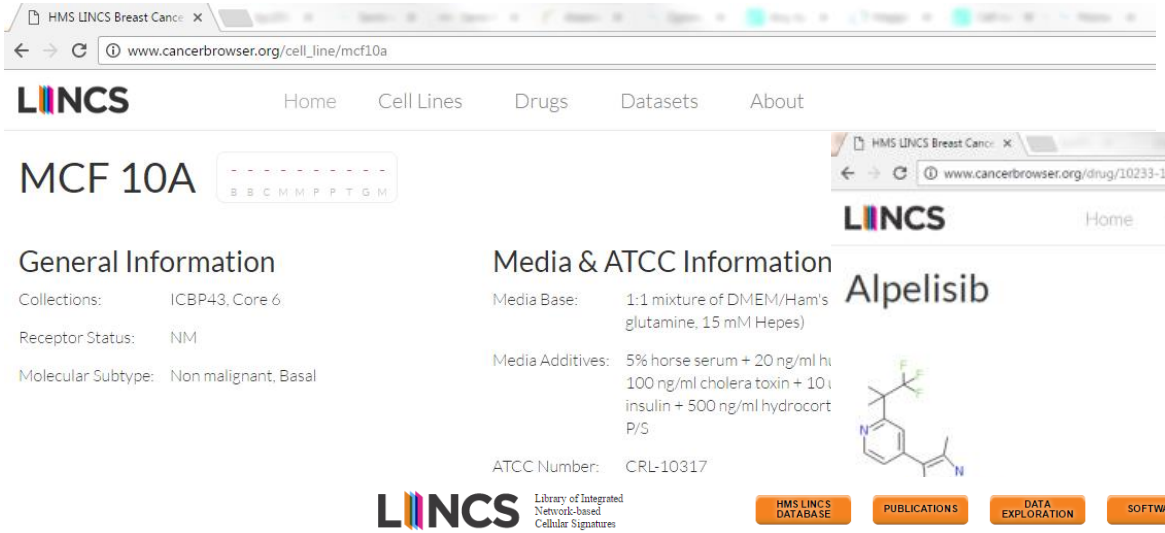
24 kinase inhibitors

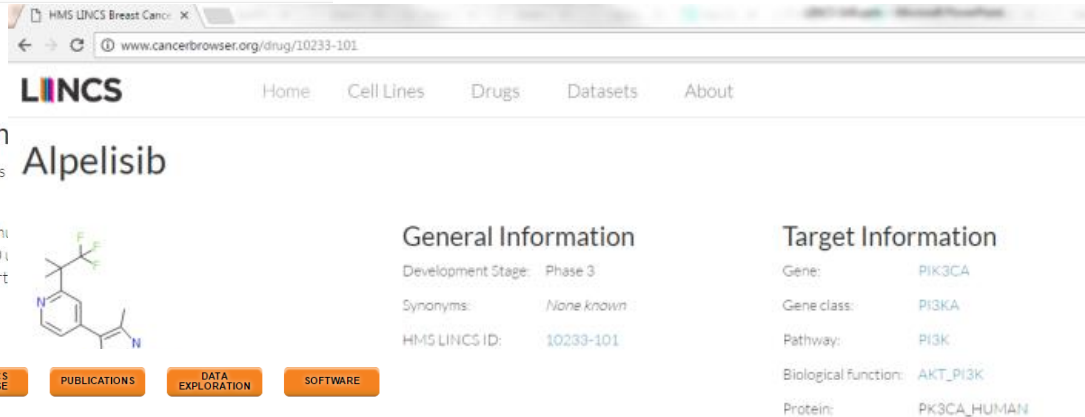
4 misc inhibitors

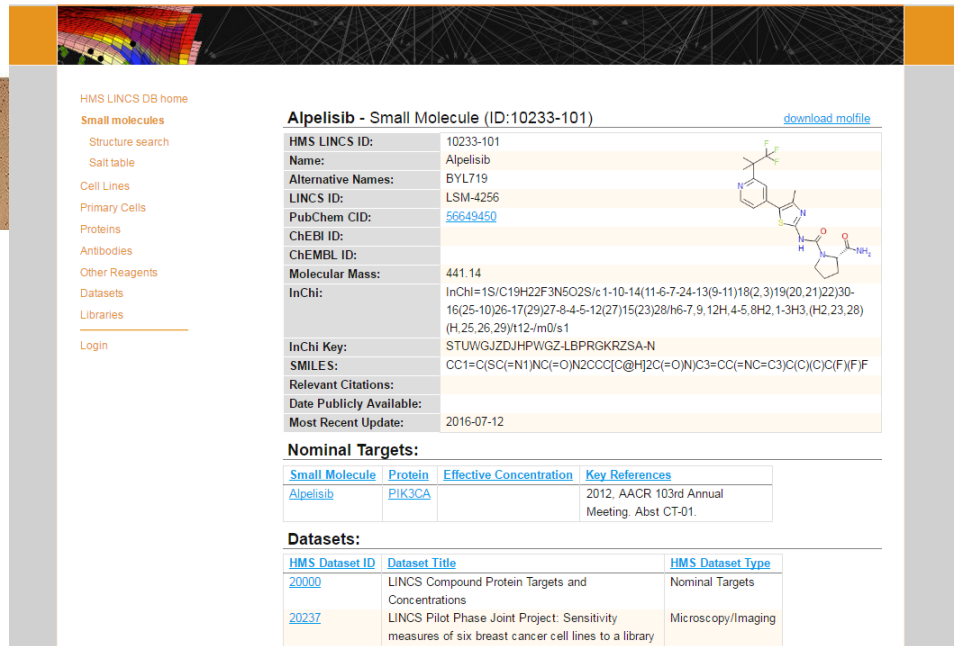
3 chemo

4 DNA damage

LINCS cell line and compound resources

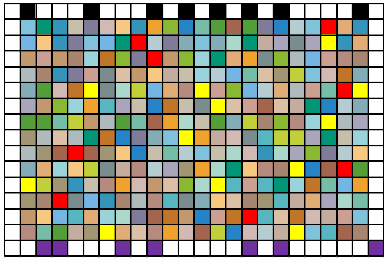


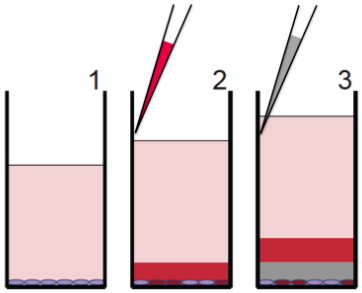


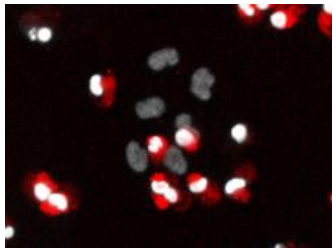


http://www.cancerbrowser.org/
http://lincs.hms.harvard.edu/db/

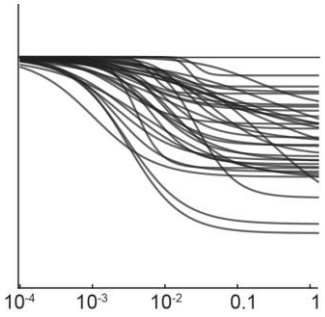
Experimental workflow



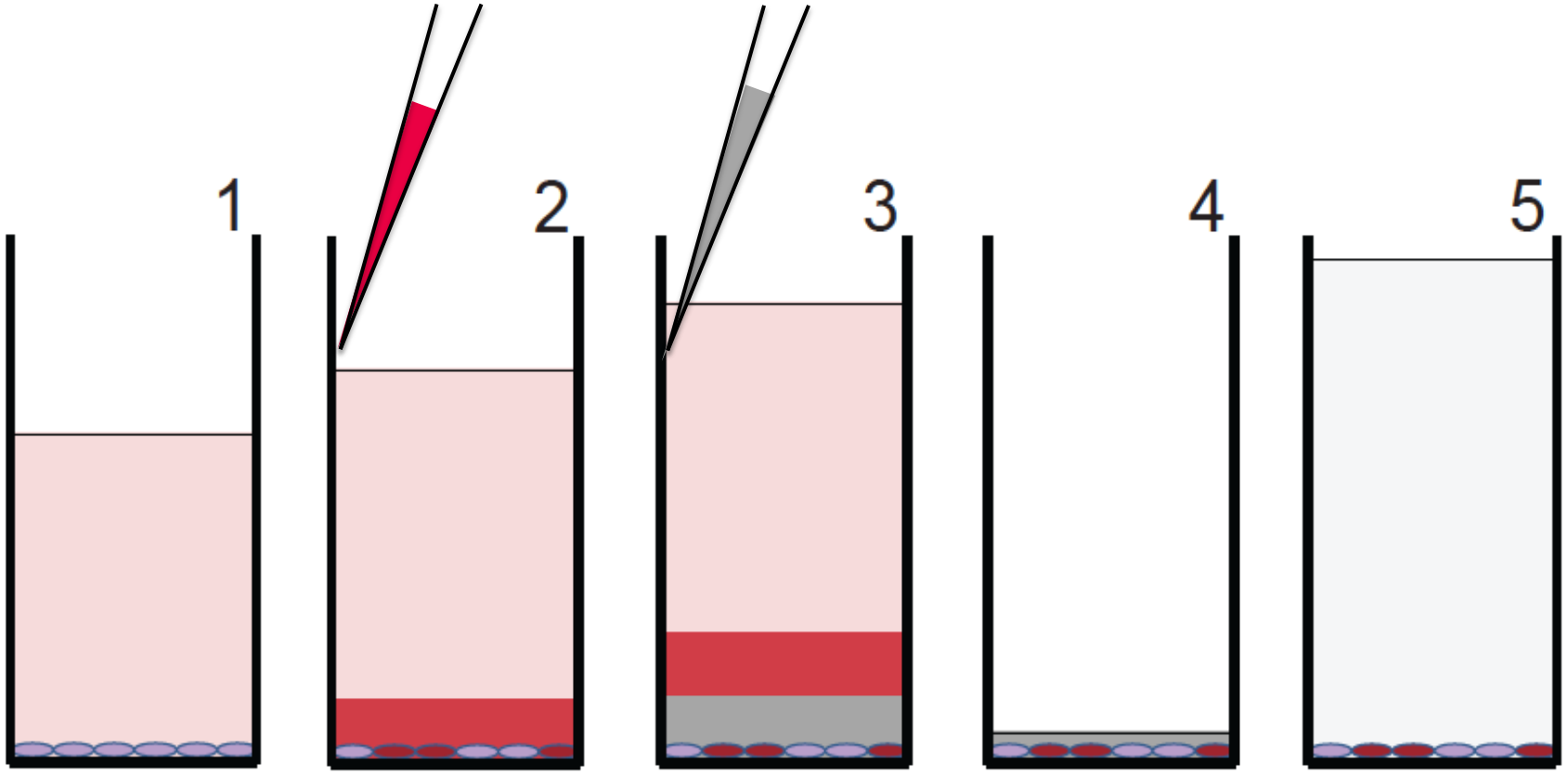




