ICSB Workshop: Drug Response Measurement and Analysis

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Acknowledgements

HARVARD MEDICAL SCHOOL | Laboratory of Systems Pharmacology

- Jeremy Muhlich
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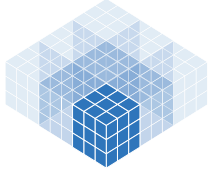
http://github.com/datarail

GRcalculator.org

- Nick Clark (U Cincinnati)

Funding:



NIH LINCS PROGRAM

- NIH LINCS grant U54-HL127365

Testing therapeutics in vitro

- Fundamental limitations of clinical research:
  - Each patient is different
  - Tumors are heterogeneous
  - Response is hard to quantify
  - Only one drug or drug combination can be tested

- Advantages of cell lines:
  - Multiple assays can be run in parallel
  - Experiments can be reproduced
  - Responses can be studied at multiple levels

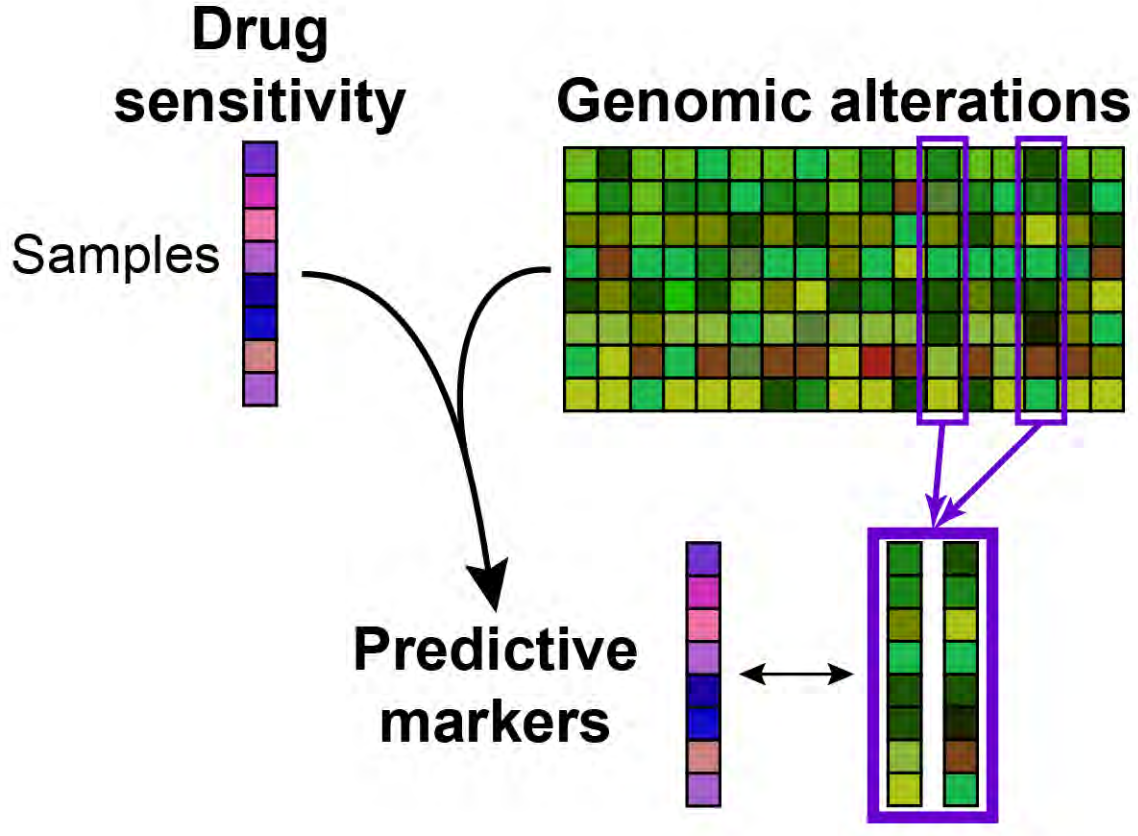
Cell lines as biological system to test drugs

- Measuring, quantifying, and understanding ability to inhibit proliferation and induce cell death.

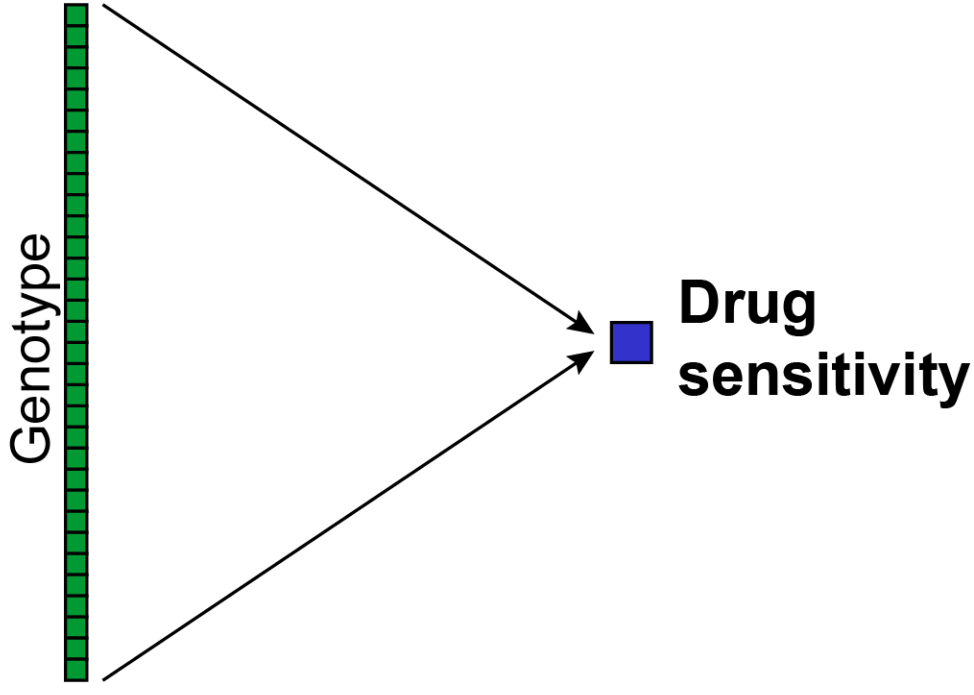
- Study effects across different models (cancer cells, healthy tissues).

- Combine phenotypic readouts with molecular markers to study mechanisms of action

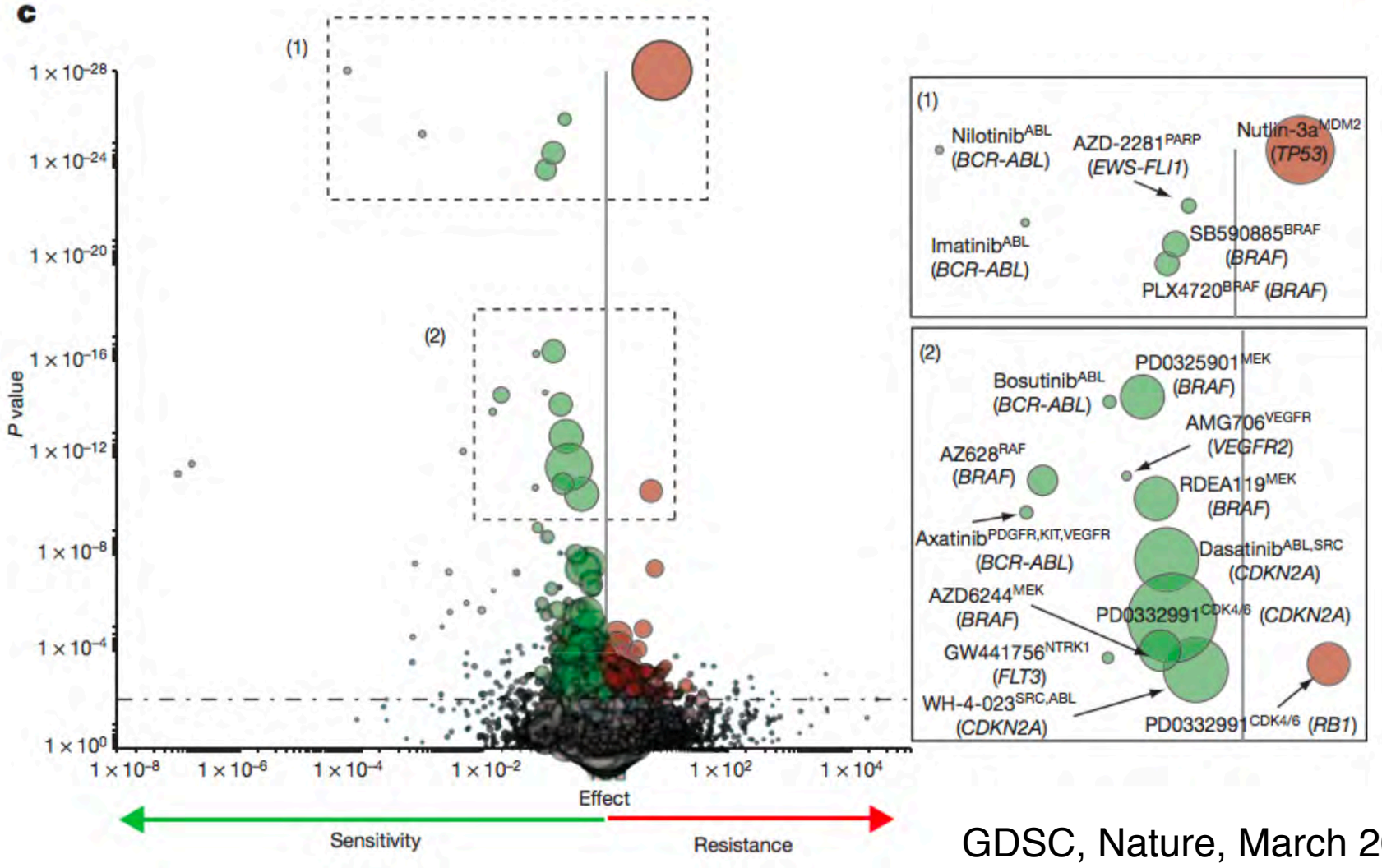
Current approaches focus on mapping drug sensitivity to genotype using screening data



Current approaches assume that genotype and drug sensitivity are directly connected

