Using 21st century tools to support compound development: A case study with potential cancer therapeutics

BIOMED21
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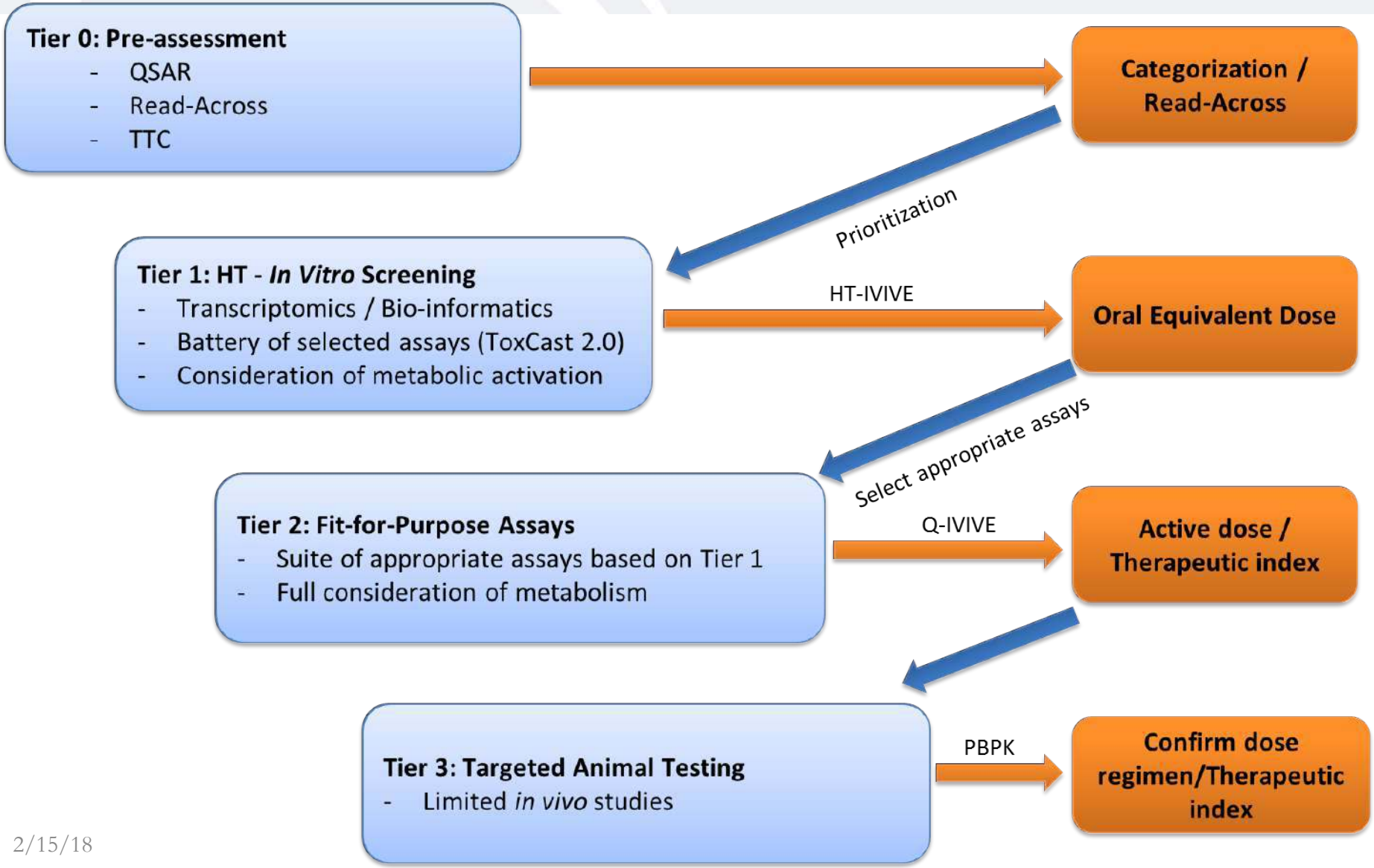
Tiered testing strategy



Computational Toxicology

QSAR and cheminformatics for prioritization and addressing data gaps

Transcriptomics supports mode of action studies

In vitro assay development

Fit-for purpose cell based assays –defining safe exposures for risk based decision making

Pharmacokinetics and IVIVE

Human relevant metabolism

Translating in vitro concentrations to in vivo doses

An example with cancer as a therapeutic target

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- QSAR-type strategy for chemical design
  - Targeting a particular molecular interaction based on a previous compound with known MoA
- In vitro:
  - Compound inhibits proliferation of cancer lines
    - Induces cell death in hematopoetic cell lines
  - No effect on non-cancer (primary) cells
- In vivo:
  - Compound inhibits tumor growth
  - No obvious off-target toxicity, other than GI distress at high doses
- Preliminary data indicates targeted interaction is not occurring

- What is the MoA?