t	Treatment	Dose (uM)	Cell count	Dead cell count
72	Staurosporine	1	5091	1833
72	Staurosporine	1	5929	2137
72	Staurosporine	1	5663	2021
72	DMSO	0	8000	297
72	Staurosporine	0.316	6613	1142
72	DMSO	0	7732	329
72	Staurosporine	1	5463	2473
72	DMSO	0	8746	88
72	Staurosporine	0.316	6168	1496
72	Staurosporine	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

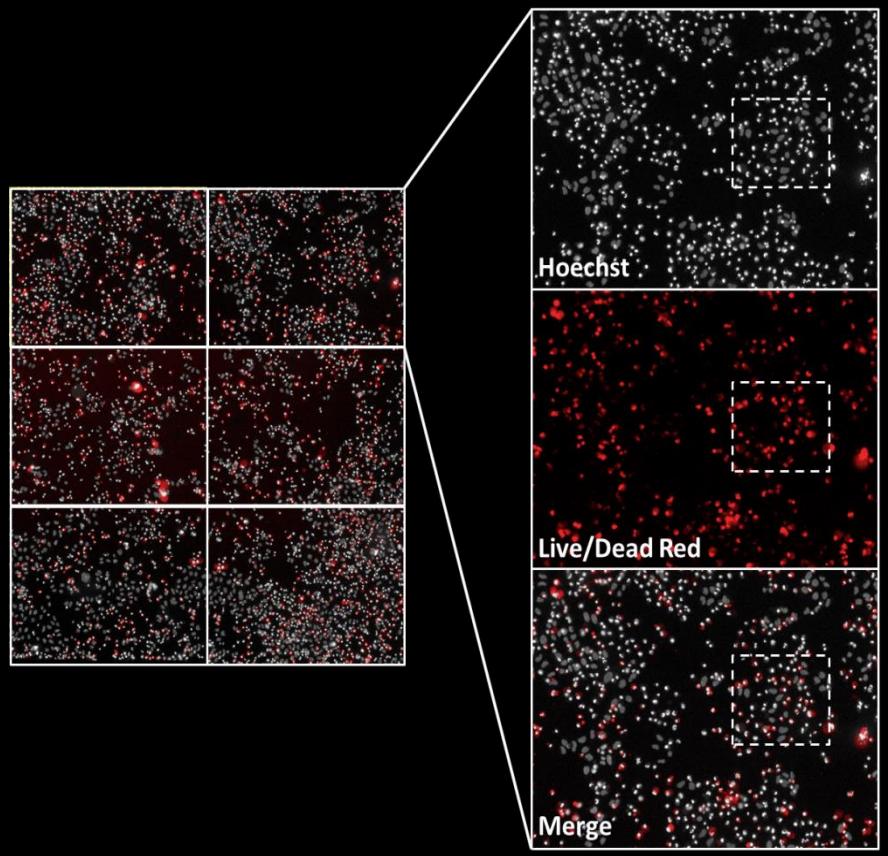


Dye-drop assay protocol

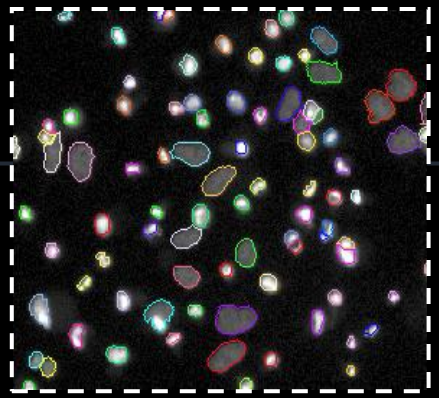


Niepel*, Hafner* et al., Curr Protoc Chem Biol, in press (June 2017)