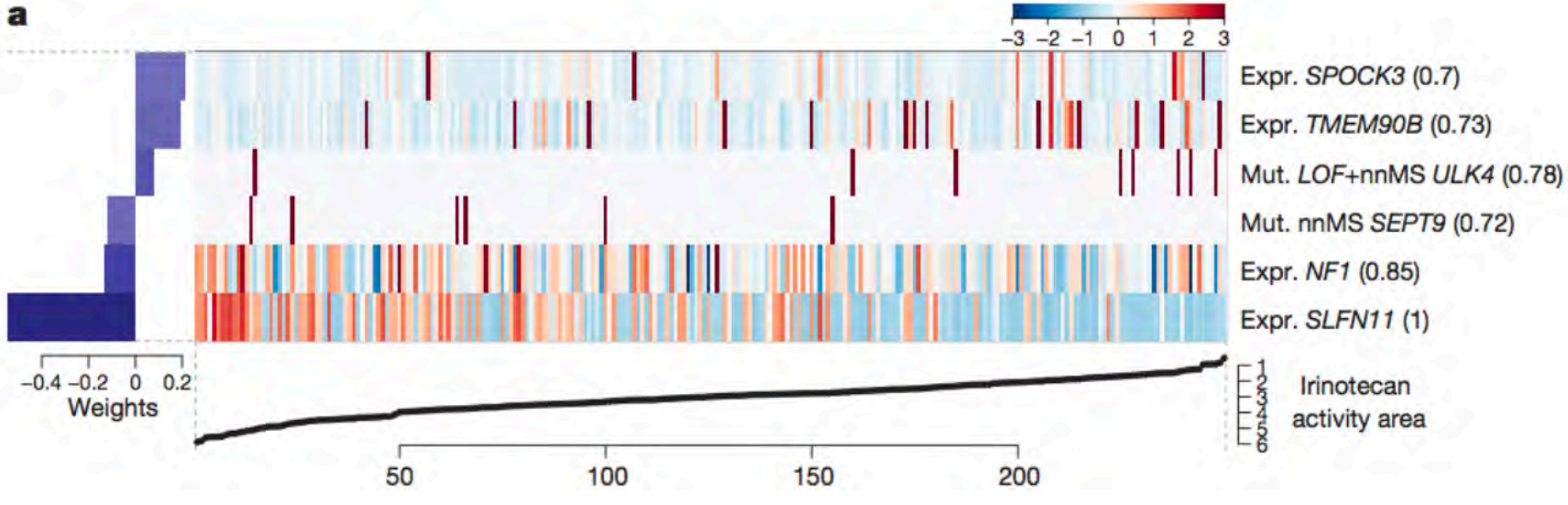


Large drug response datasets: CCLE, CTRP, GDSC, gCSI



GDSC, Nature, March 2012

Large drug response datasets: CCLE, CTRP, GDSC, gCSI



CCLE, Nature, March 2012

Current approaches require reproducible drug sensitivity studies

Inconsistency in large pharmacogenomic studies

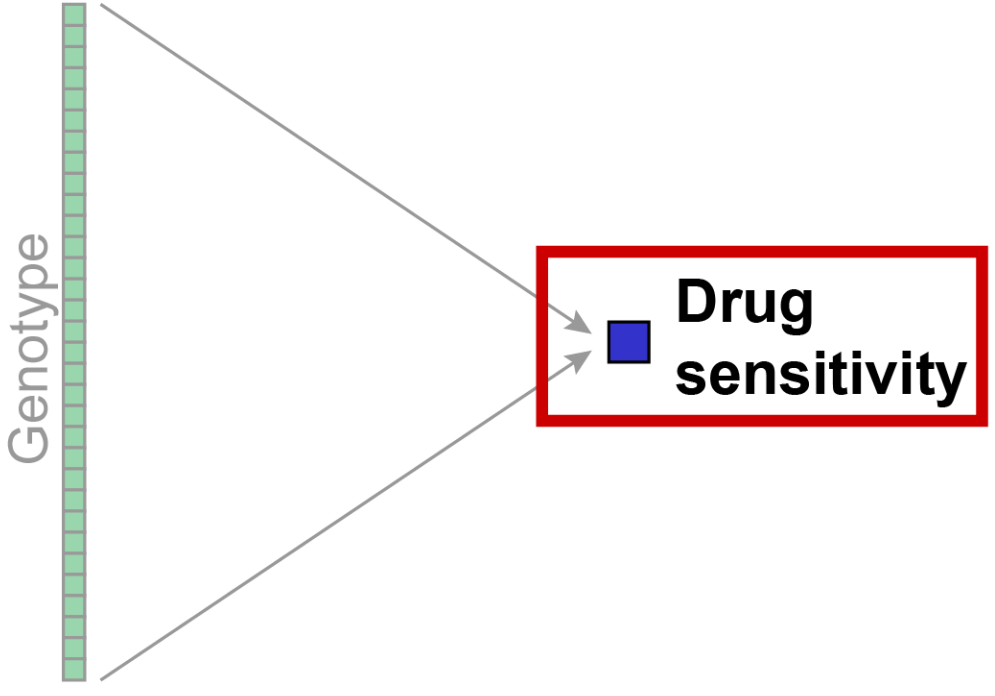
Benjamin Haibe-Kains, Nehme El-Hachem, Nicolai Juul Birkbak, Andrew C. Jin, Andrew H. Beck, Hugo J. W. L. Aerts & John Quackenbush

Affiliations | Contributions | Corresponding author

Nature **504**, 389–393 (19 December 2013) | doi:10.1038/nature12831

1. CCLE & GDC, Nature, Dec 2015
2. Haverty et al., Nature, May 2016
3. Bouhaddou et al. Nature, Dec 2016
4. Mpindi et al., Nature, Dec 2016
5. Safikhani et al., Nature, Dec 2016
6. Geeleher et al., Nature, Dec 2016

Advancing precision medicine requires improved proper quantification of drug sensitivity



How to best measure the response phenotype?

ICSB Workshop: Drug Response Measurement and Analysis

Part 1: New metrics of drug response

Part 2: Best practices for experimental design, execution and analysis

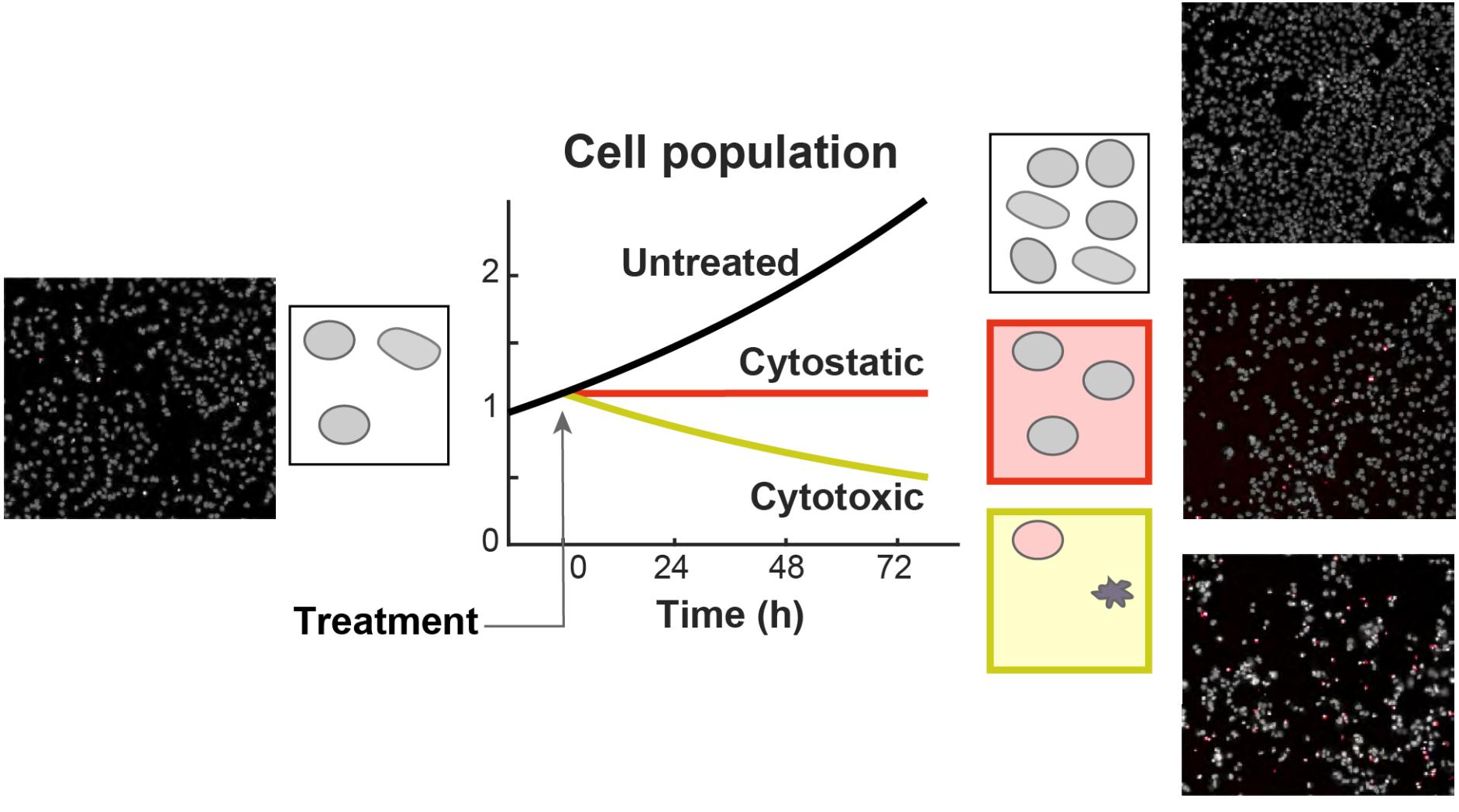
Part 3: Assessing drug synergy in combination therapies