Two-pronged approach

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- Big data:
  - Genome-wide association
  - Transcriptomics
  - Metabolomics

- High content imaging to evaluate cellular response and phenotype
  - Beginning with general cell morphology
  - Moving into more targeted hypothesis testing

Designing assays for MoA testing

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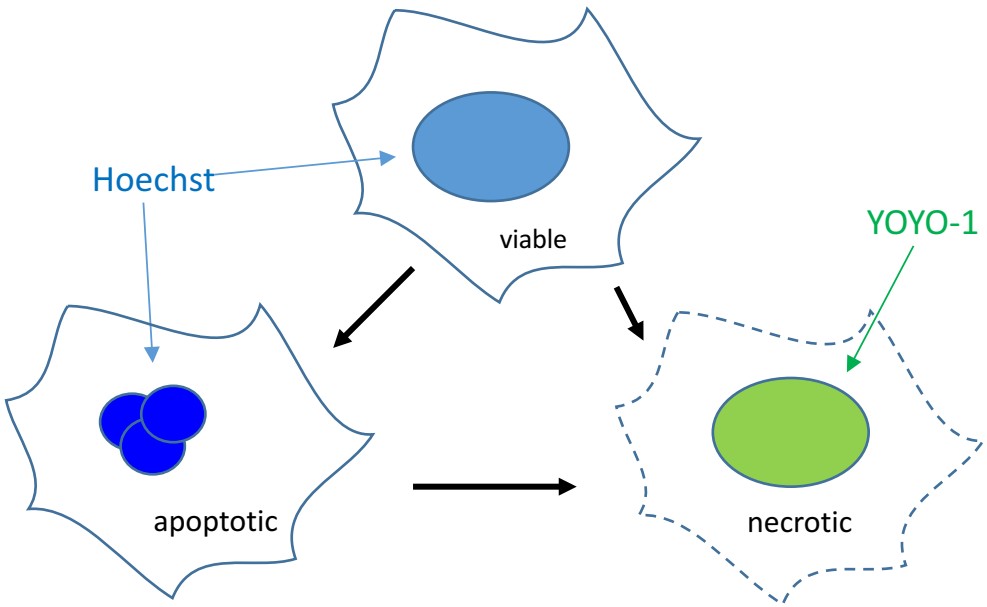
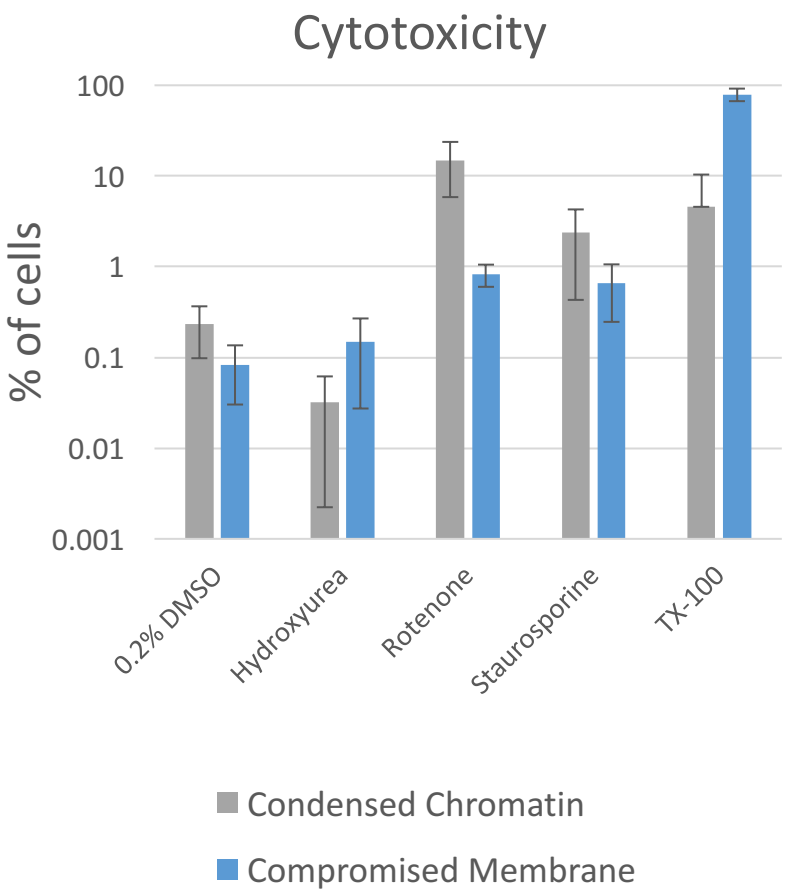
- What we knew:
  - Assayed for effectiveness in 99 cancer cell lines:
  - Measured ATP as an assay for viability.
  - Kills all lymphomas.
  - Slows growth in solid tumor lines with variable effectiveness
  - No effects on non-cancer cells
- Test system:
  - Intact human cells, representing those that were sensitive and insensitive to drug
  - Identified key events that could affect "viability"
    - Cytotoxicity, cell stress, cell cycle arrest, etc.
  - Used compounds with varying efficacy and controls with known MoA