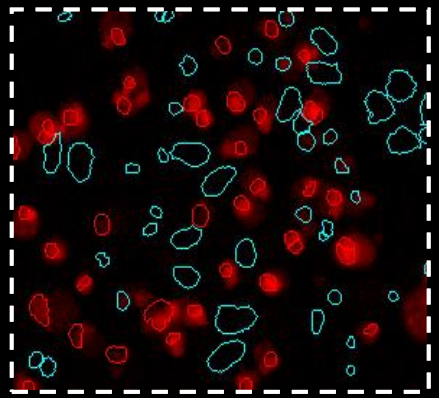
Image analysis



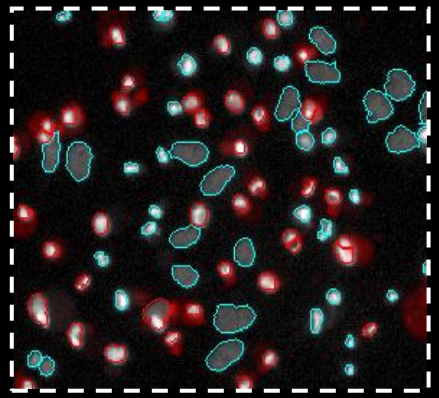
1. Segment nuclei



2. Measure LDR signal

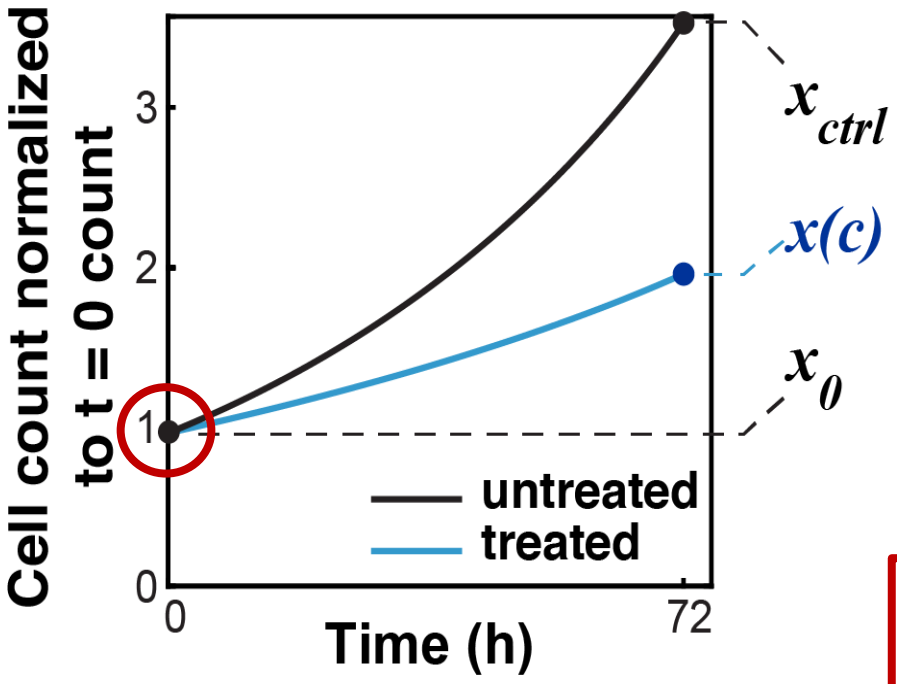


3. Classify live/dead cells



Well	Row	Column	Cell Line	Time point	Treatment	Dose (uM)	Cell count	Dead cell count	Cell count t=0
C2	3	2	MCF10A	72	Staurosporine	1	5091	1833	1954
C3	3	3	MCF10A	72	Staurosporine	1	5929	2137	1954
C4	3	4	MCF10A	72	Staurosporine	1	5663	2021	1954
C5	3	5	MCF10A	72	DMSO	0	8000	297	1954
C6	3	6	MCF10A	72	Staurosporine	0.316	6613	1142	1954
C7	3	7	MCF10A	72	DMSO	0	7732	329	1954
C8	3	8	MCF10A	72	Staurosporine	1	5463	2473	1954
D2	4	2	MCF10A	72	DMSO	0	8746	88	1954
D3	4	3	MCF10A	72	Staurosporine	0.316	6168	1496	1954
D4	4	4	MCF10A	72	Staurosporine	0.1	7941	636	1954
D5	4	5	MCF10A	72	DMSO	0	8529	360	1954
D6	4	6	MCF10A	72	Staurosporine	0.316	6994	1157	1954
D7	4	7	MCF10A	72	DMSO	0	8872	160	1954
D8	4	8	MCF10A	72	DMSO	0	9166	73	1954
C2	3	2	MCF10A	72	Staurosporine	1	5091	1833	1954
C3	3	3	MCF10A	72	Staurosporine	1	5929	2137	1954
C4	3	4	MCF10A	72	Staurosporine	0.1	5663	2021	1954
C5	3	5	MCF10A	72	DMSO	0	8000	297	1954
C6	3	6	MCF10A	72	Staurosporine	0.316	6613	1142	1954
C7	3	7	MCF10A	72	DMSO	0	7732	329	1954
C8	3	8	MCF10A	72	Staurosporine	1	5463	2473	1954
D2	4	2	MCF10A	72	DMSO	0	8746	88	1954
D3	4	3	MCF10A	72	Staurosporine	0.316	6168	1496	1954
D4	4	4	MCF10A	72	Staurosporine	0.1	7941	636	1954
D5	4	5	MCF10A	72	DMSO	0	8529	360	1954
D6	4	6	MCF10A	72	Staurosporine	0.316	6994	1157	1954
D7	4	7	MCF10A	72	DMSO	0	8872	160	1954
D8	4	8	MCF10A	72	DMSO	0	9166	73	1954
C2	3	2	MCF10A	72	Staurosporine	1	5091	1833	1954
C3	3	3	MCF10A	72	Staurosporine	1	5929	2137	1954
C4	3	4	MCF10A	72	Staurosporine	1	5663	2021	1954
C5	3	5	MCF10A	72	DMSO	0	8000	297	1954
C6	3	6	MCF10A	72	Staurosporine	0.316	6613	1142	1954
C7	3	7	MCF10A	72	DMSO	0	7732	329	1954
C8	3	8	MCF10A	72	Staurosporine	1	5463	2473	1954
D2	4	2	MCF10A	72	DMSO	0	8746	88	1954
D3	4	3	MCF10A	72	Staurosporine	0.316	6168	1496	1954
D4	4	4	MCF10A	72	Staurosporine	0.1	7941	636	1954
D5	4	5	MCF10A	72	DMSO	0	8529	360	1954
D6	4	6	MCF10A	72	Staurosporine	0.316	6994	1157	1954
D7	4	7	MCF10A	72	DMSO	0	8872	160	1954
D8	4	8	MCF10A	72	DMSO	0	9166	73	1954
C2	3	2	MCF10A	72	Staurosporine	1	5091	1833	1954
C3	3	3	MCF10A	72	Staurosporine	1	5929	2137	1954
C4	3	4	MCF10A	72	Staurosporine	1	5663	2021	1954
C5	3	5	MCF10A	72	DMSO	0	8000	297	1954
C6	3	6	MCF10A	72	Staurosporine	0.316	6613	1142	1954
C7	3	7	MCF10A	72	DMSO	0	7732	329	1954
C8	3	8	MCF10A	72	Staurosporine	1	5463	2473	1954
D2	4	2	MCF10A	72	DMSO	0	8746	88	1954
D3	4	3	MCF10A	72	Staurosporine	0.316	6168	1496	1954
D4	4	4	MCF10A	72	Staurosporine	0.1	7941	636	1954
D5	4	5	MCF10A	72	DMSO	0	8529	360	1954
D6	4	6	MCF10A	72	Staurosporine	0.316	6994	1157	1954
D7	4	7	MCF10A	72	DMSO	0	8872	160	1954
D8	4	8	MCF10A	72	DMSO	0	9166	73	1954