ICSB Workshop: Drug Response Measurement and Analysis

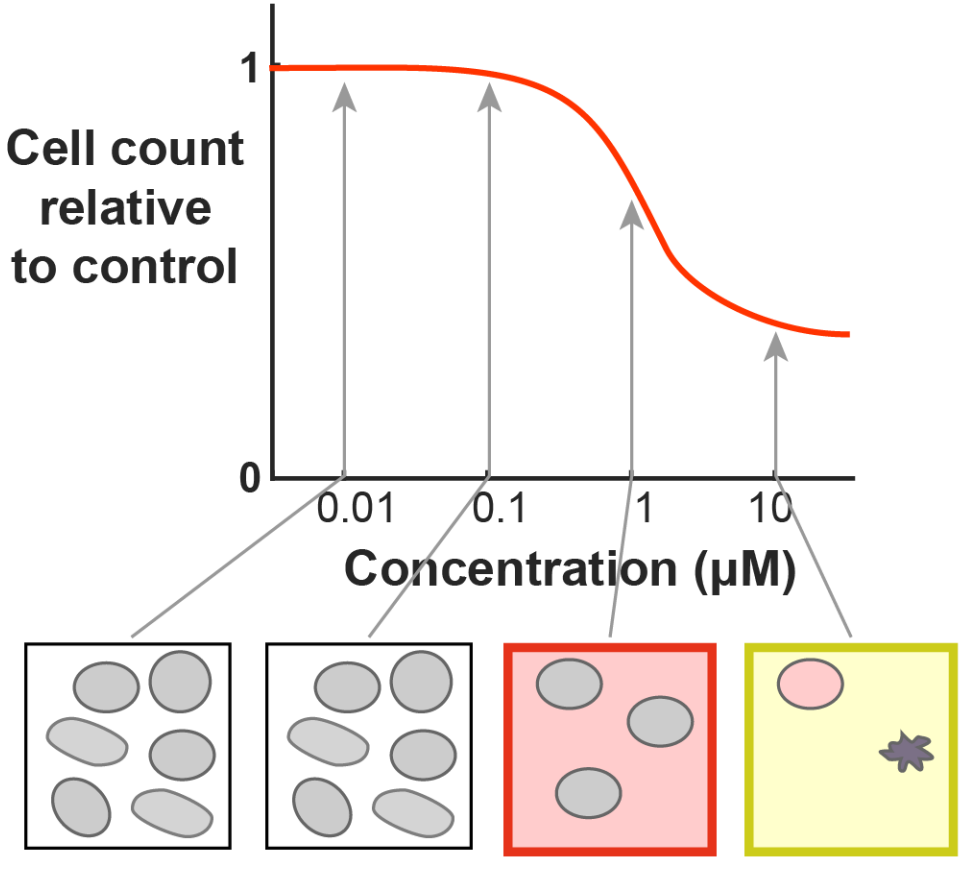
Part 1: New metrics of drug response

Marc Hafner

Measuring drug response is essential in pharmacology



Drug response is assayed at multiple doses



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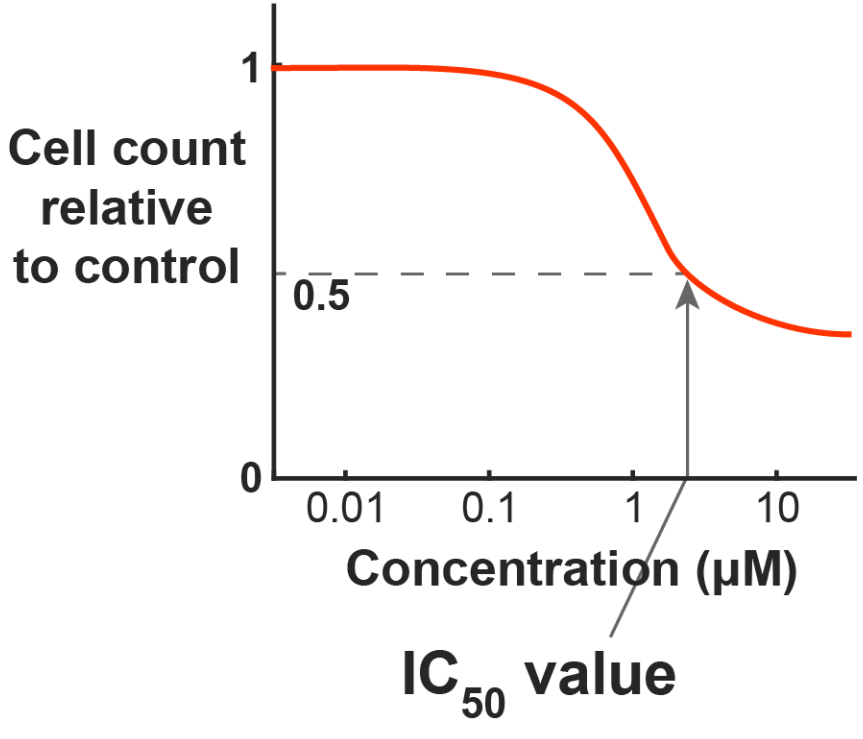
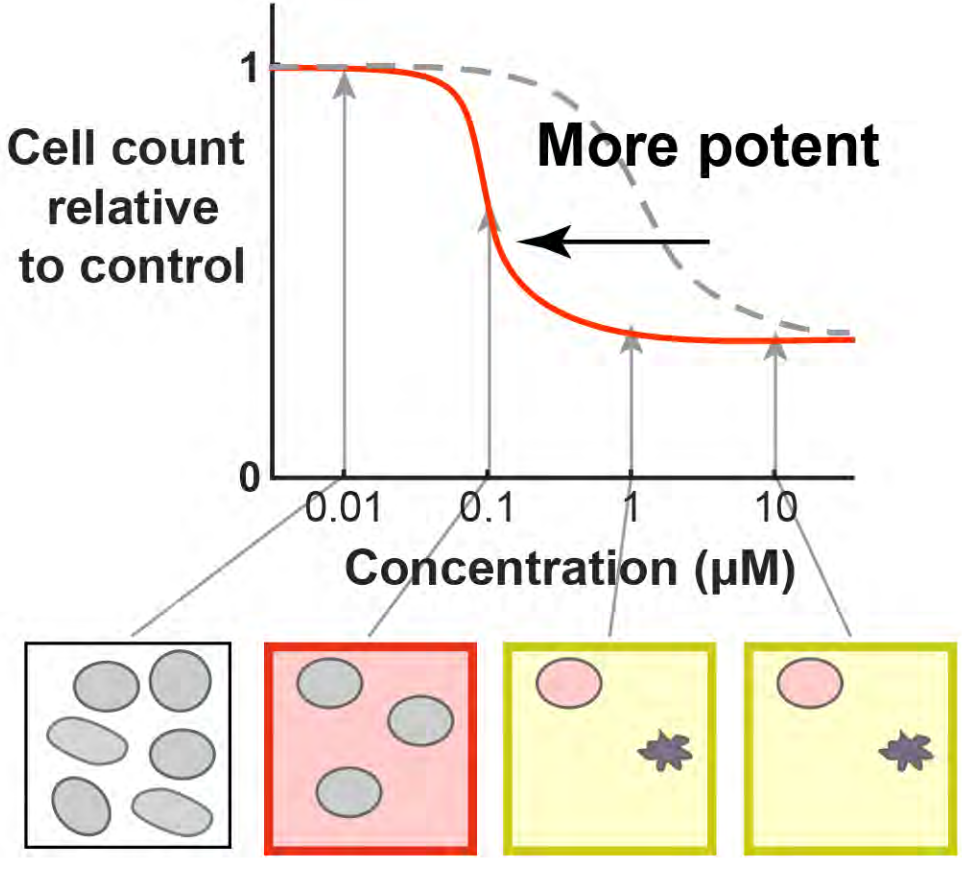


IC_{50} value is the concentration at which the relative cell count is 0.5.

Dose response curves vary across cell lines

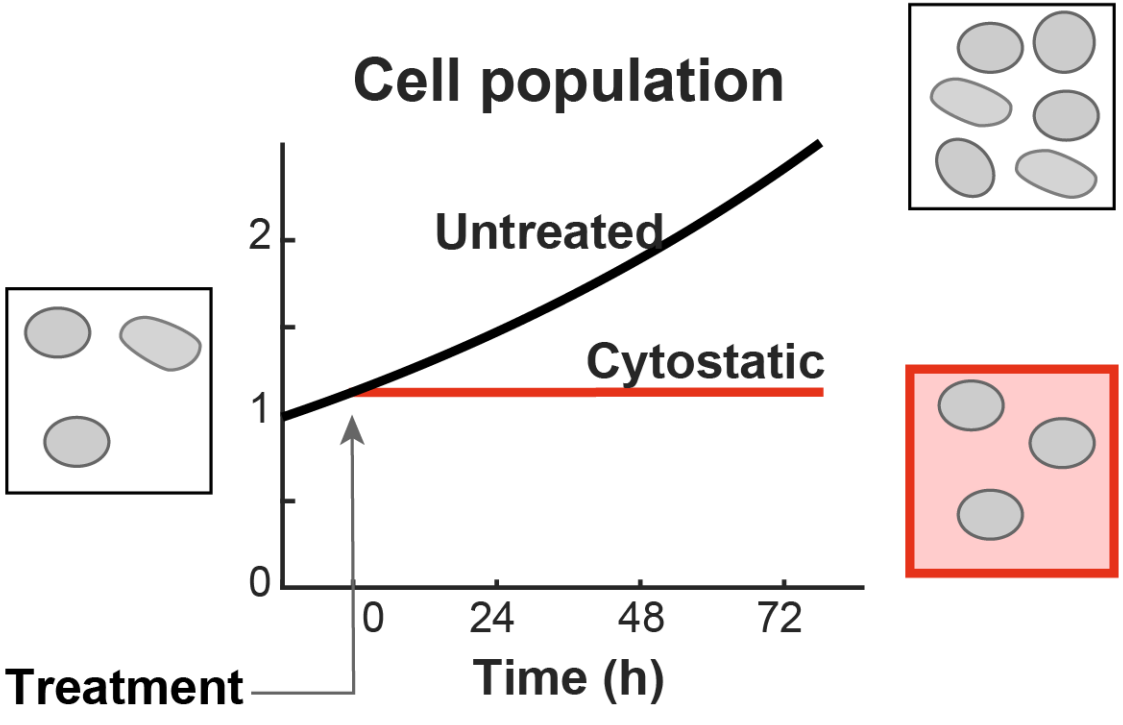


Normalization by the untreated control

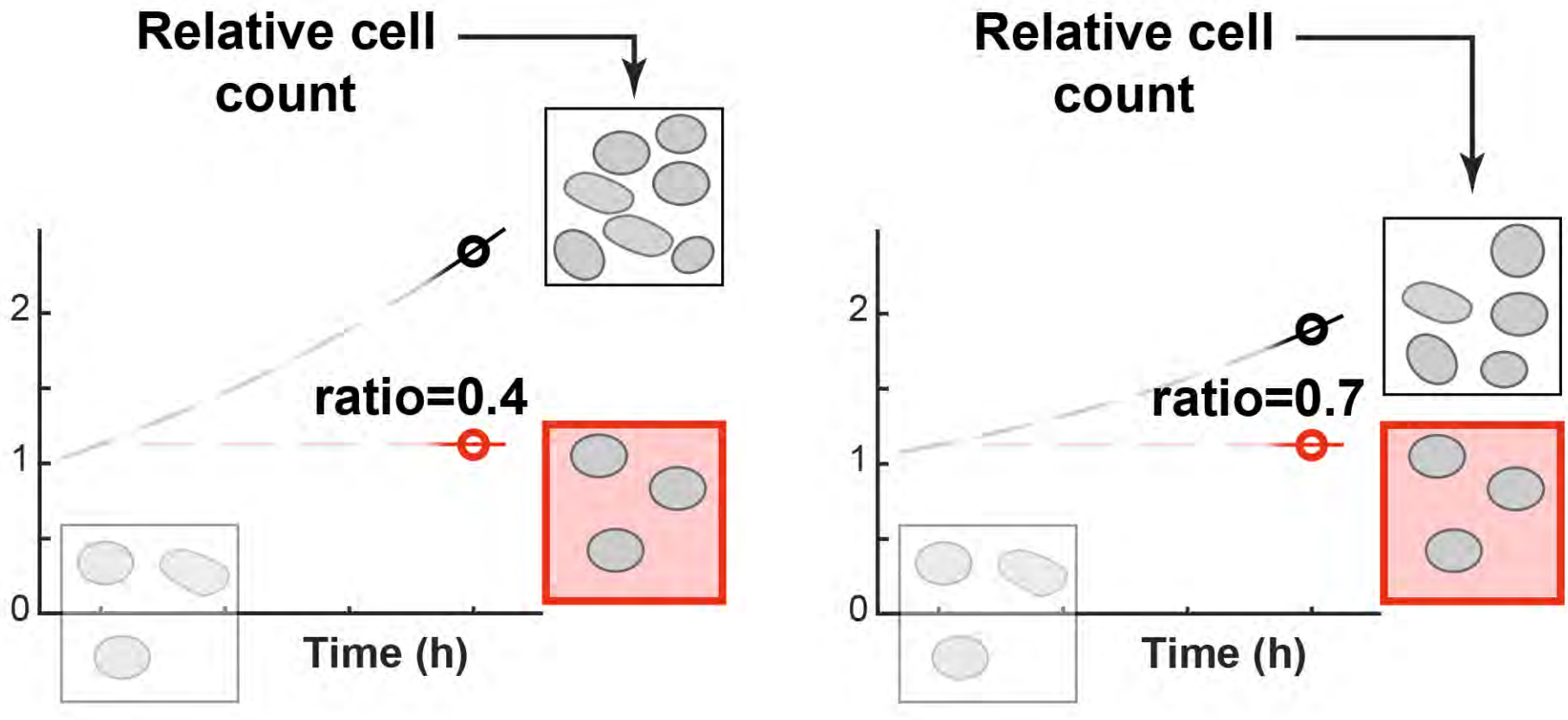
Relative cell count is valid when the untreated control remains the same:

- Phenotype is not related to cell growth
- Untreated cells do not grow
- Short assays during which growth is negligible