Cytotoxicity

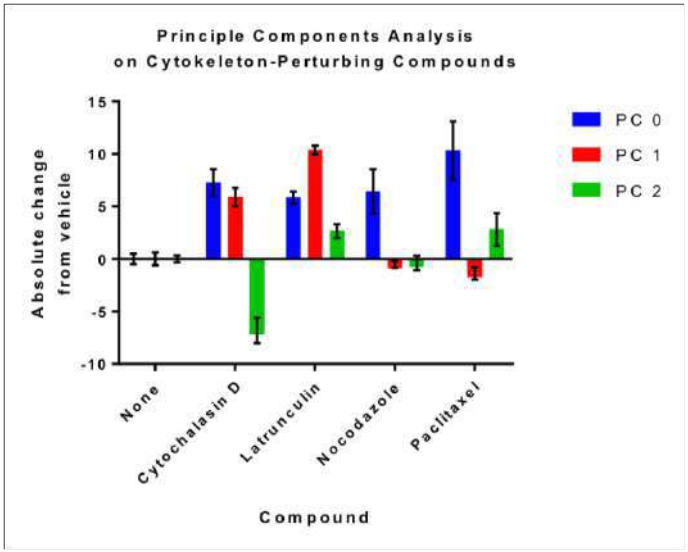
- Multiplexed high-content assessment of cell counts, membrane integrity, and nuclear size, morphology, and texture.



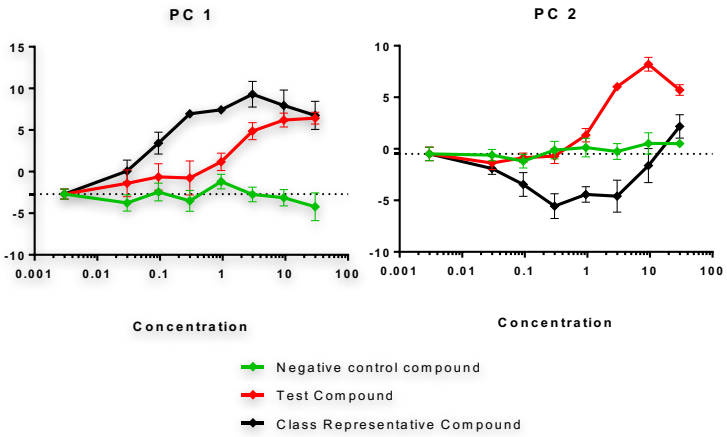


Cytoskeleton and Cellular Morphology

- **Highly multiplexed high-content assessment of morphology, intensity, and texture measurements with regard to the actin and microtubule cytoskeletons.**
- **Compound class clustering**

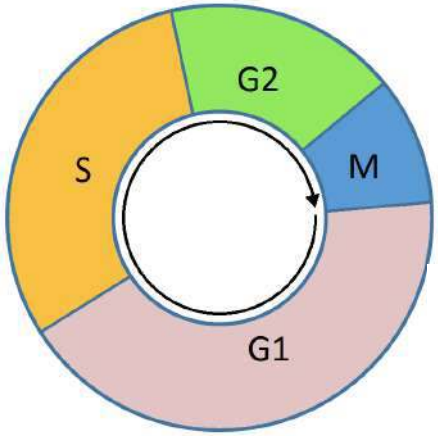


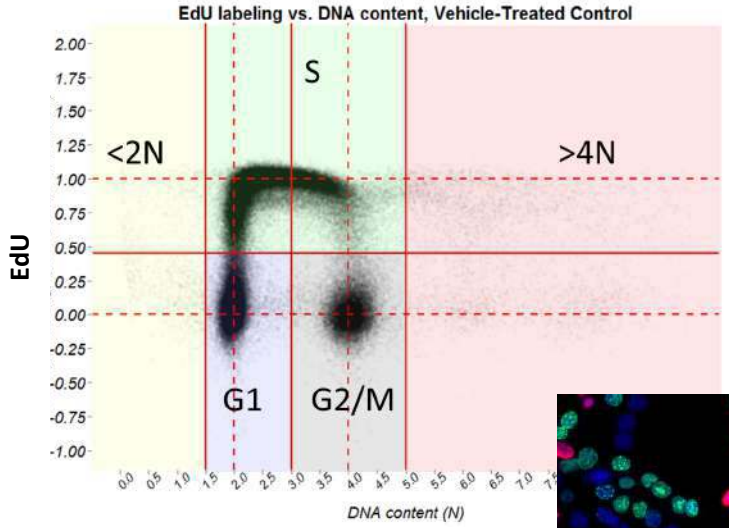
Dose response analysis on principle components reveal differences in mechanism of action