GR values rely on three measures of cell count



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

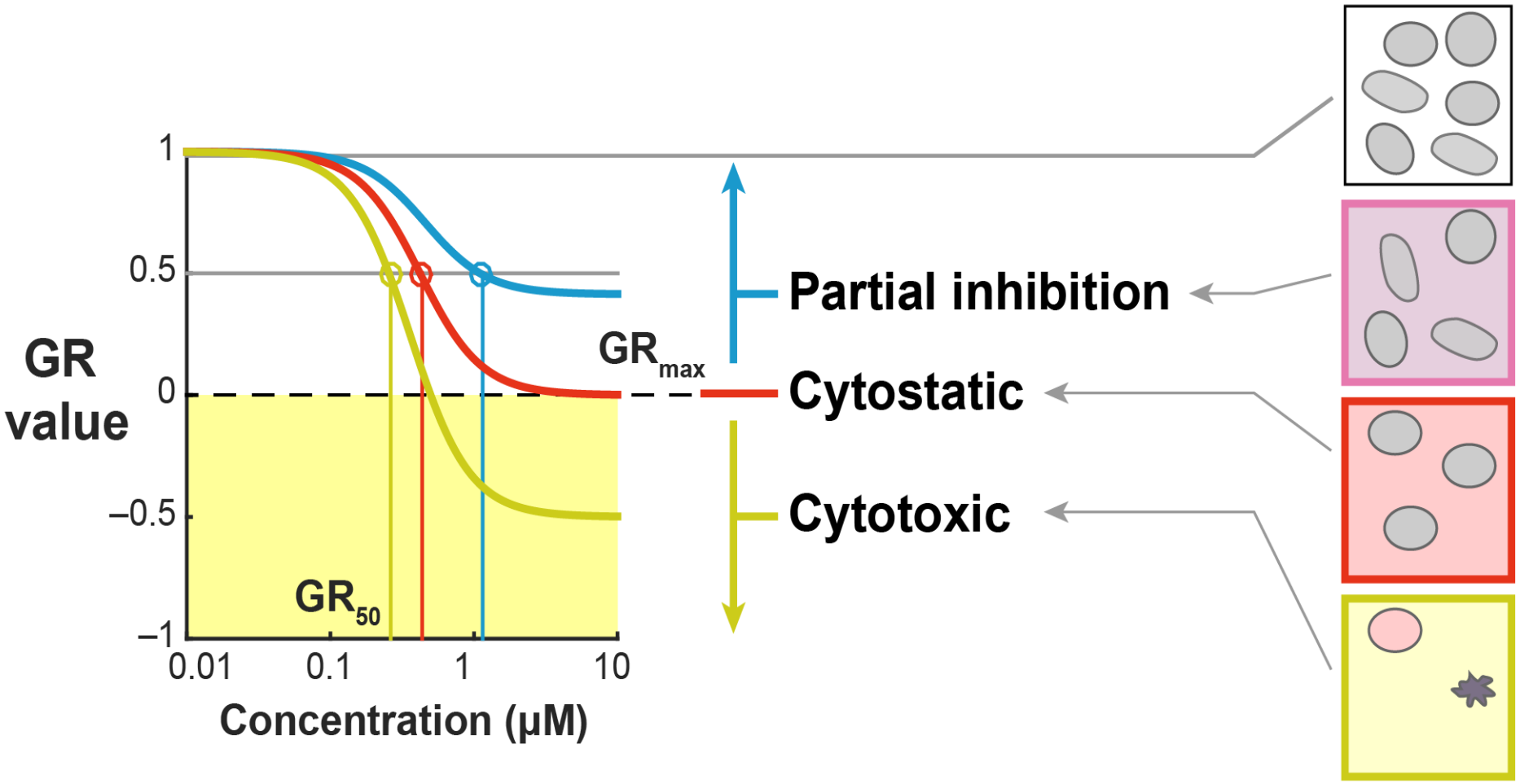
$x(c)$ is the treated cell count

x_{ctrl} is the control cell count

x_0 is the cell count at the time of treatment

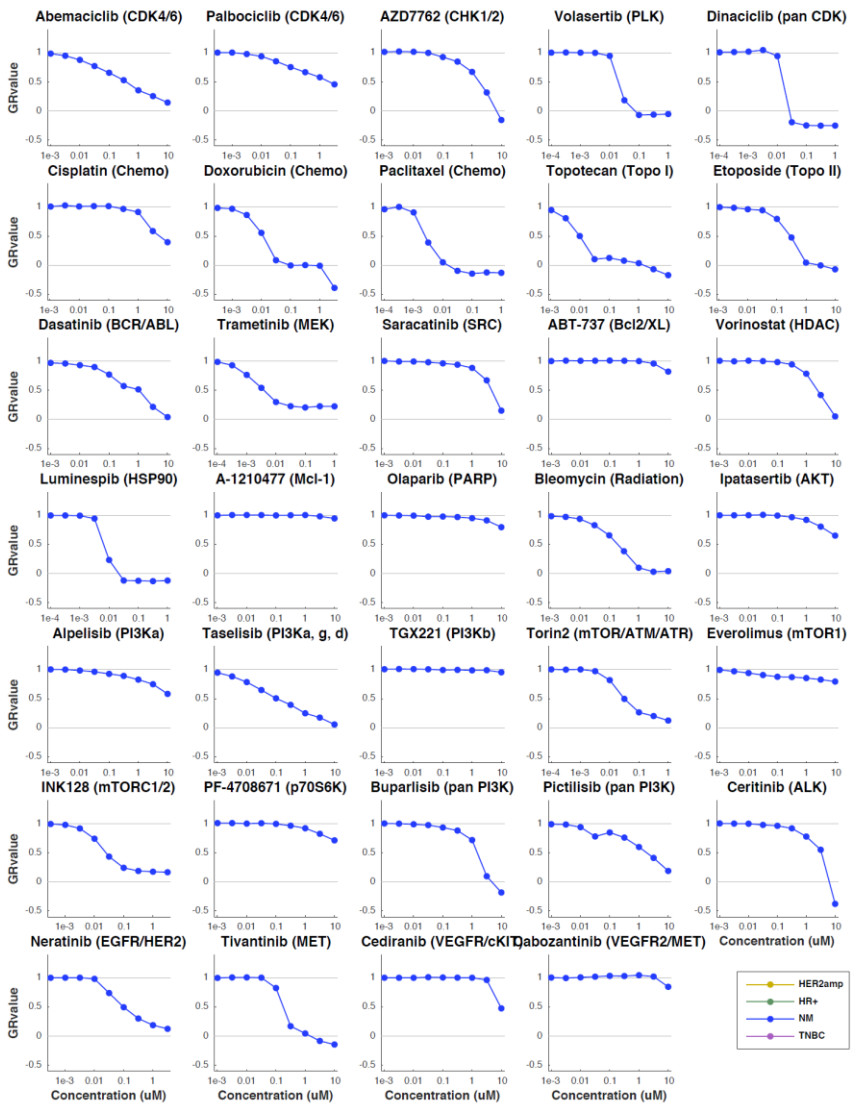
Hafner*, Niepel* et al. Nat Methods, 2016, 13:521-7

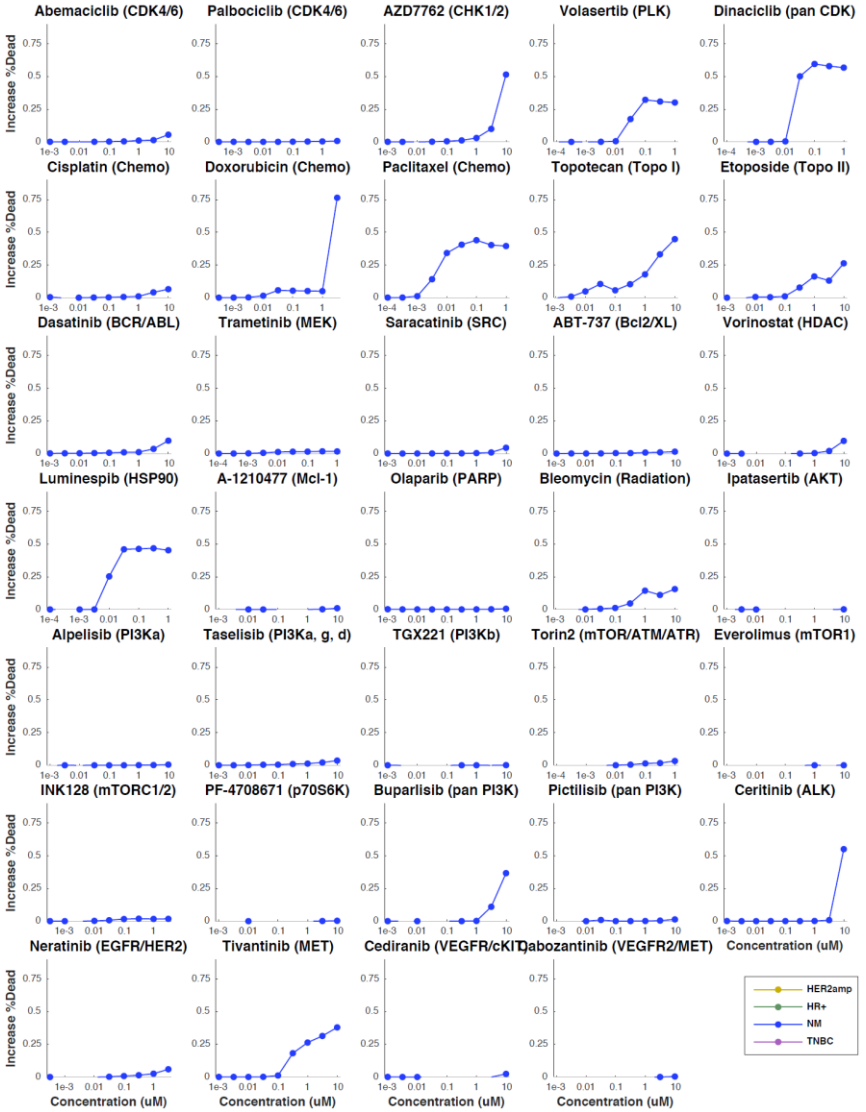
GR values are independent of the division rate and directly relate to the phenotype