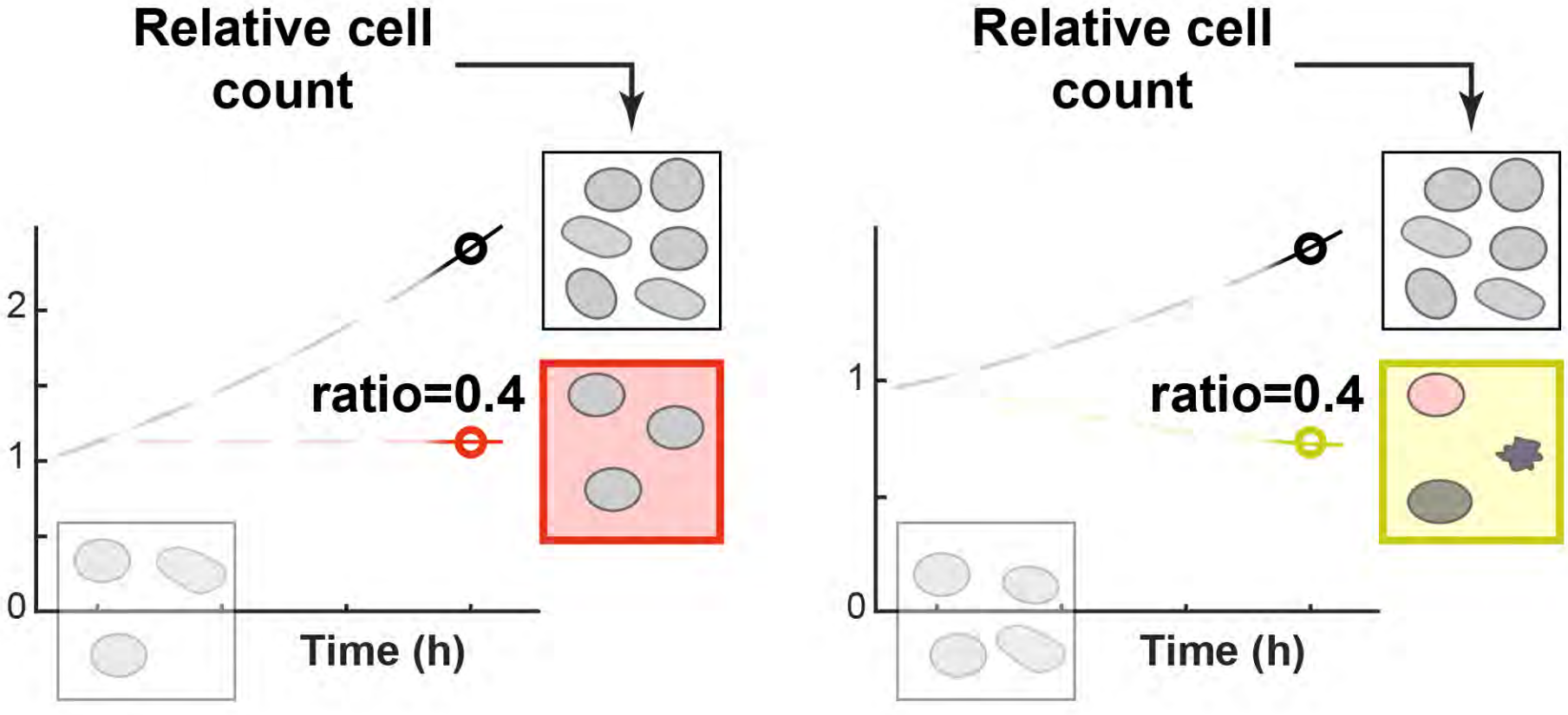
Assays that have a growing population



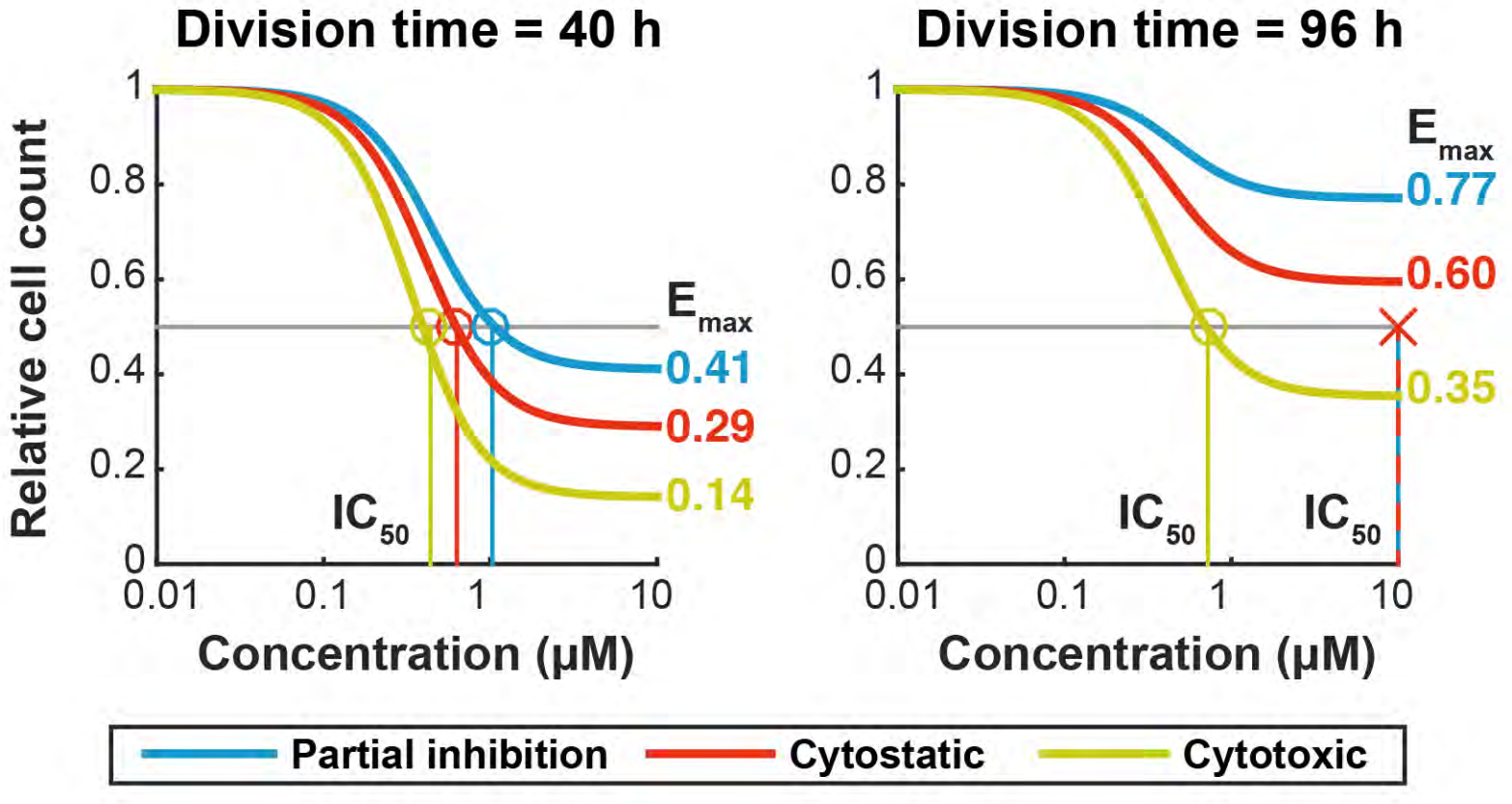
Relative cell count is biased by division rate



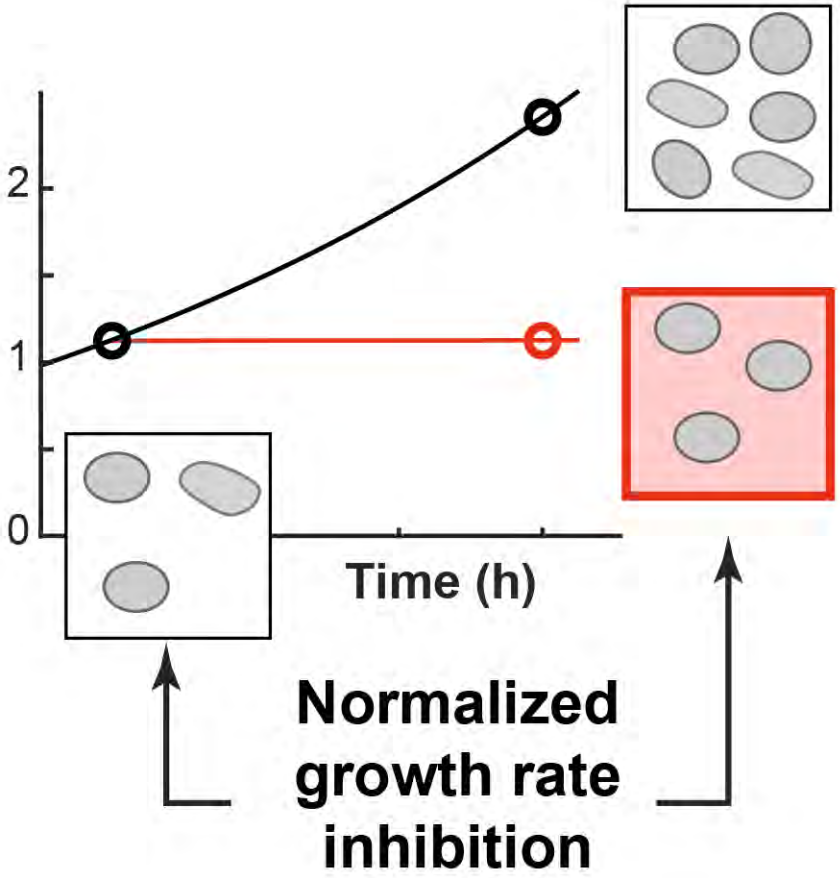
Relative cell count does not distinguish underlying phenotypes

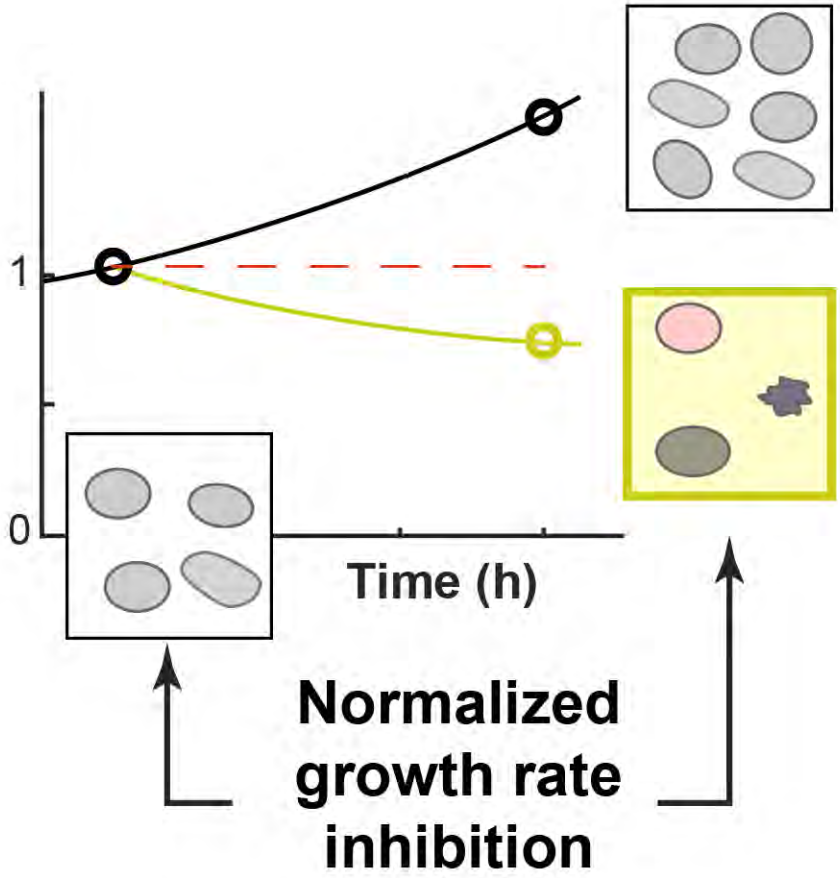


Slow growing cell lines look systematically more resistant when using relative cell count