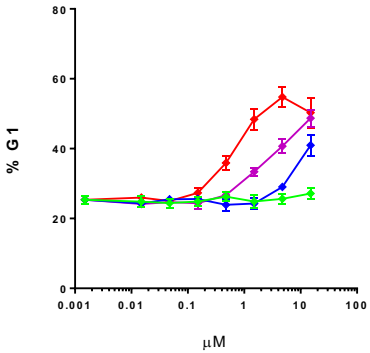


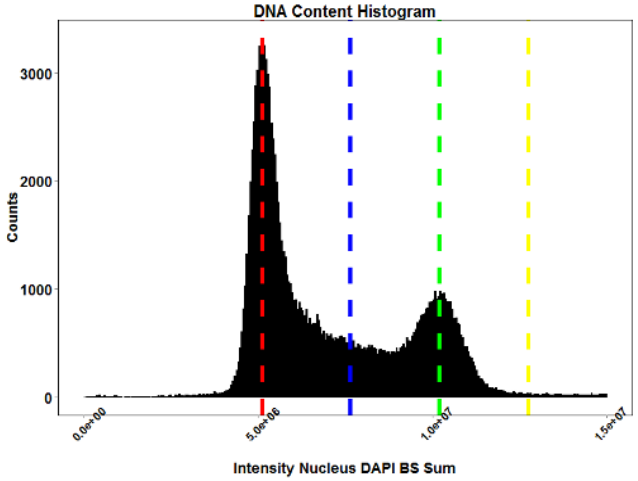
Cell Cycle Analysis

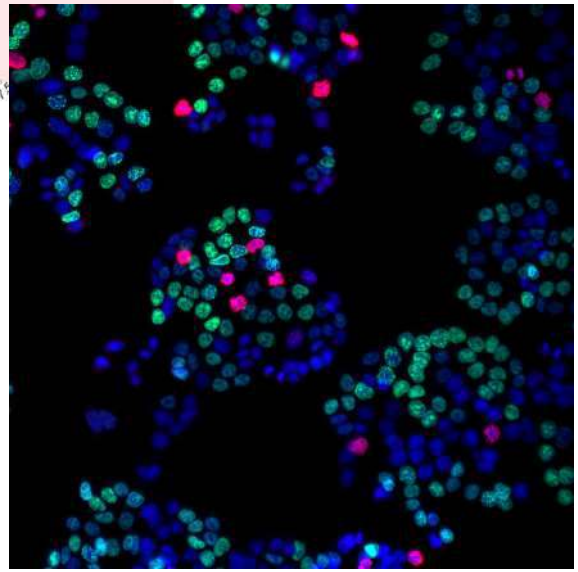
- High content assessment of cell cycle profile (G1, S, G2, M) in adherent or suspension cells.
- Dose response studies