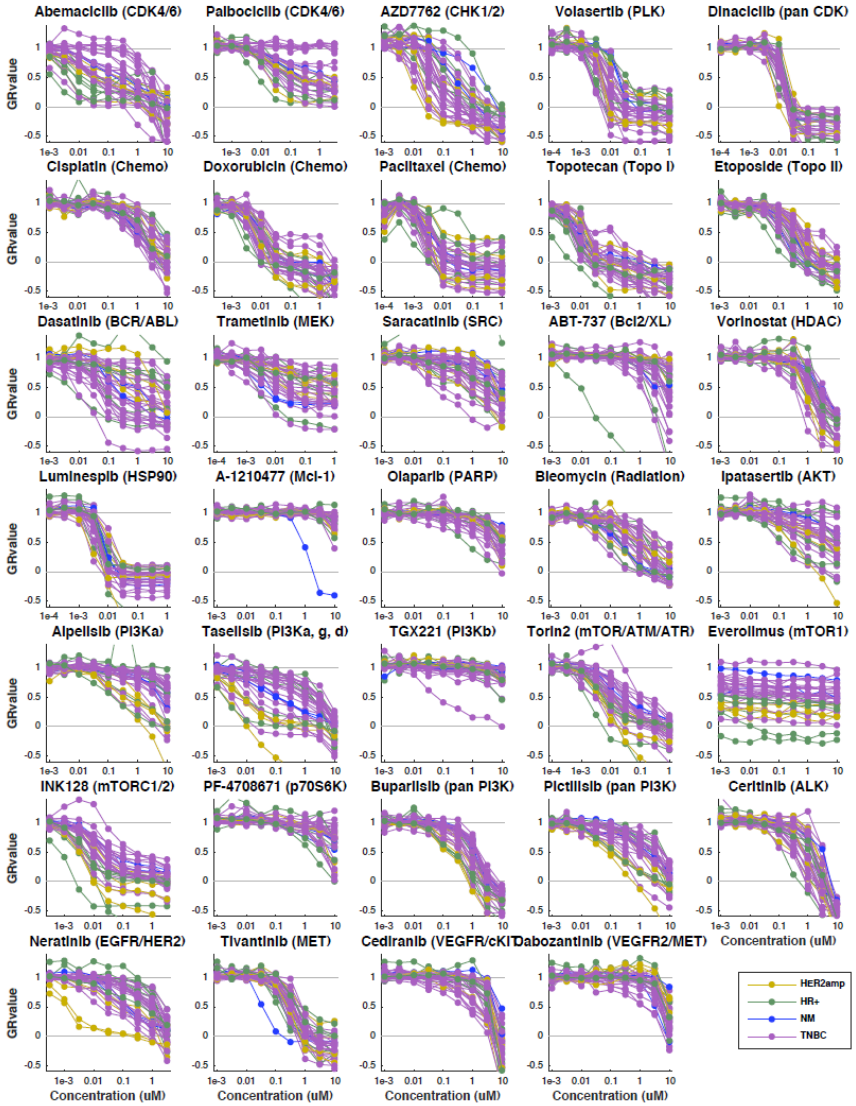
Hafner*, Niepel* et al. Nat Methods, 2016, 13:521-7.