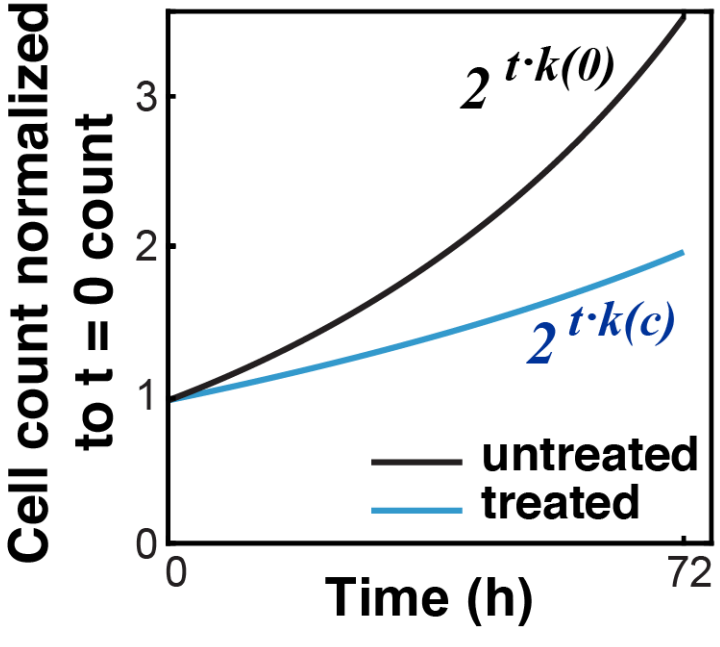


New unbiased metrics that define these underlying phenotypes are needed





Normalized growth rate inhibition (GR) value

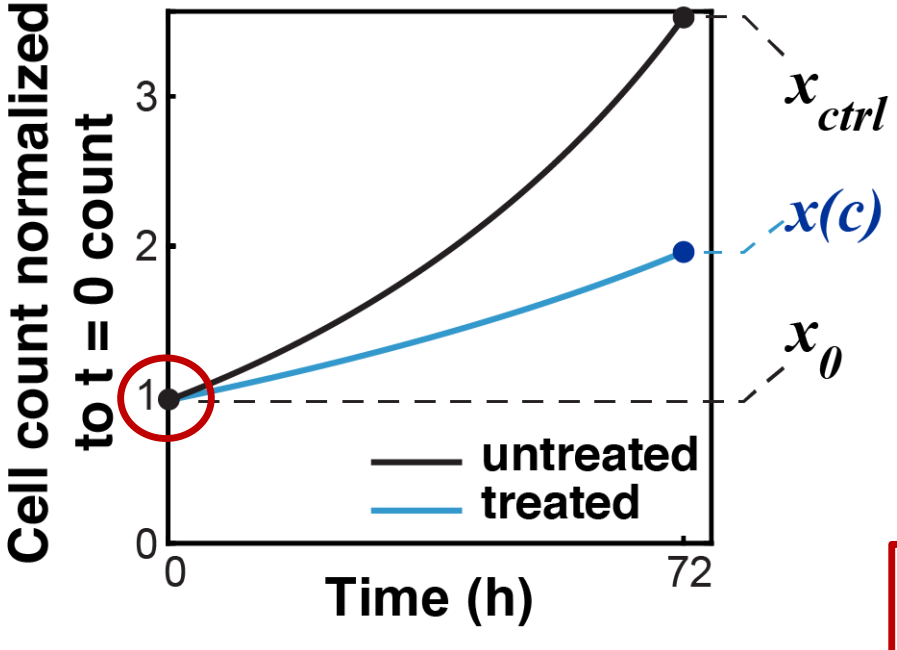


$$GR(c) = 2^{k(c)/k(0)} - 1$$

k(c) is the treated growth rate

k(0) is the control growth rate

GR values rely on three measures of cell count



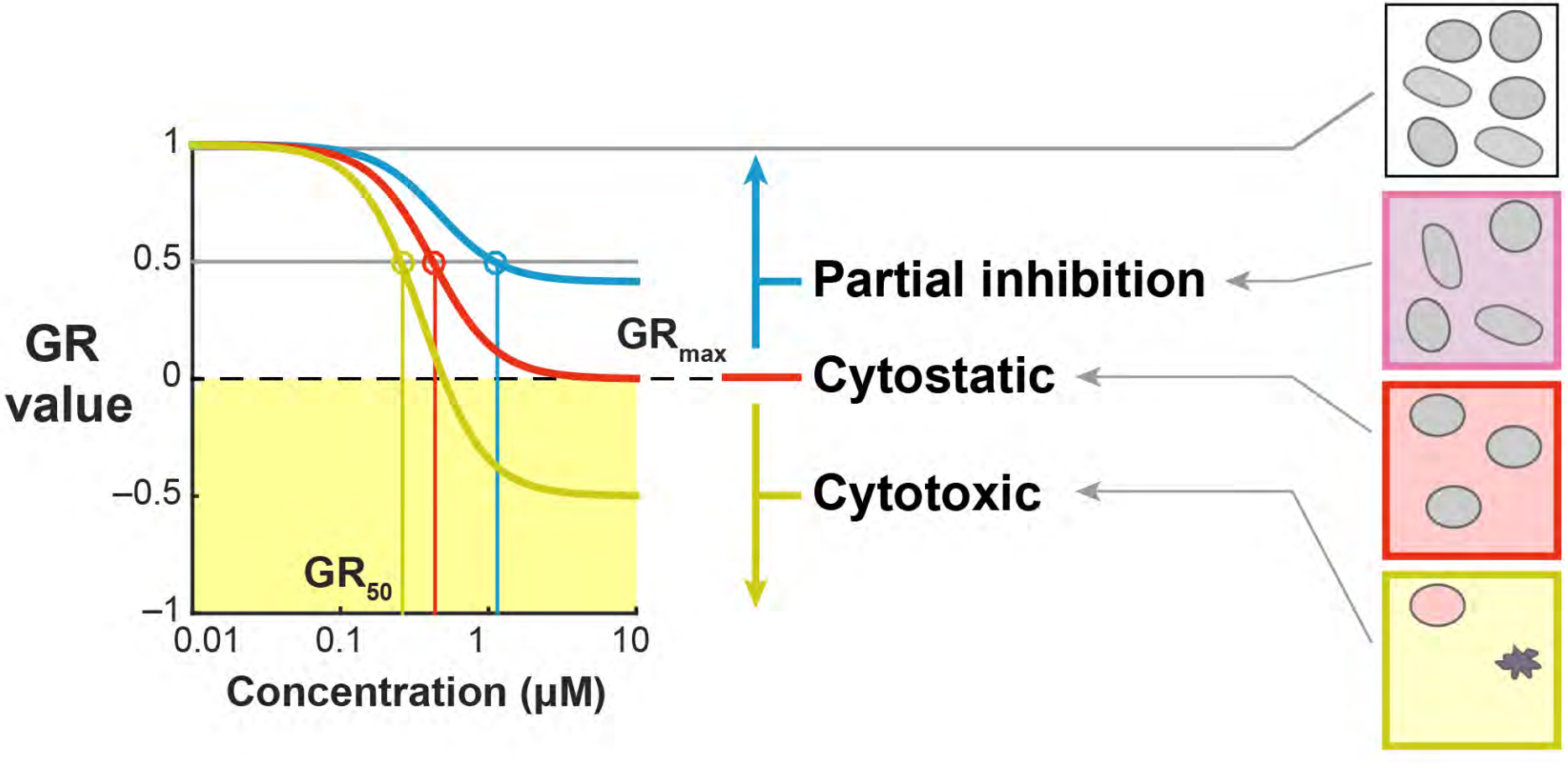
$$GR(c) = 2^{\frac{\log_2(x(c)/x_0)}{\log_2(x_{ctrl}/x_0)}} - 1$$

$x(c)$ is the treated cell count

x_{ctrl} is the control cell count

x_0 is the cell count at the time of treatment

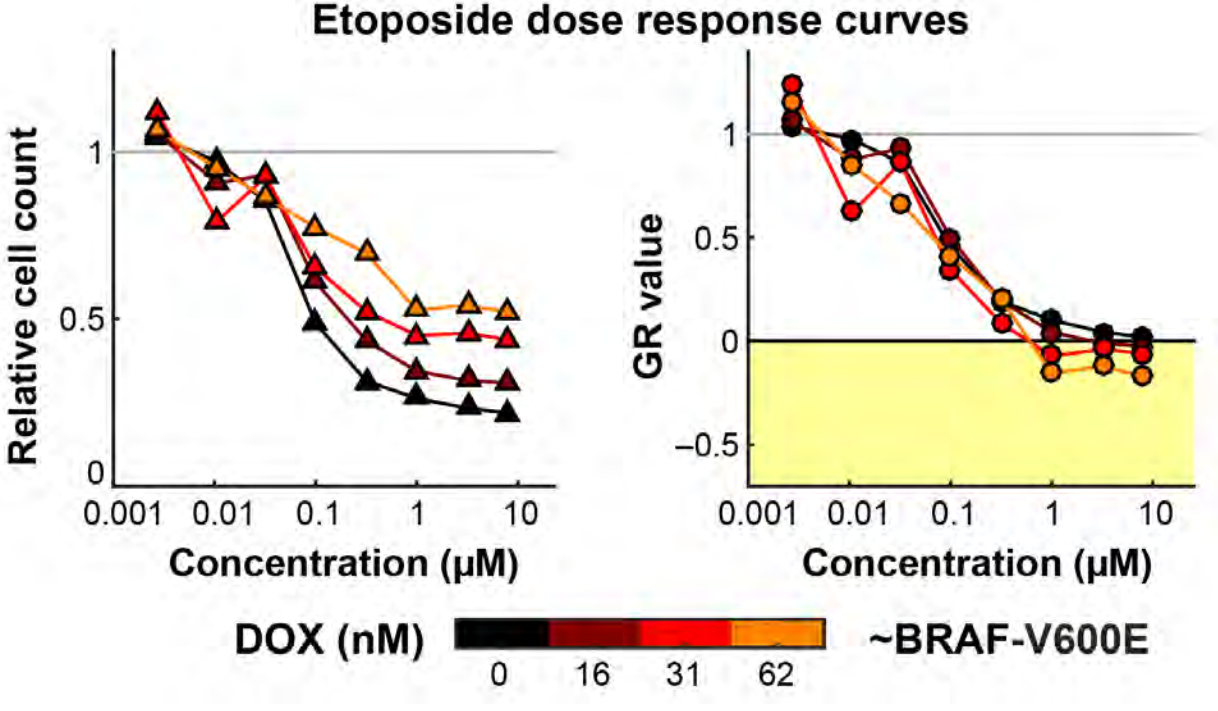
GR values are independent of the division rate and directly relate to the phenotype

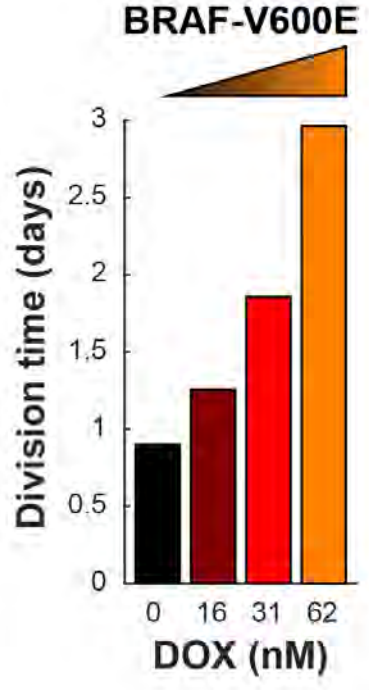


Hafner*, Niepel* et al. Nat Methods, 2016, 13:521-7

Genetic alterations affect division time independently of drug sensitivity

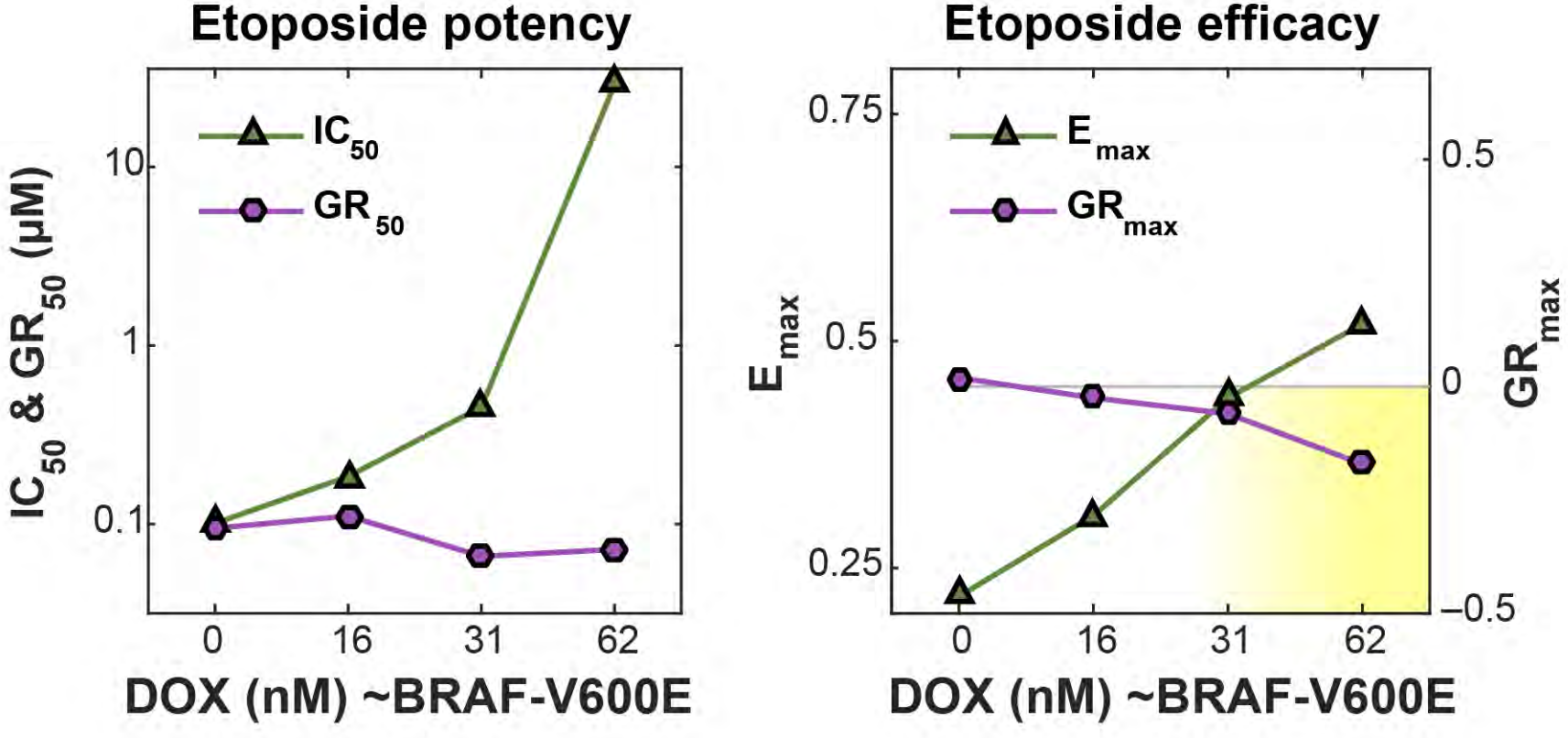
Etoposide sensitivity in HME RPE-1 cells expressing $BRAF^{V600E}$.