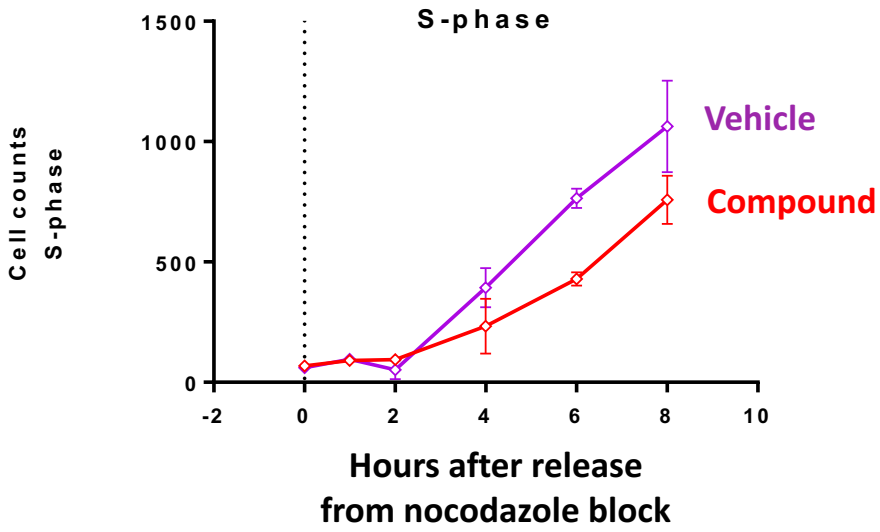


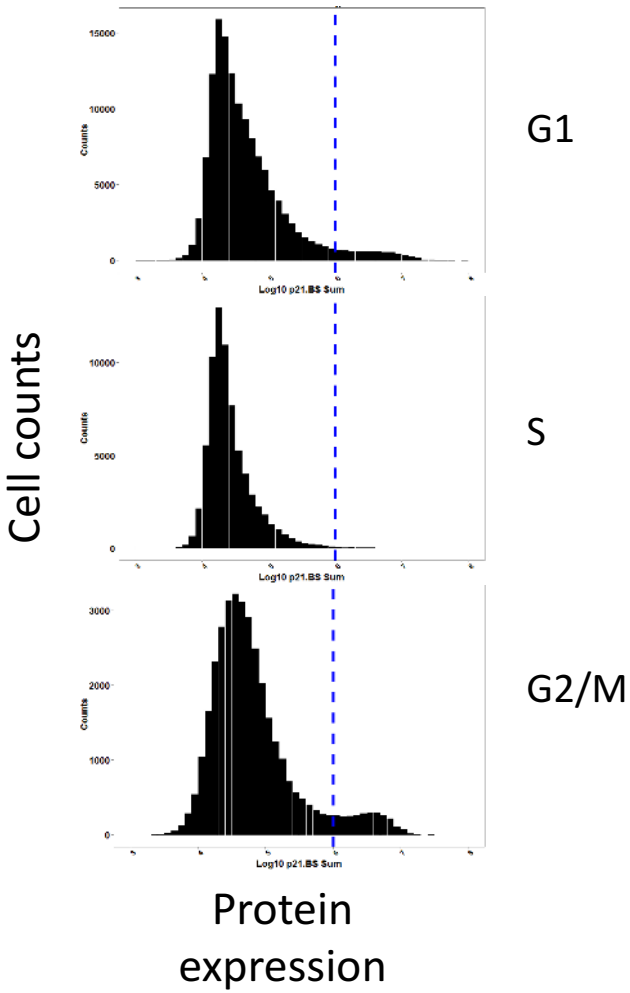




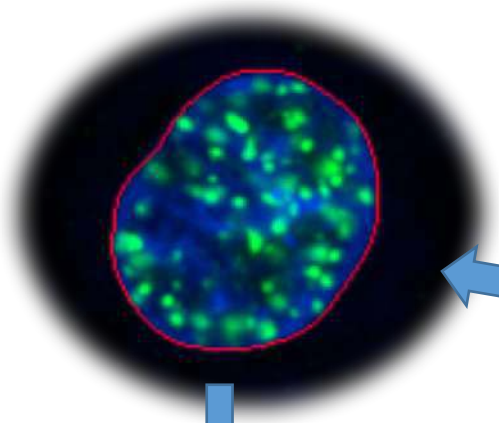
Cell Cycle Analysis

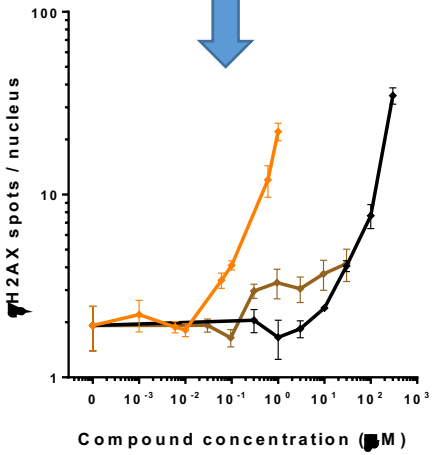
- Multiplexed single cell analysis of cell counts, morphology, protein expression, translocation, or modification, correlated with cell cycle phase analysis.
- Synchronization and kinetic cell cycle progression studies.

