Profiling the relative drug sensitivities of varied cell lines simultaneously

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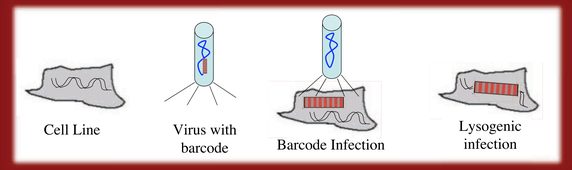
Introduction

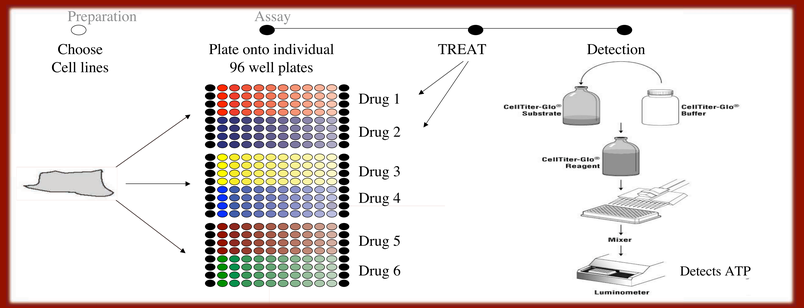
One of the goals of the Cancer Program at the Broad Institute is to determine which chemical compounds target which cell lines, and then to understand the genetic mutations that make these cell lines sensitive. For instance, Gefitinib (Iressa™) targets a particular subset of lung cancer cell lines with an Epidermal Growth Factor Receptor (EGFR) point mutation. A mistake in diagnosis of this particular subset can lead to a possibly fatal lung disease, unusual bleeding, and extreme fatigue. It is therefore imperative to understand which cell lines interact with which compounds. The current approach to find new treatments is to grow up cell lines, treat with 1-2 drug per plates and read cell viability using a traditional method like **Cell Titer Glo** (CTG), which is accurate but low throughput.

Our goal is to develop an accurate high throughput method to observe all the various behaviors between all cell lines and drugs.

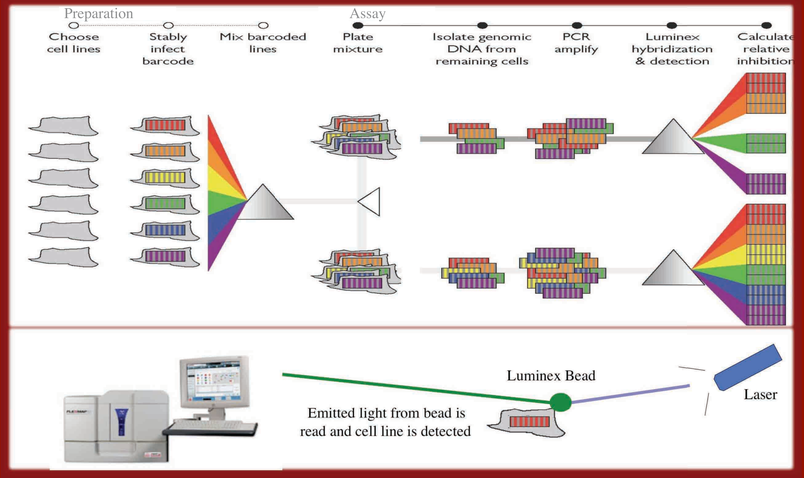
PRISM (Profiling Relative Inhibition Simultaneously in Mixtures)
The PRISM method has been piloted in the Golub Lab on lung and melanoma cell lines. Whereas a traditional method like CTG measures the sensitivity of one cell line at a time, PRISM is a method of measuring individual cell line viability in a mixture of many different cells. Currently, it can read up to 80 cell lines at once using xTAG technology developed by Luminex Inc. By attaching a unique DNA 'barcode' to each cell of each cell lines, Luminex can detect the number of barcodes and measure cell line viability.



Methods



CTG:	Grow individual cell lines	Plate 1 cell line per plate	2 drugs per plate	2 minute protocol
	Culturing	Plating	Treatment	Detection
PRISM:	Grow all cell lines together	Plate all cells in each well	8 drugs per plate	1 day protocol



Materials

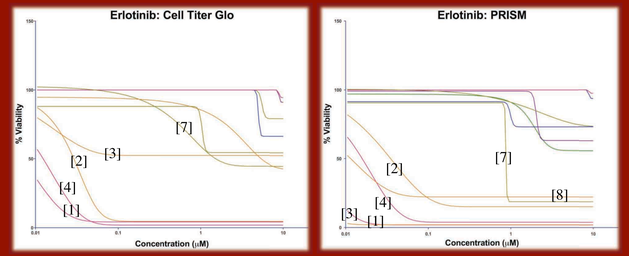
Drug	Target	Expectation
Erlotinib (Tarceva™)	EGFR point mutation	EGFR mutant cell lines should die
Gefitinib (Iressa™)	EGFR point mutation	Similar reaction as Erlotinib
Paclitaxel (Taxol™)	Mitotic Spindles	Target all
Ampicillin	Bacterial Cell Walls	Ineffective on all (Negative control)
Staurosporine	Inhibits ATP binding sites	Target all (Positive control)
Puromycin	Inhibits Translation	Target all (Positive Control)