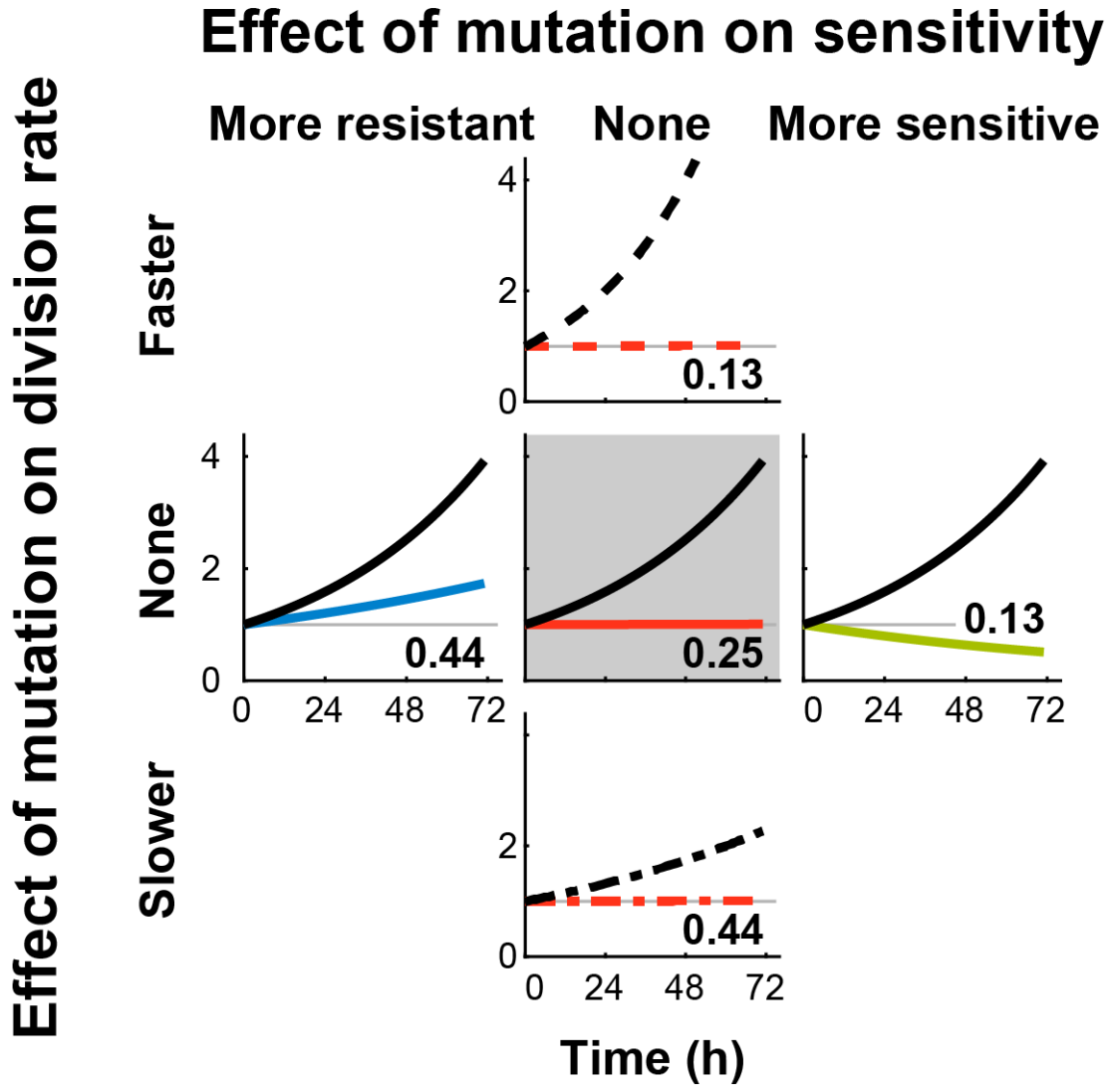


Thanks to Jia-Yun Chen for the cell line

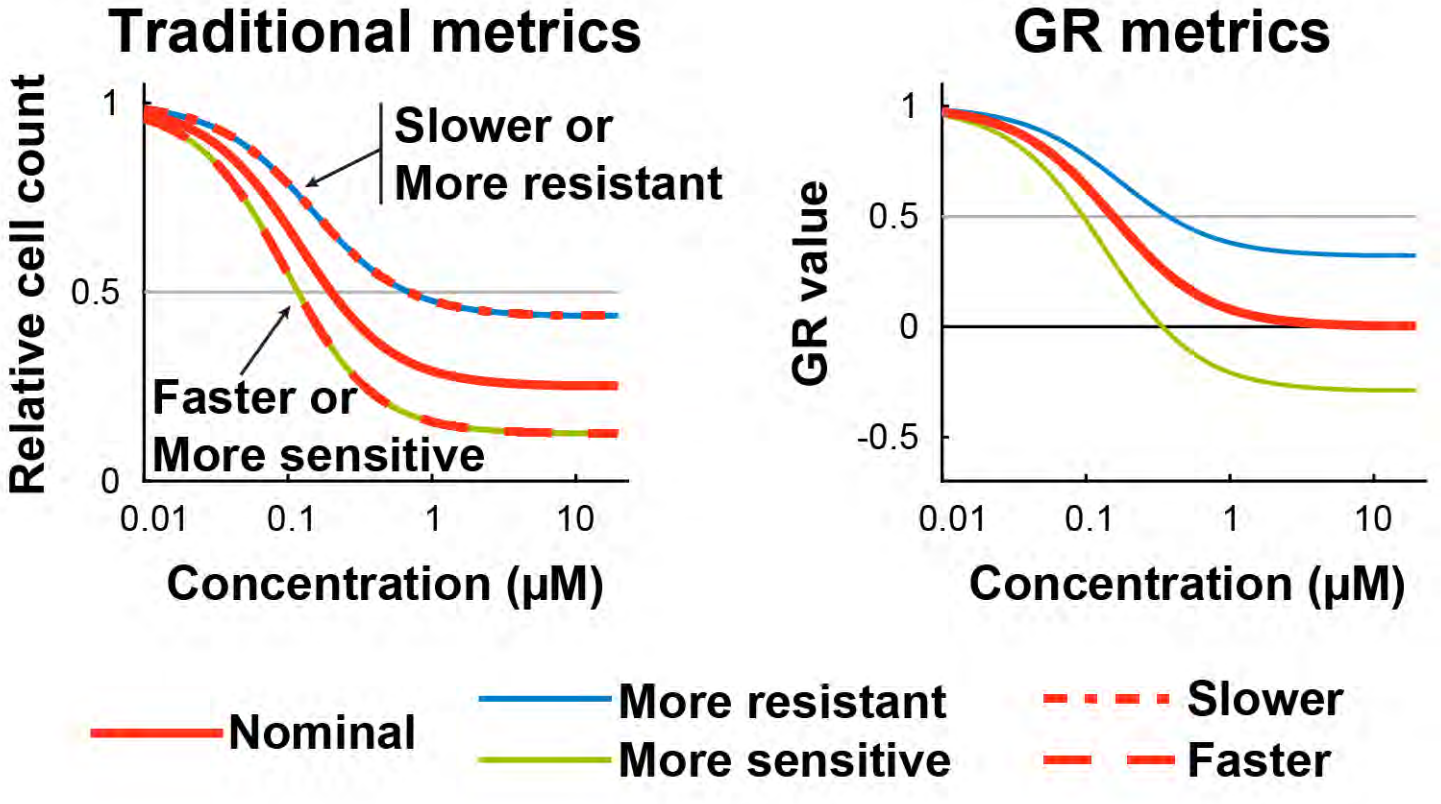
Genetic alterations affect division time which biases traditional sensitivity metrics



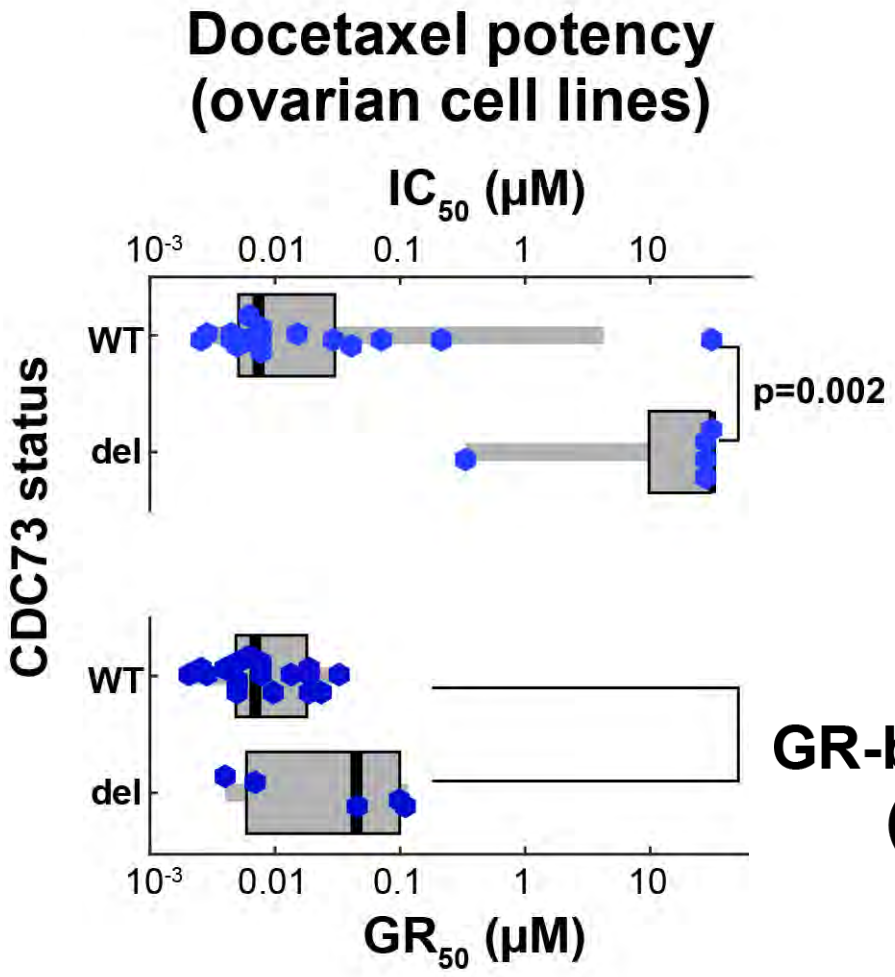
Model of interaction between mutation, division time and drug sensitivity



Division rate and sensitivity are confounded in traditional metrics of drug response



False-positive associations between IC_{50} and genotype are common

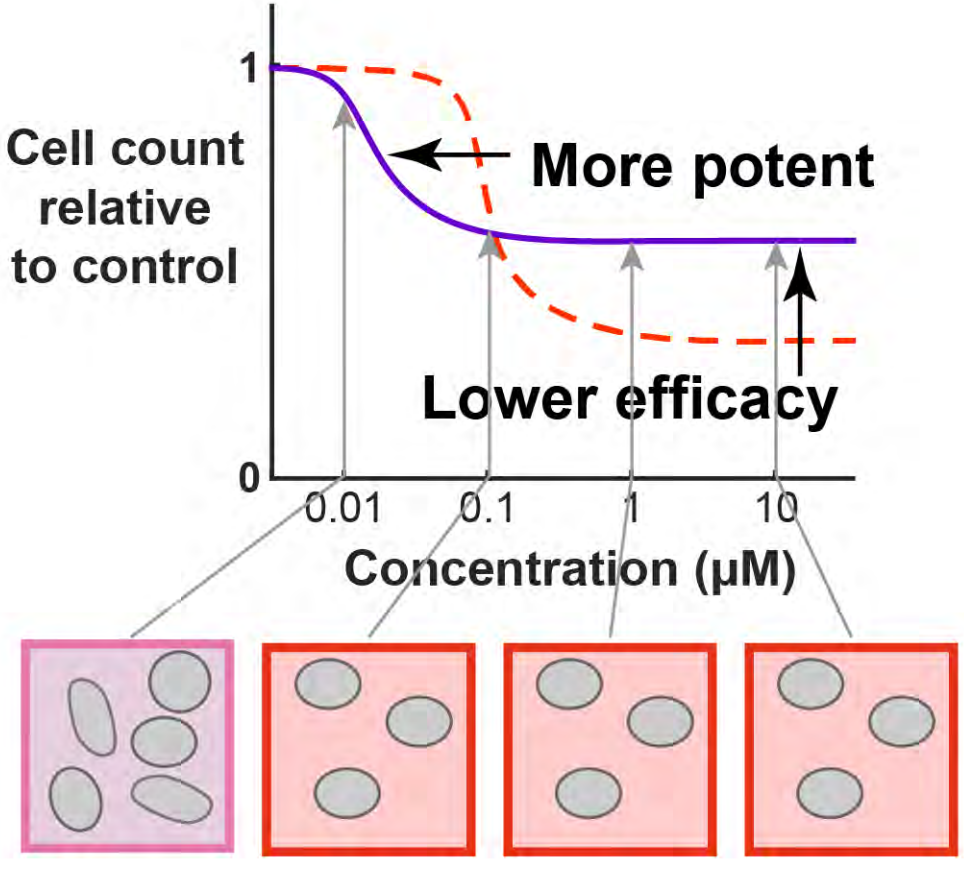


CDC73-loss slows growth, which artificially increases IC_{50} values.