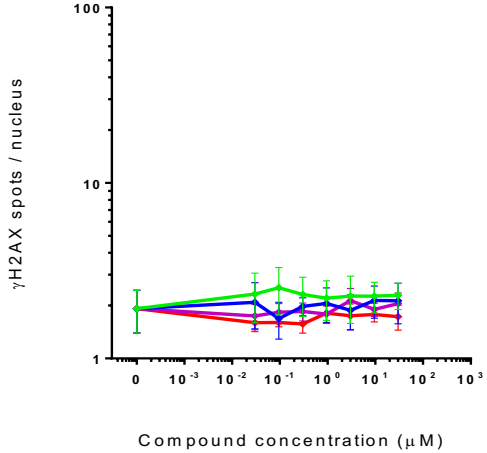


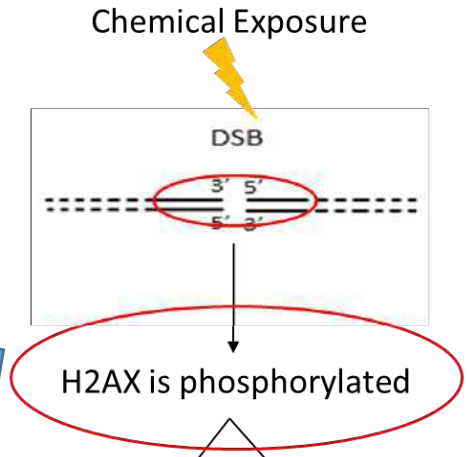


Genotoxicity









DSBs are repaired

DSBs are not repaired

Cell Death

MN formation

Cell Death

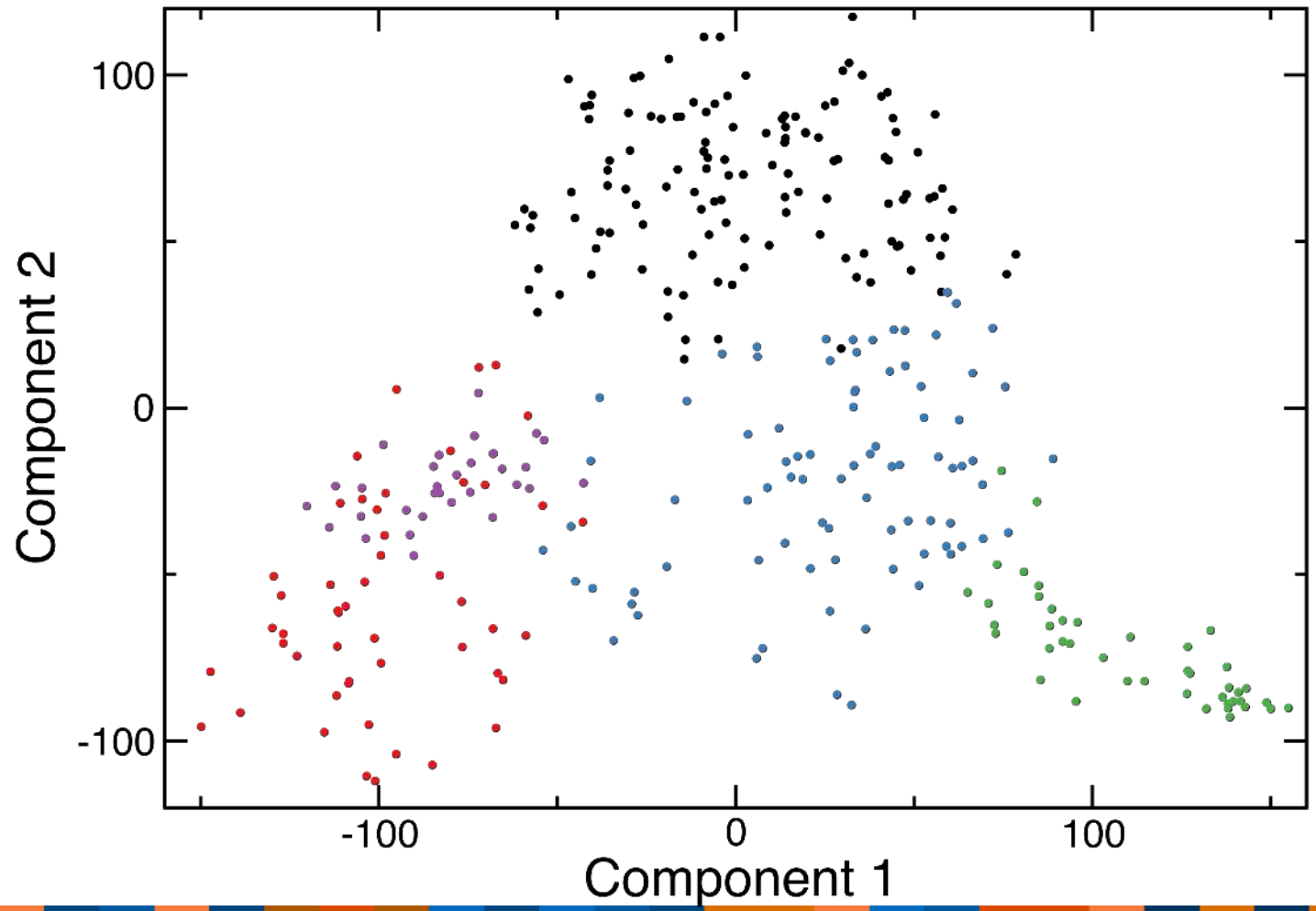
Transformation

- Suite of assay approaches targeting multiple points in the DNA damage and repair sequence in human cells:
  - For the assessment of dose-response, point-of-departure, and adaptive response to chemically-induced DNA damage.

Using genome-wide associations to inform mode of action

- Compound differentially affects hematopoietic and solid tumors
- Can we use a panel of cell lines to advance our understanding of compound mechanism
- What are the genomic characteristics that impact sensitivity of cell lines to the compound of interest?
- Are there mutations, deletions, or amplifications in specific genes, or differences in basal expression, that predict sensitivity, and what do these tell us about the compound's interaction with tumor cells?