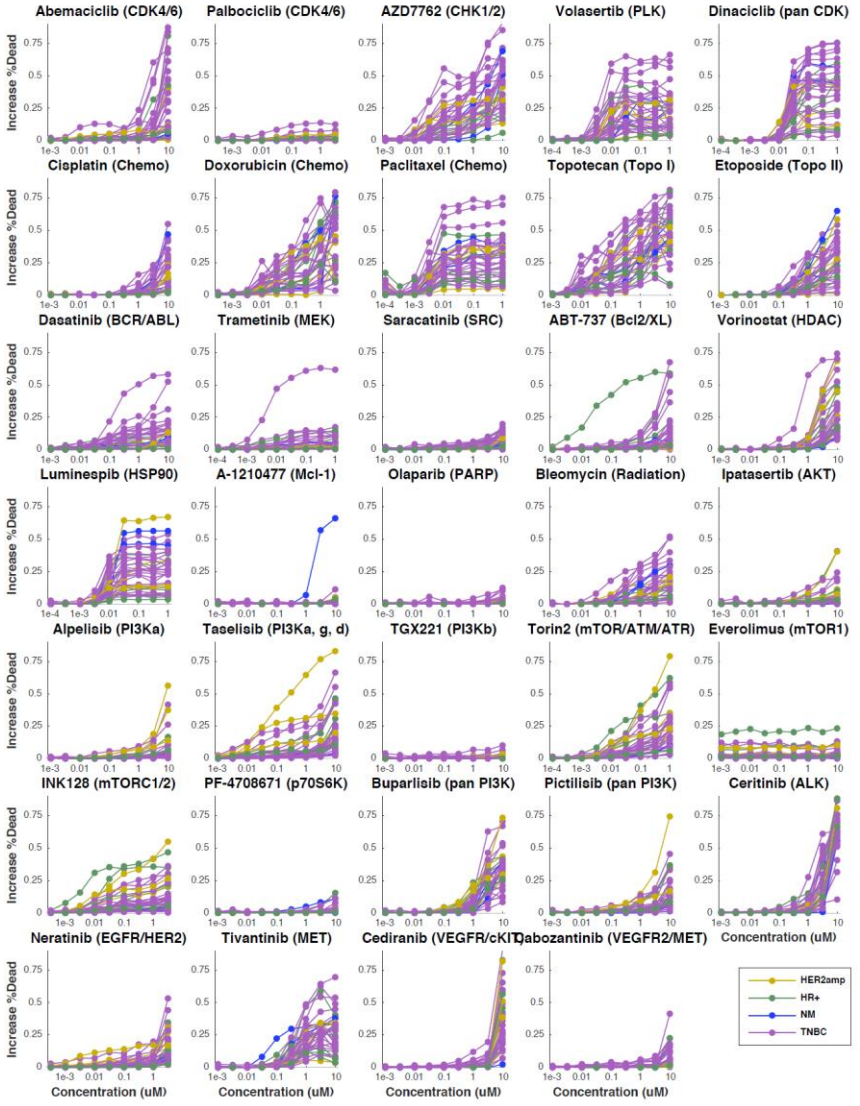
Dose response results – MCF10A cells



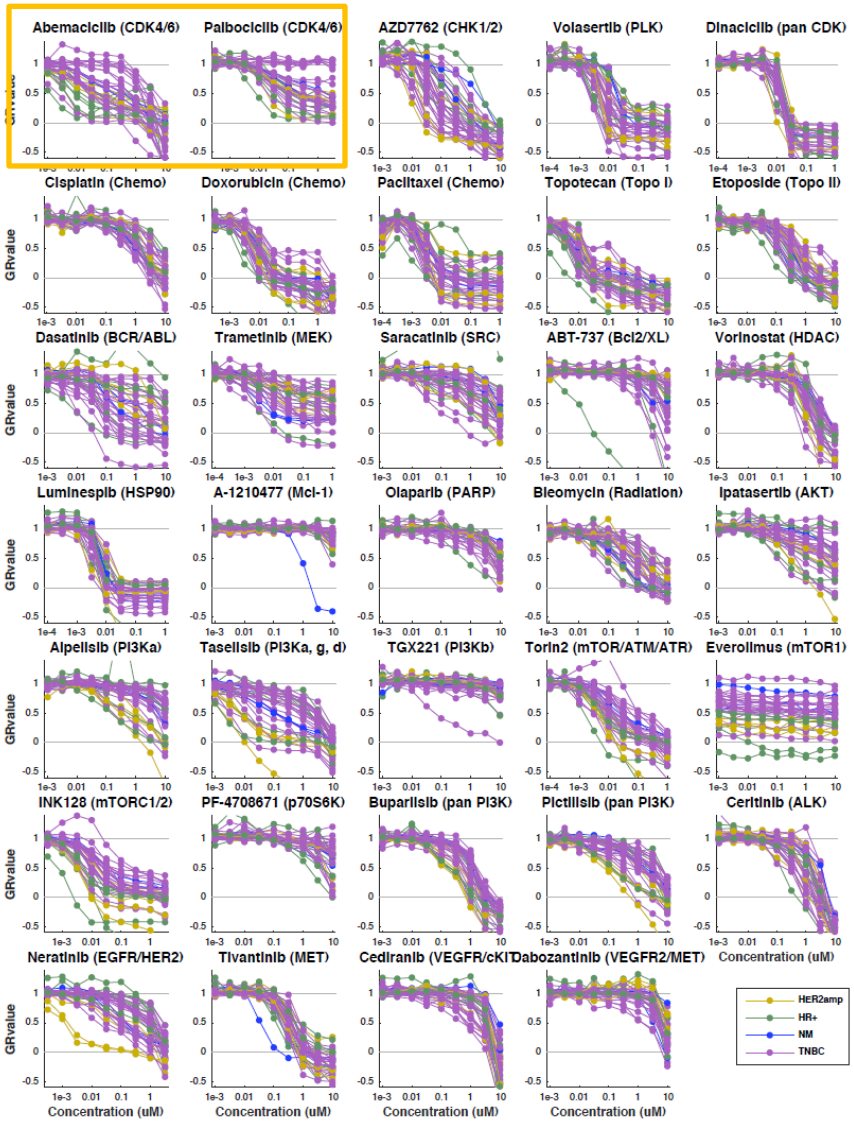


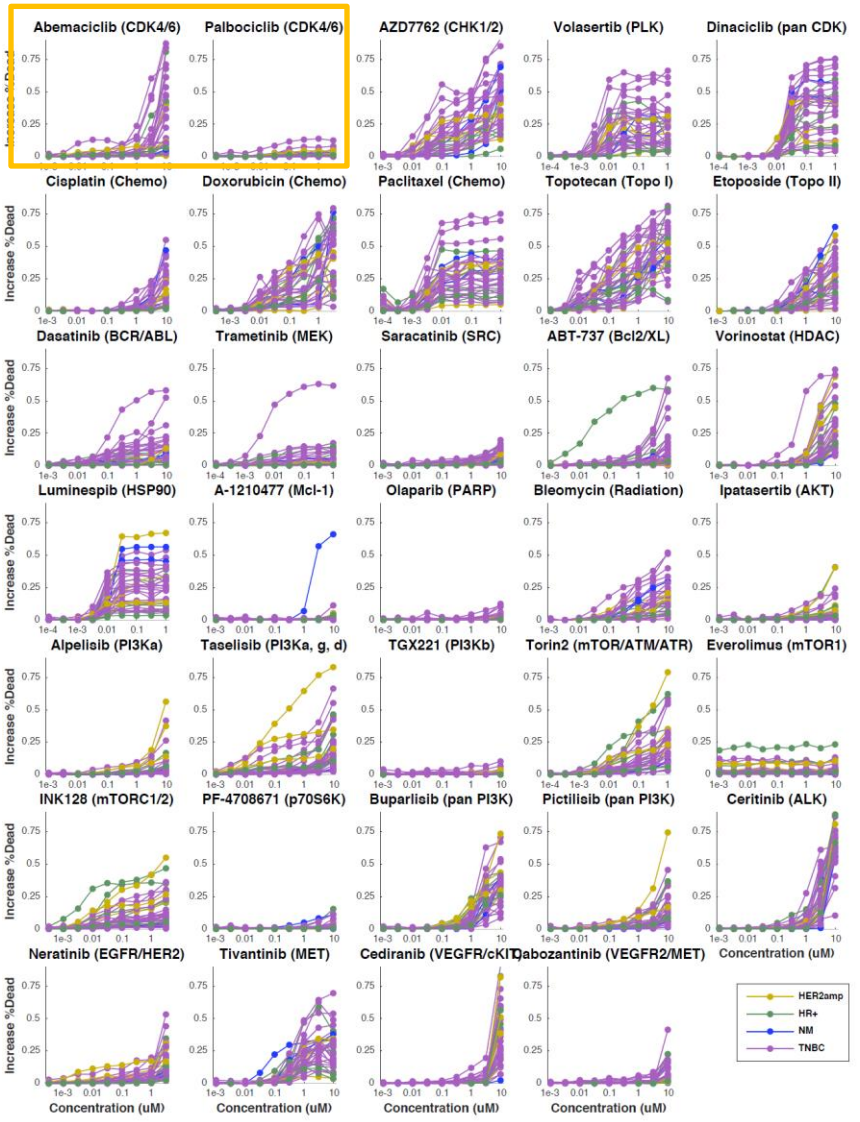
Dose response results – all cell lines



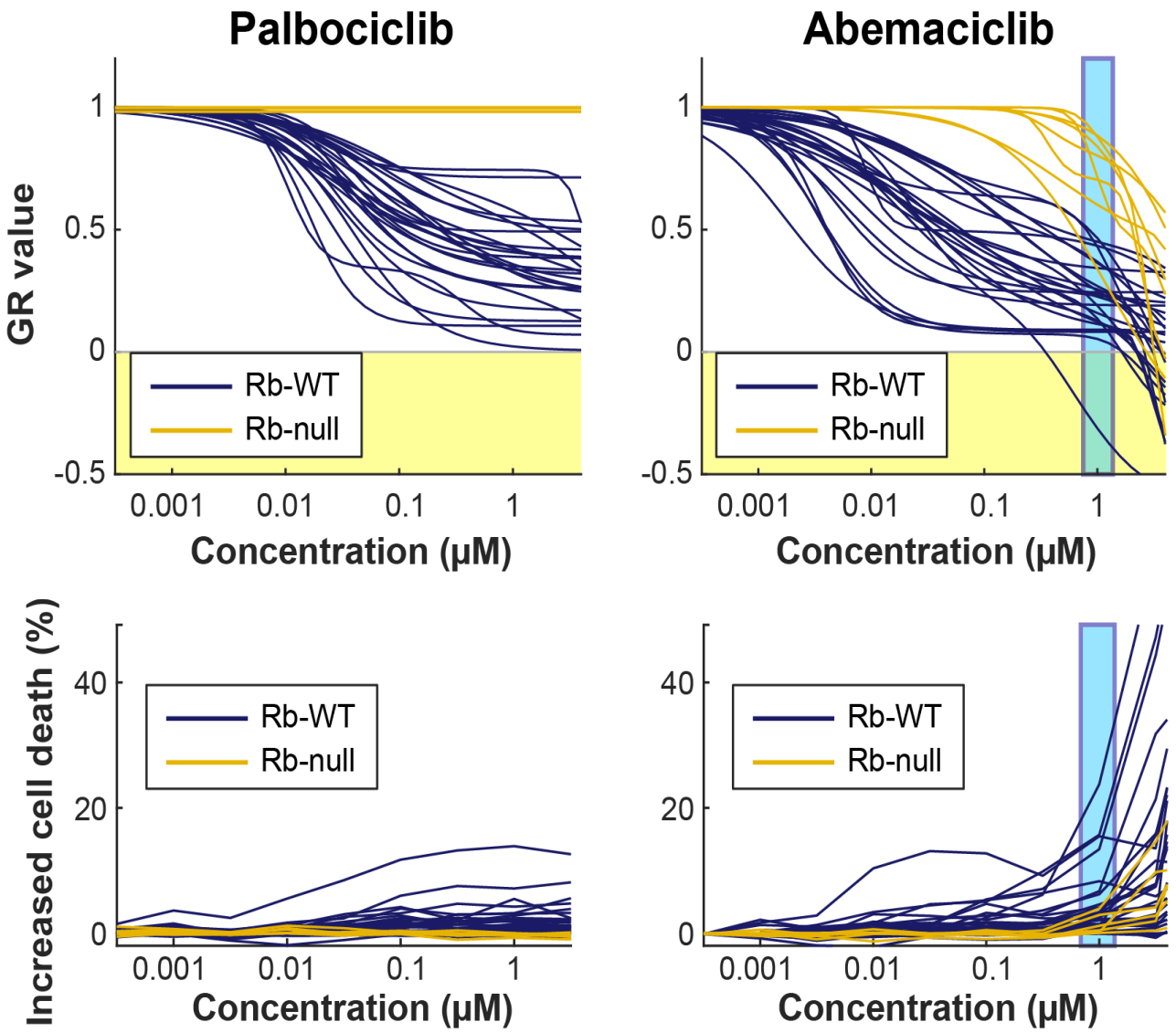


Dose response results – CDK4/6 inhibitors





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

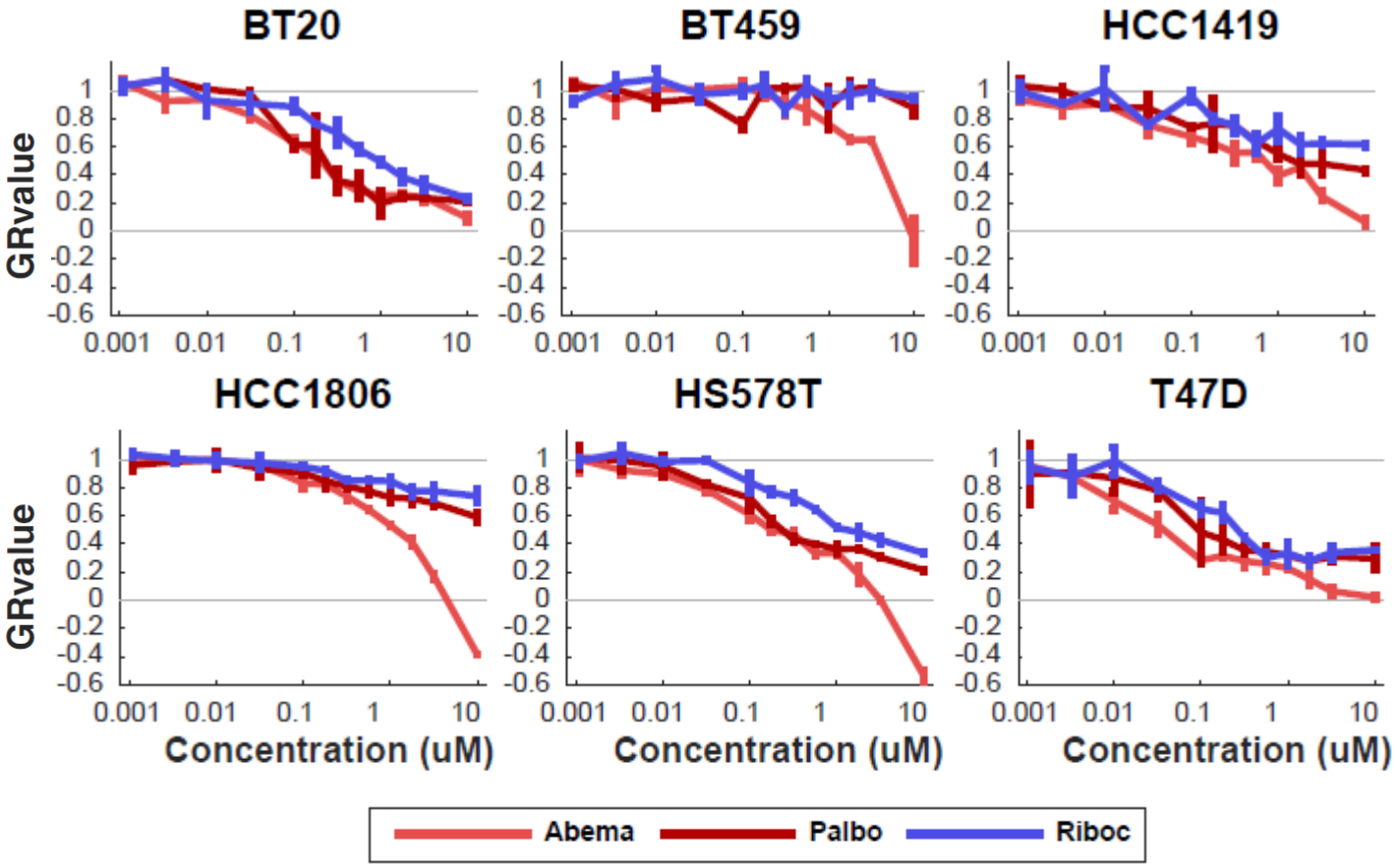


Clinical characteristics of CDK4/6 inhibitors