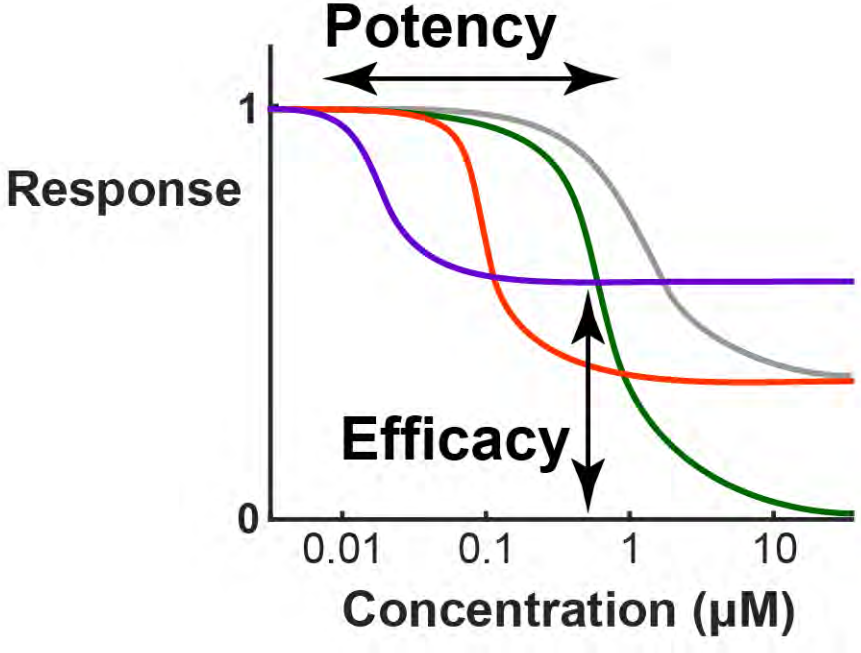
GR-based (GR_{50})	Traditional (IC_{50}) FDR<0.15	Traditional (IC_{50}) non-sign.
FDR<0.15	49	50
non-sign.	73	58276

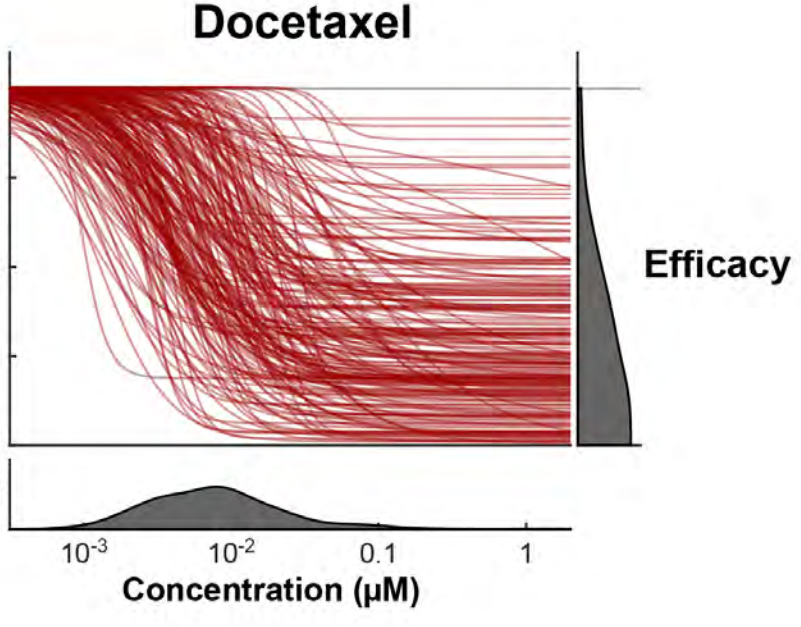
Data from Haverty et al., Nature 2016, 533, 333-7

Response can vary both potency and efficacy



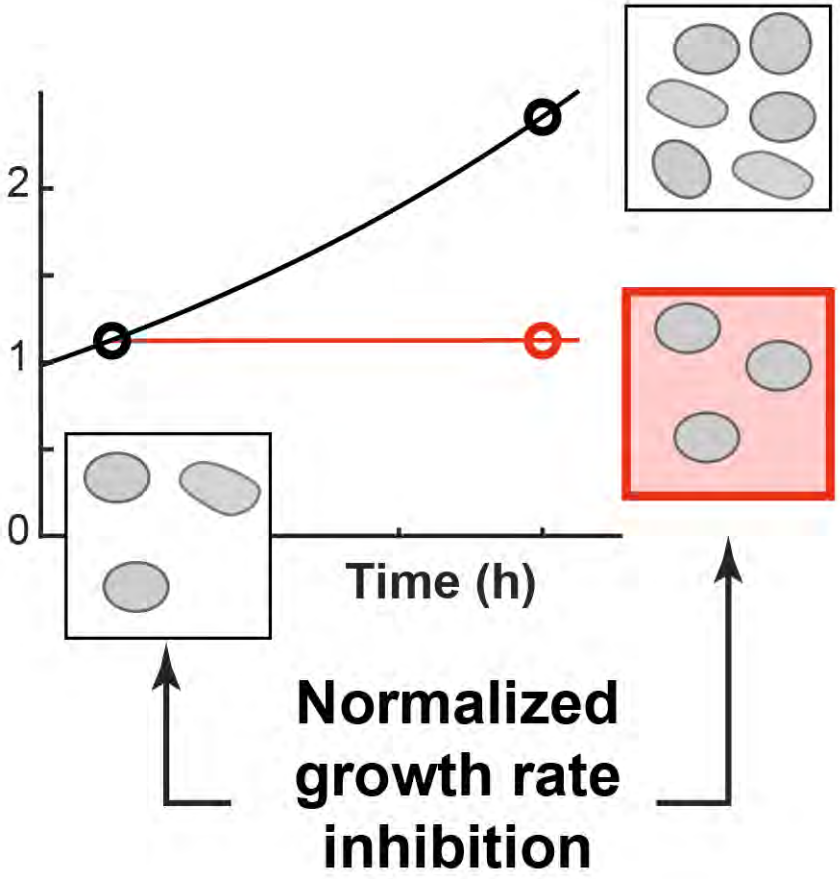
Response diversity occurs in both potency and efficacy across cell lines and drugs