Landscape of cancer cell line genetic diversity



Cell line sensitivity to genotoxins depends on basal gene expression and mutation status

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P53 mutation status

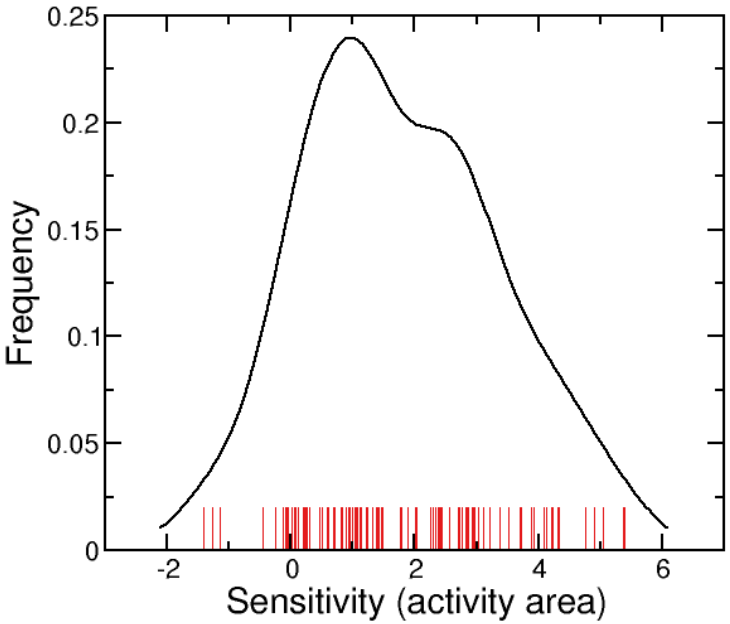
Expression cluster	WT	Mut	Del	Amp	Mut/Amp	Total
0	0.145	0.304	0.25		0.54	0.252
	(70)	(139)	(15)		(1)	(225)
1	-0.674	-0.535	-0.326	0.295	0.171	0.026
	(82)	(80)	(12)	(2)	(1)	(177)
2	-0.674	0.535	-0.326			-0.573
	(48)	(44)	(12)			(109)
3	0.168	0.075	0.123			0.126
	(28)	(22)	(3)			(53)
4	0.161	0.141	0.213	-0.233	1.82	0.169
	(27)	(40)	(3)		(1)	(72)
Total	-0.039	0.072	0.025	0.119	0.843	0.028
	(255)	(330)	(45)	(3)	(3)	(636)

Sensitivity to etoposide, mitomycin C, and bleomycin

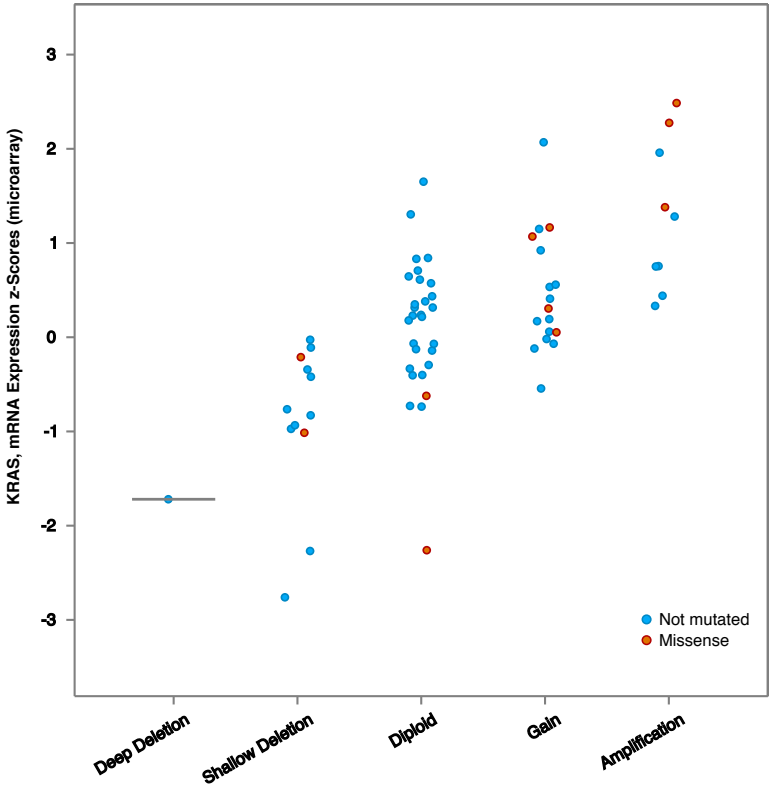
Defining the domain of genetic variability in cell lines

Concentration response for target compound was run in a panel of 82 cancer cell lines with available genotype/expression data