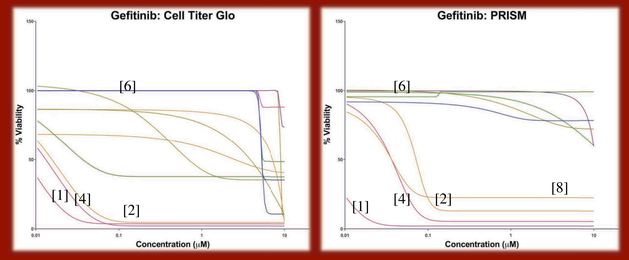
Genetic Background	*Cell Lines	Expectation
*All cell lines are non small cell lung carcinomas		
EGFR Point Mutations	HCC827 [1] HCC4006 [2] HCC2279[3] PC-9 [4] LouNH91 [5]	Should be targeted by Erlotinib and Gefitinib
Copy Number EGFR Mutations	H1975 [6] H820 [7]	Resistant to Erlotinib/Gefitinib
BRAF Mutations	HCC364 [8] H1755 [9]	Should not be targeted by any specific drug
K-RAS mutations	H2009 [10] H460 [11]	Should not be targeted by the drugs (would be targeted by (Erbitux™ & Vectibix™)
Non specific mutations	H23 [12] H1299 [13] DV-90 [14]	Specific drugs should not kill these

Results & Discussion

Accuracy
The PRISM method was 84% as accurate as the CTG method. Accuracy was defined by comparing the IC50 values of both the PRISM method and CTG method, setting a cut off value for sensitivity, and measuring the correlation between both CTG and PRISM. For these purposes, we assumed that CTG was 100% accurate, which is unlikely.

Efficiency
The PRISM method was much more efficient. During a pilot study of lung cancer cell lines, the PRISM method used only 5 plates to measure 80 cell lines and 40 drugs in 11 serial dilutions. It would have taken Cell Titer Glo approximately 1600 plates to produce the same amount of results.





Erlotinib
- EGFR Point Mutation Cell Lines [1], [2], [3], and [4] all showed a sensitivity to Erlotinib defined by an IC50 less than 0.1μM. (Cell line [5]'s IC50 could not be calculated due to its very large standard of error.)

- EGFR Copy Number Mutation Cell line [7] showed its resistance to Erlotinib with an IC50 of about 0.9μM in both the PRISM and CTG assays.

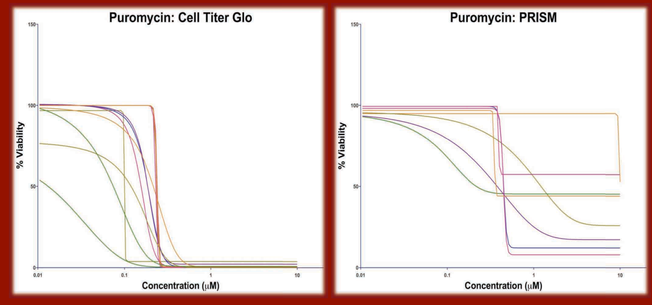
Gefitinib
- EGFR Point Mutation Cell Lines [1], [2], and [4] all showed a sensitivity to Gefitinib defined by an IC50 less than 0.1μM. (Cell Lines [3] and [5]'s IC50's could not be calculated due to their very large standards of error.)

- EGFR Copy Number Mutation Cell Line [6] showed resistance to Gefitinib in both the PRISM and CTG assays.

Erlotinib and Gefitinib

- The BRAF Mutant Cell line [8] showed a sensitivity to both Gefitinib and Erlotinib which seemed contradictory as this mutation is supposed to confer resistance. This only occurred in the PRISM assay.

A Remaining Challenge
- Puromycin IC50 values are documented between 0.1μM and 0.5μM. The CTG method accurately measured these values, while Luminex measured a wider and higher range of IC50 values.



Conclusions
- Currently, PRISM is best used for a rough estimation of cell line viability following drug treatment.

- PRISM detects sensitivities; it can be used as a high throughput method to single out certain cell line and drug combinations that could be followed up on using the CTG method or another more accurate method.

Future Applications

The long term goal of developing an efficient high throughput assay for cancer treatments is to:

- **understand how particular drugs target specific classes of mutations**
- **understand how certain groups of drugs respond to certain sets of cell lines**
- **cluster cell lines with similar responses to drugs based on genetic mutations**

Acknowledgements

I would like to thank my amazing mentors, Channing Yu, Joshua Gould, and Griselda Metta Yvone who have taught and guided me throughout the summer. Also a special thanks to the Golub Lab and everybody in the Cancer Program who has hosted me these past weeks. Lastly, I would like to thank Megan Rokop, Kate MacSwain, and Allison Martino for this opportunity and their counsel.