Drug	Palbociclib (Pfizer) (PD0332991, Ibrance)	Ribociclib (Novartis) (LEE011)	Abemaciclib (Eli Lilly) (LY2835219)
IC_{50} (*in vitro* 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)

Sherr at el., Cancer Discovery 2015

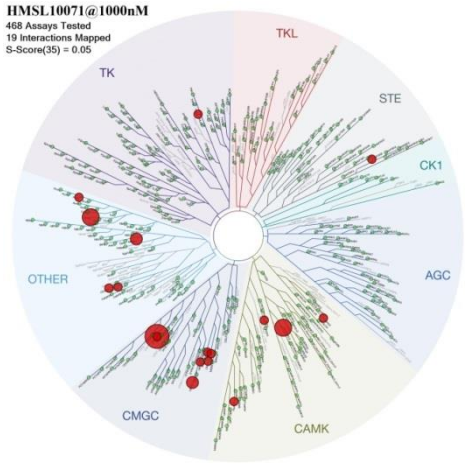
How does ribociclib compare?



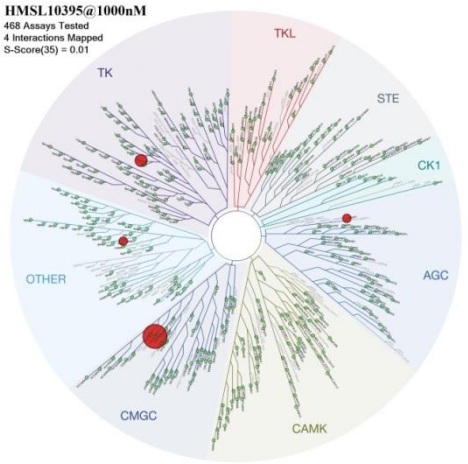
Abemaciclib remains the outlier with dose escalation.

Does polypharmacology play a role?