Target compound effect varied broadly in these cells



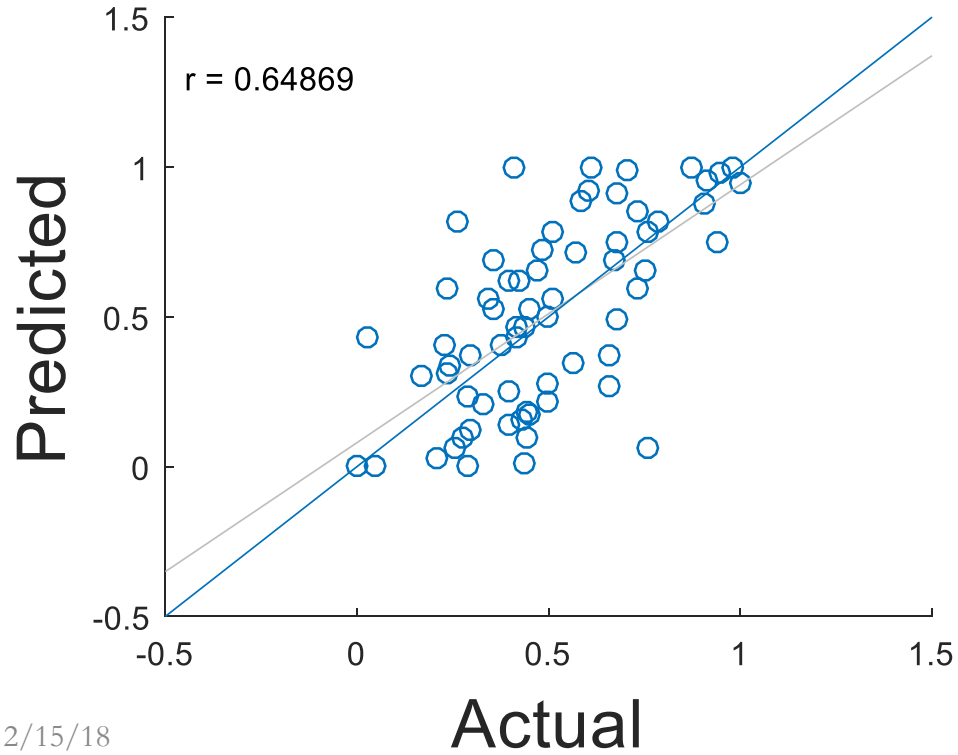
Copy number, expression, and mutation status are interrelated

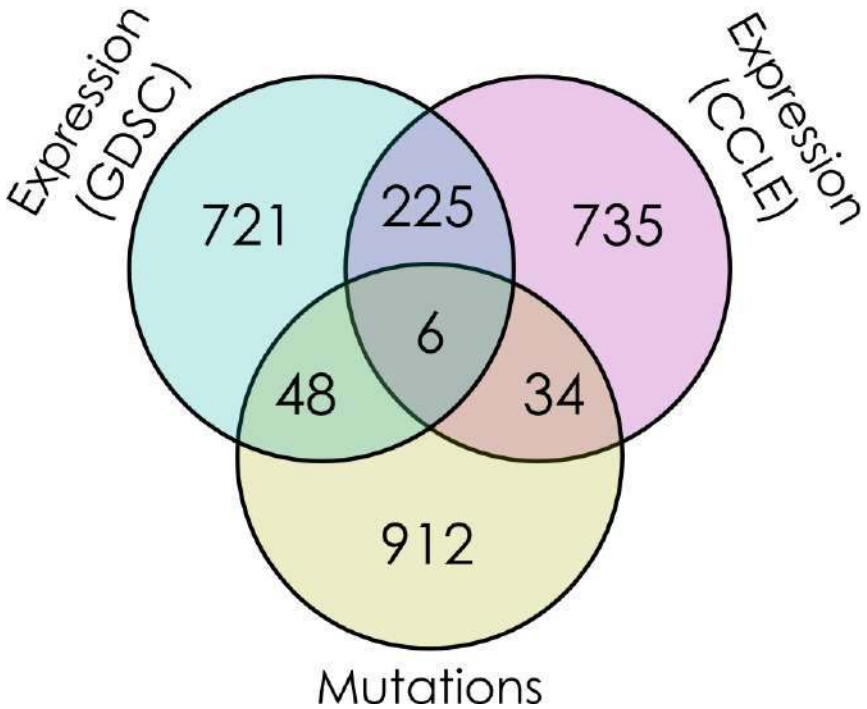


Machine learning to identify genetic features

We used Random Forest regression and Support Vector Machine models to predict the drug efficacy using gene expression and mutation status. Our in house algorithm to find variable importance was used to find the top predictors.

Top predictors included canonical cancer markers and metabolic genes related to the hypothesized mode of action





Acknowledgements

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