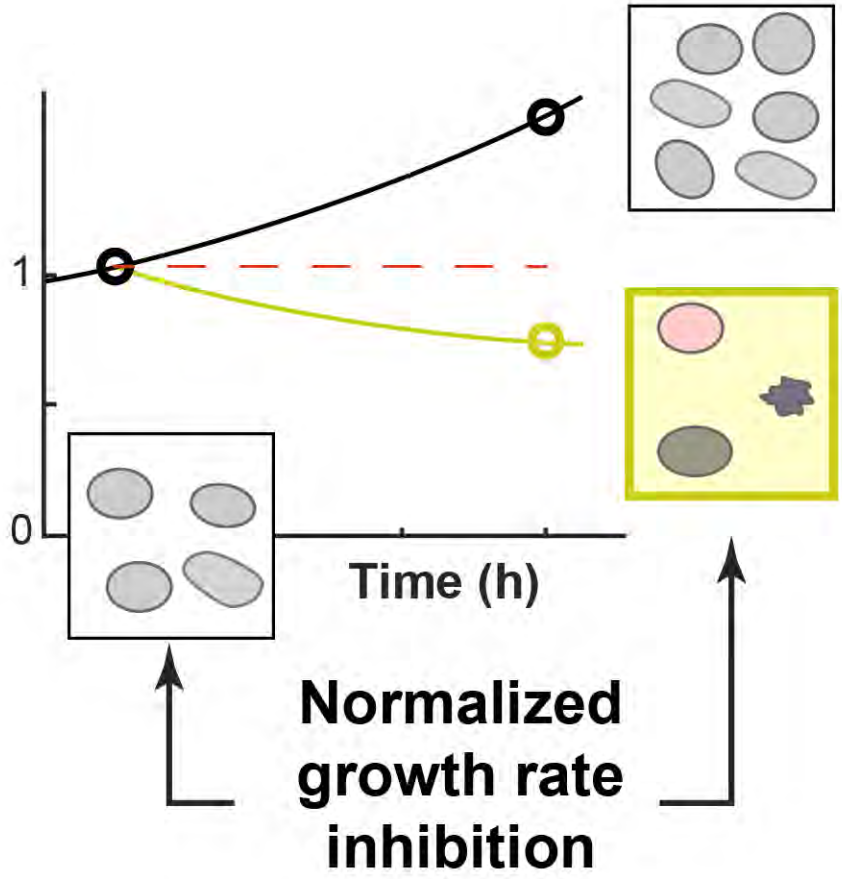




Data from Haverty et al., Nature 2016, 533, 333-7

The GR metrics capture the underlying phenotypes





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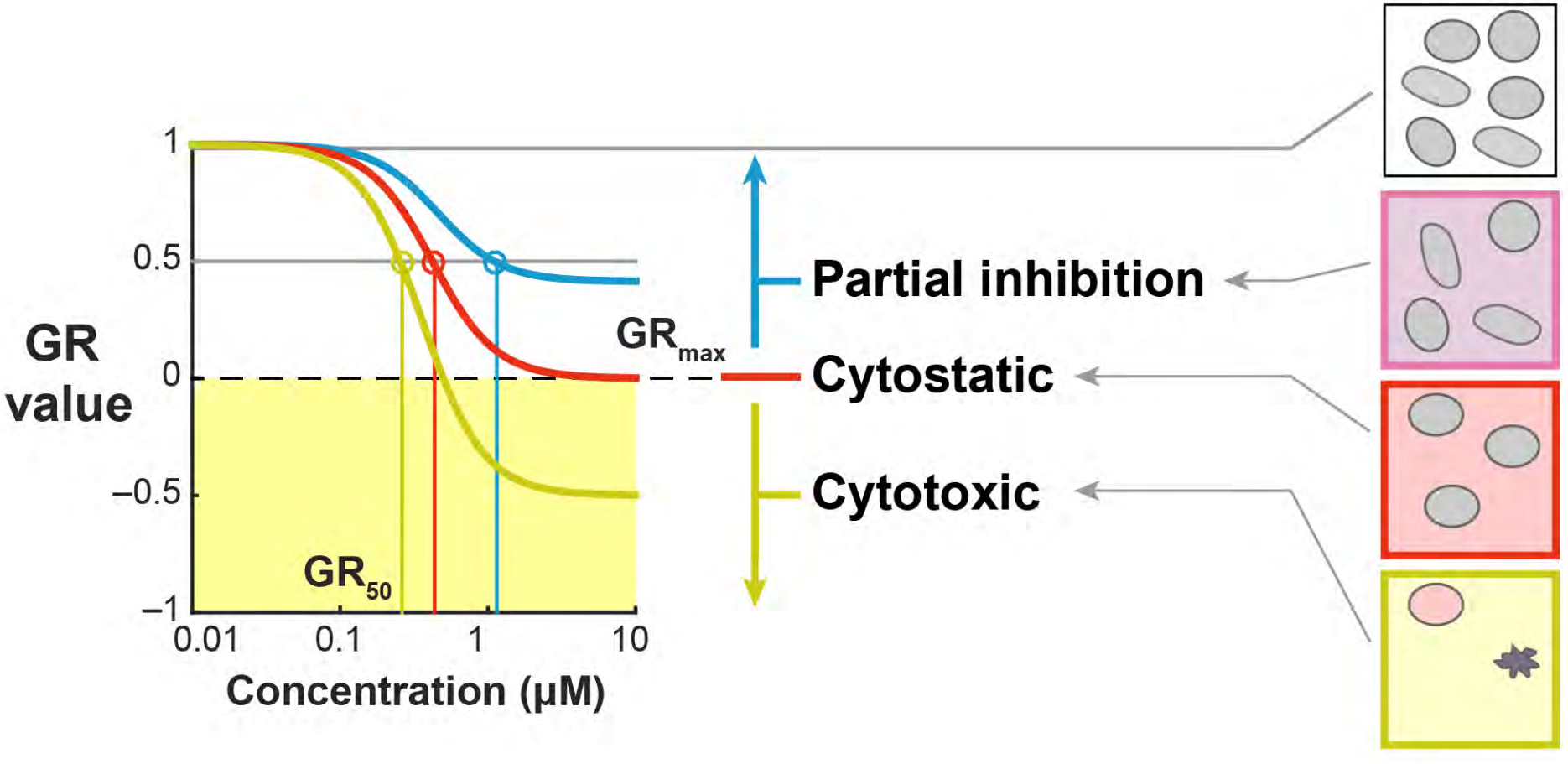
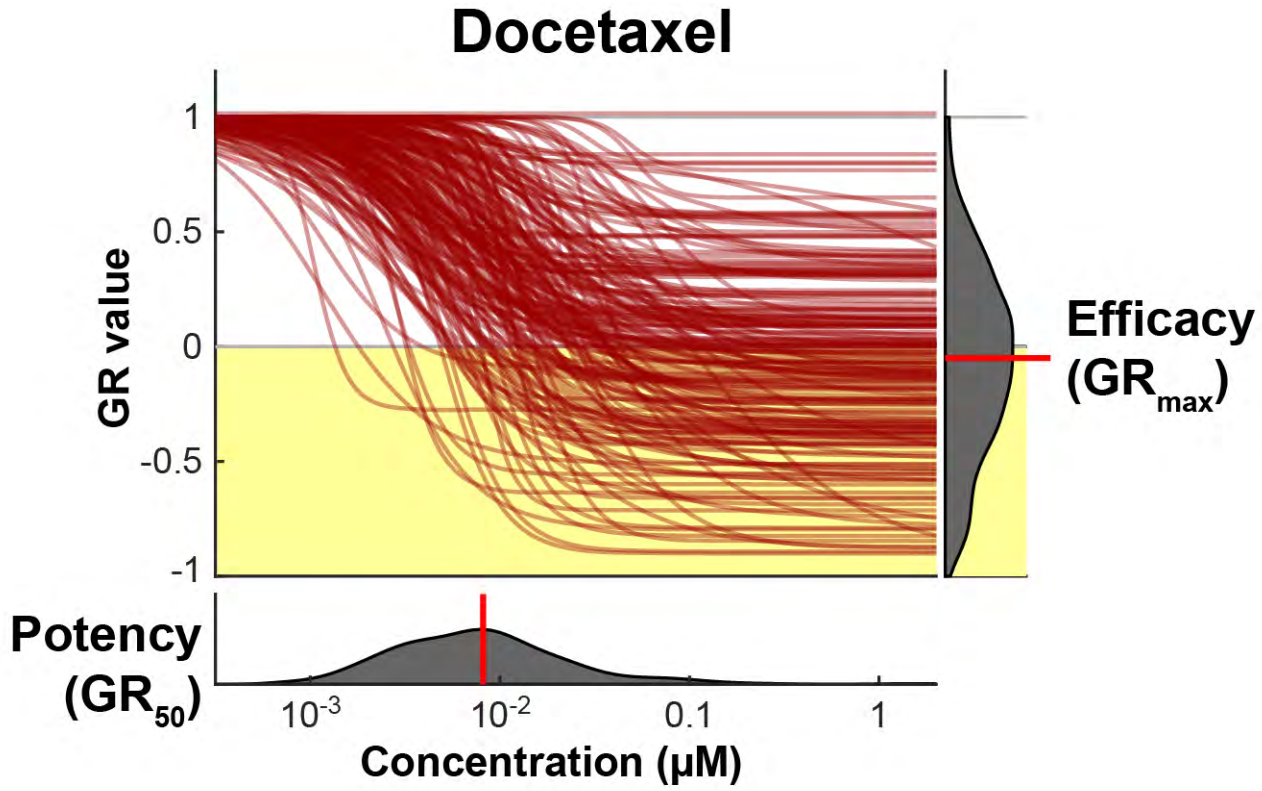


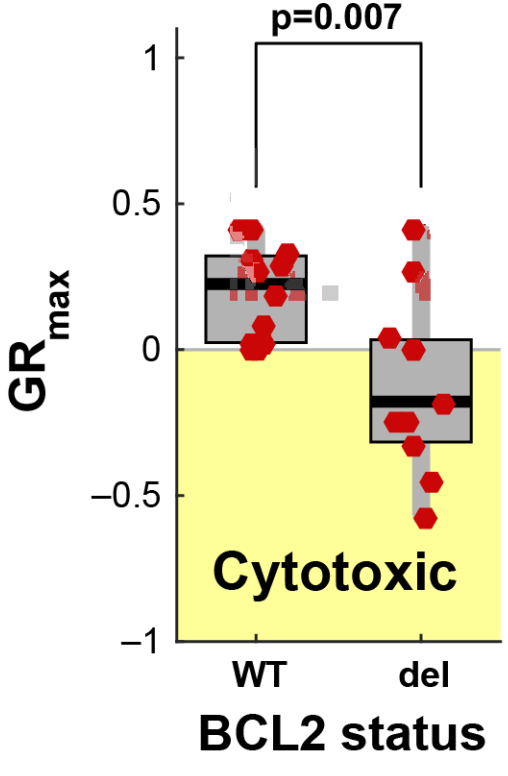
Docetaxel efficacy spans multiple phenotypes



Data from Haverty et al., Nature 2016, 533, 333-7

Efficacy (GR_{max}) correlates with genotype

Docetaxel efficacy (ovarian cell lines)



Potency(GR_{50})

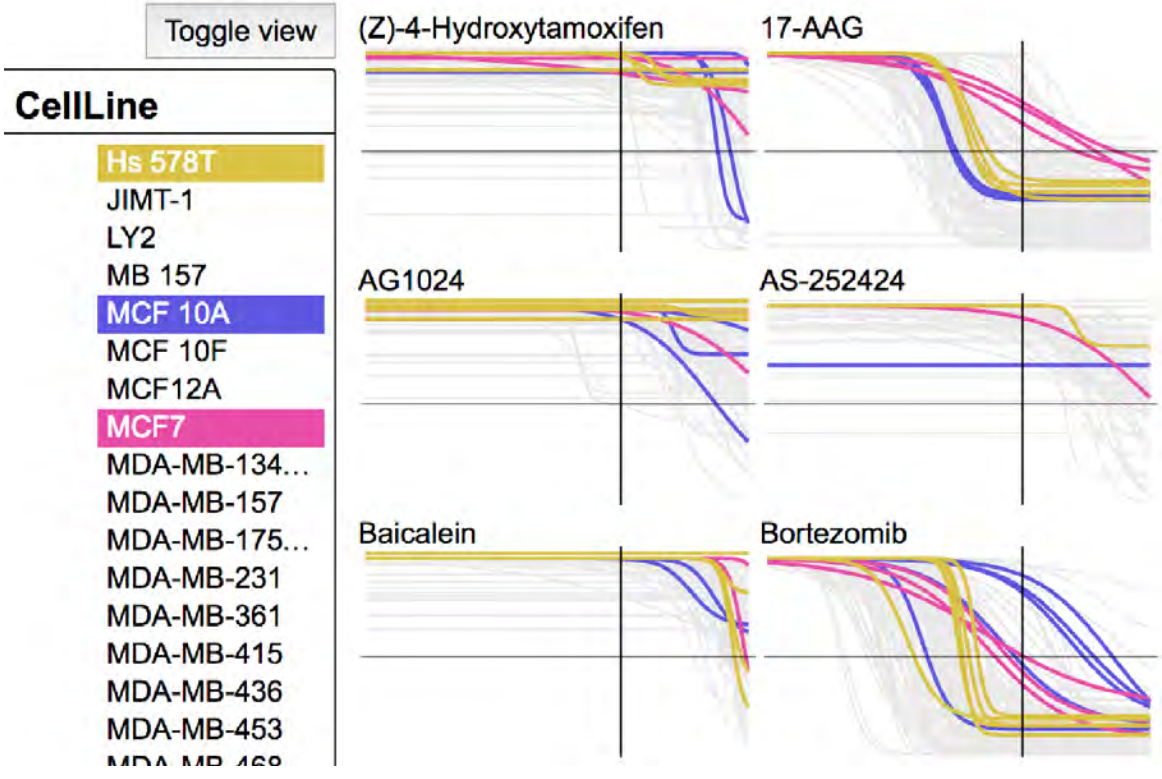
Efficacy (GR_{max})	FDR<0.15	non-sign.
FDR<0.15	18	81
non-sign.	81	58268

Hafner, et al., Nat Biotech, 2017, 35:500-2

Data processing and basic analyses can be performed on GRcalculator.org

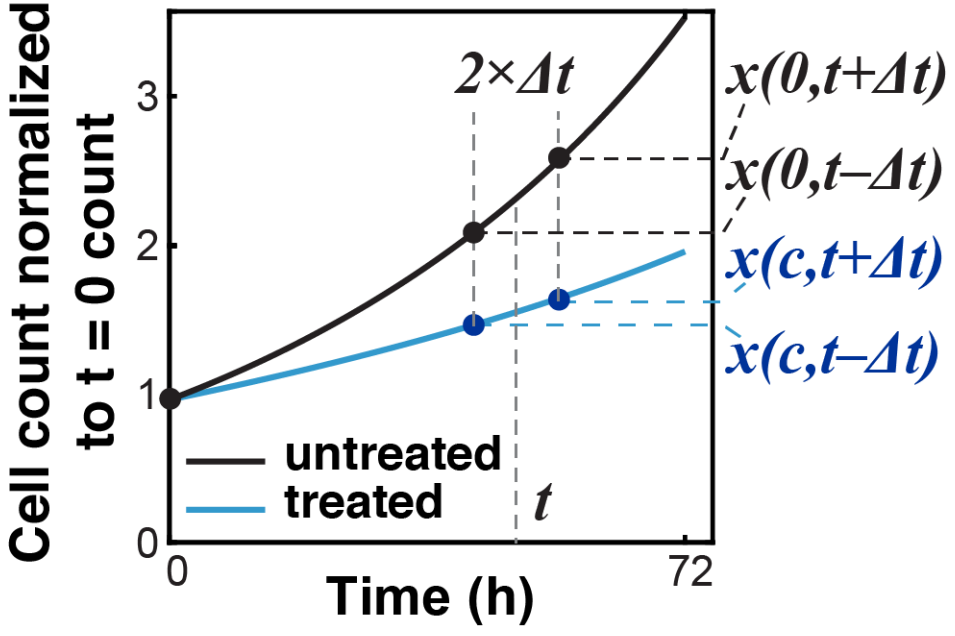
GRcalculator.org

Clark*, Hafner* et al.,
BMC Cancer, accepted



Time-dependent GR values and metrics

$$GR(c,t) = 2^{\frac{\log_2(x(c,t+\Delta t/2)/x(c,t-\Delta t/2))}{\log_2(x(0,t+\Delta t/2)/x(0,t-\Delta t/2))}} - 1$$



It allows evaluating $GR_{50}(t)$ and $GR_{max}(t)$ and quantifying adaptive response or late drug action.

How to measure and process the data?

- Planning, and optimization
- Automate as much as possible, know how it works
- Script the experimental design and analysis
- Use appropriate metrics for your experiment