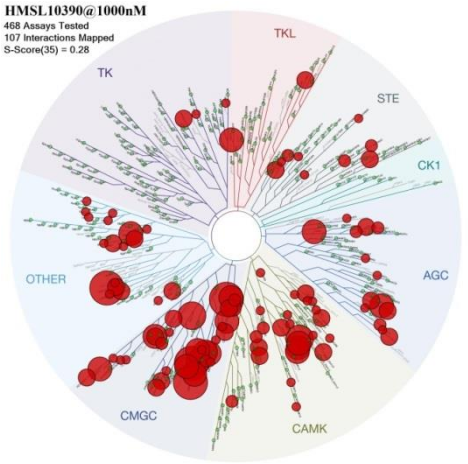
Palbociclib

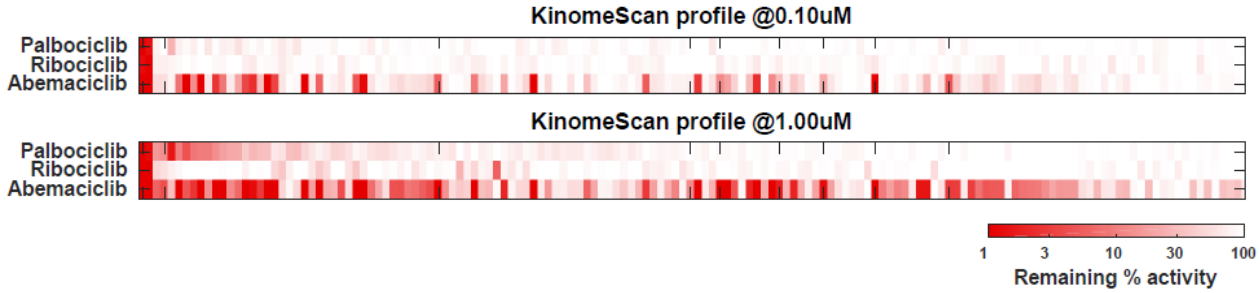


Ribociclib

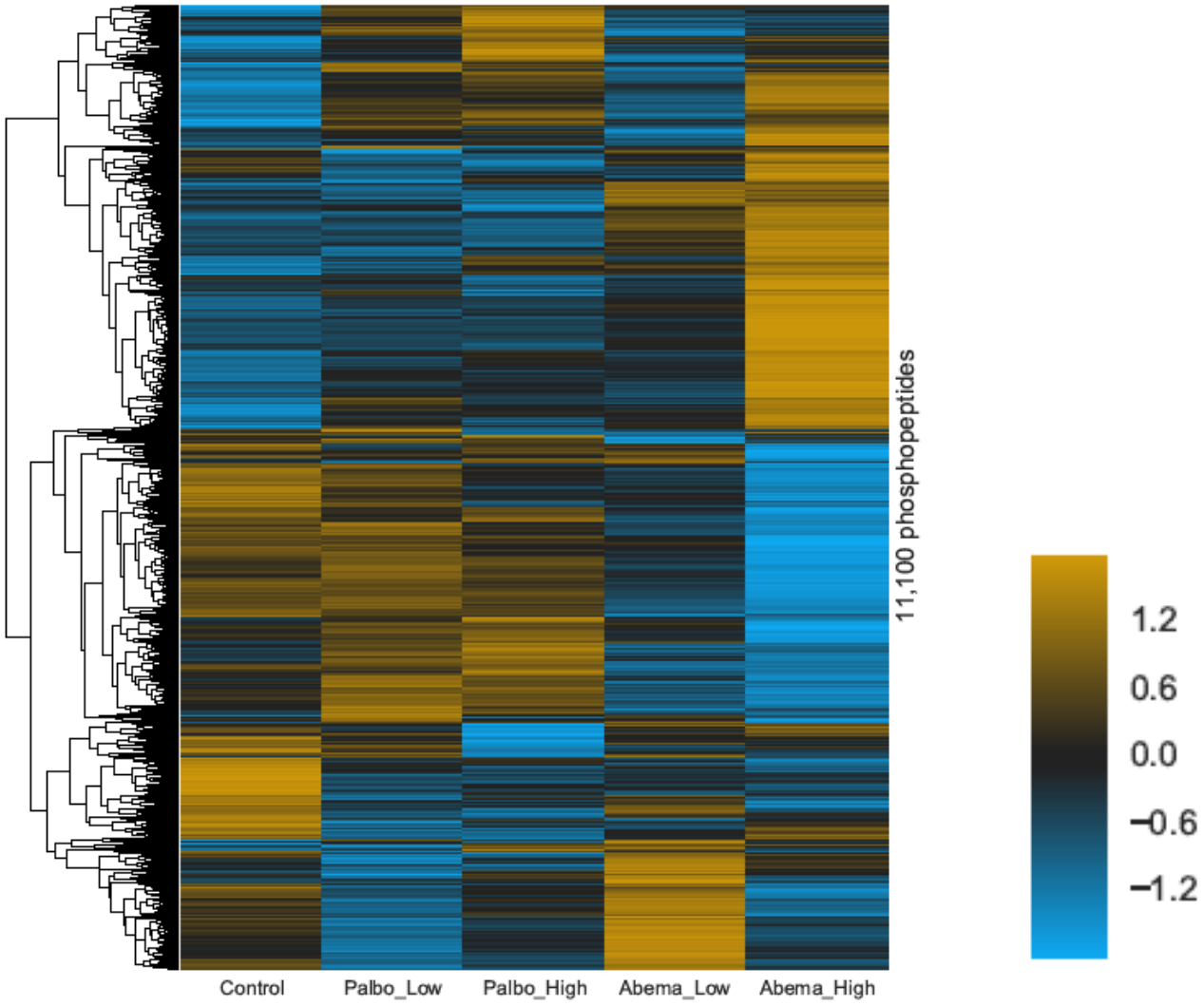


Abemaciclib

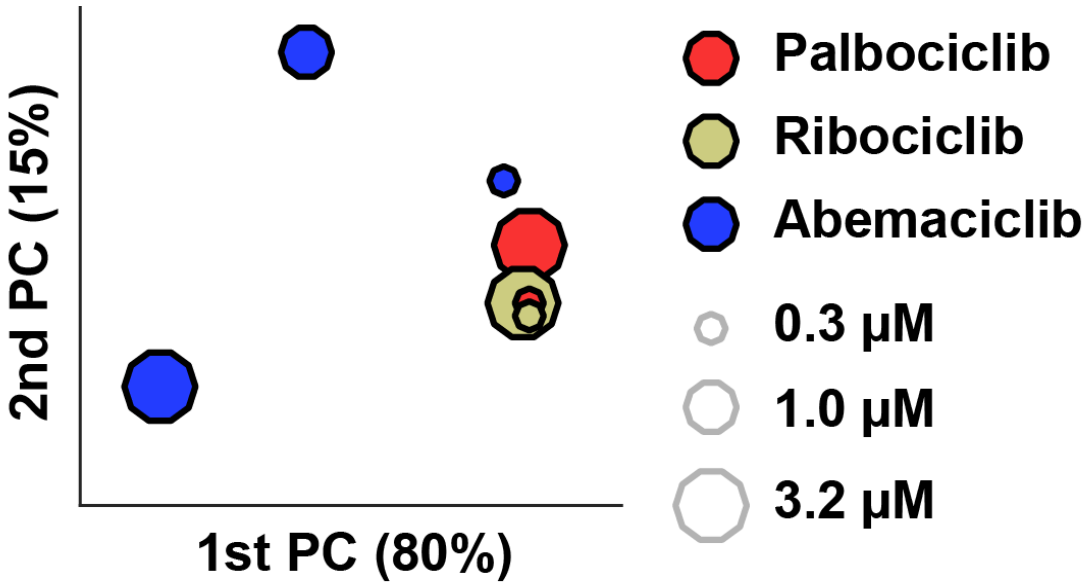




Polypharmacology results in a different phosphoproteomic profile in MCF7 cells



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



Summary

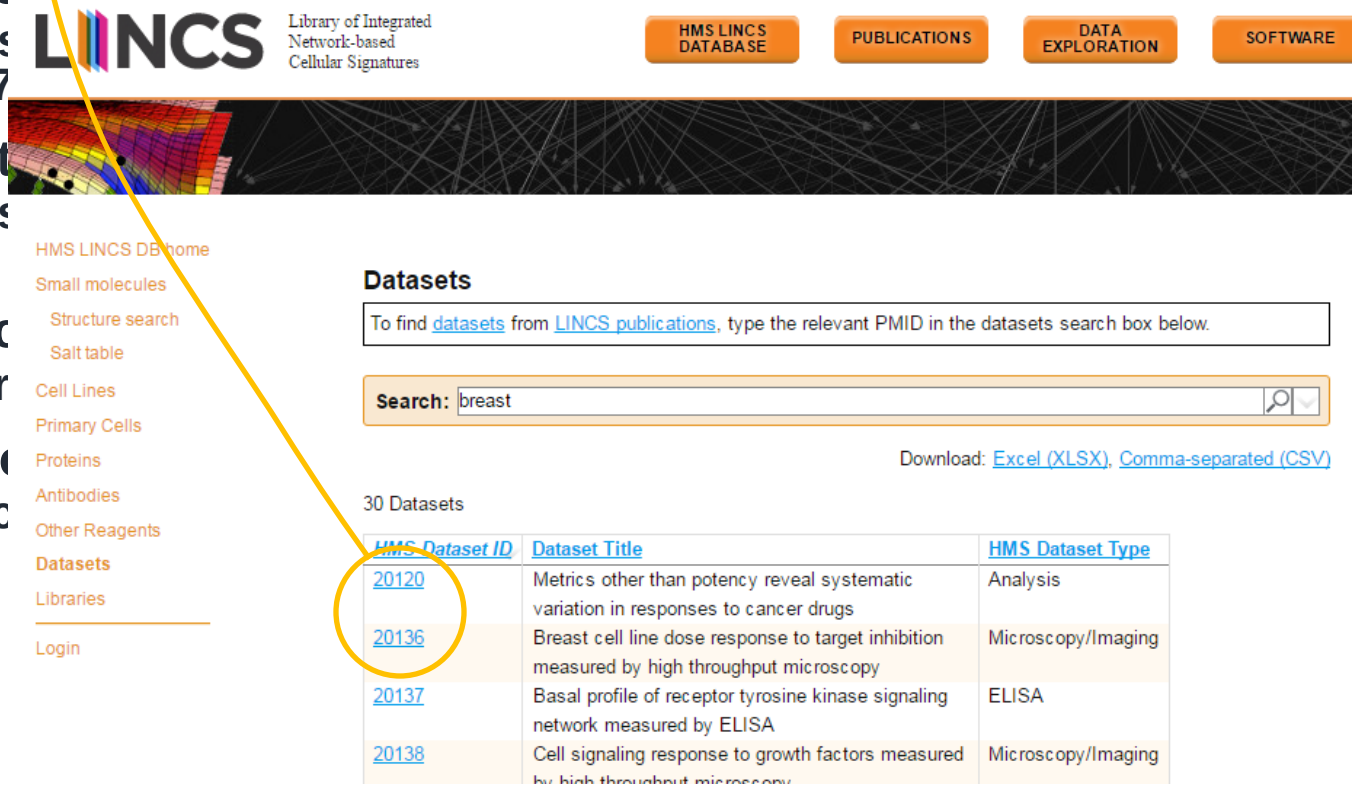
- TNBC profiling data collection is complete
  - Expansion is being planned
- First focused follow-up study associated inhibitor polypharmacology with enhanced efficacy
- Additional follow-up projects are underway
- Data will be released as a LINCS resource for future hypothesis testing and generation

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 (20237-52, 20259-60)
  - Dose responses
    treated with 107
- Density- and cont
  - Dose responses
    12 compounds
- Multiplexed cyclic
  - MCF10A cells tr
- TNBC response t
  - 6 cell lines, 28 c



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

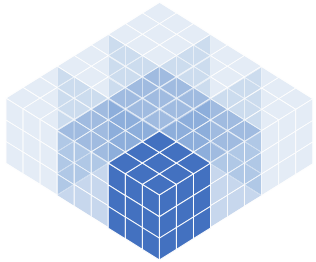
- Caroline Shamu
- Jen Smith
- Stuart Rudnicki
- Richard Siu
- Rachel Warden

Zhao lab

- Thanh Von
- Haluk Yuzugullu

Brugge lab

- Dan Stover



NIH LINCS PROGRAM