Identification of Effective Genotype-selective Drug Combinations in Melanoma

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Abstract # 5088

ABSTRACT

Resistance and partial responses to individual therapeutic agents are major obstacles in cancer treatment. In certain cases, combination therapies have been shown to be more effective, however systematic approaches to the identification of synergistic combinations of drugs are not well established. We have used a combinatorial screening platform to test an array of small molecule drug combinations. A panel of one hundred fifty agents was curated to include compounds that inhibit common receptors and signaling pathways active in melanoma, and broader groups of conventional cell cycle-active and genotoxic agents. Sixteen-point dose-effect curves were determined. Forty of the compounds exhibiting activity and representing the major classes tested were further analyzed. Three doses each were chosen, and then set up in all pair-wise combinations using a hit-picking robot, and analyzed against cell lines with activated *BRAF*, activated *RAS*, or neither mutation. Several combinations were selectively effective for melanomas containing activating mutations in *BRAF*, but were ineffective for mutant *RAS* melanomas, which were most susceptible to a small group of other drug combinations. Work is underway to validate the effective combinations in animal models, and to mine transcriptome and phosphoproteomic data associated with the cell lines to better predict sensitivity and resistance.

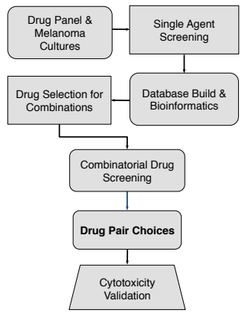
AIMS

1. Determine single and combination drug sensitivities of early passage melanoma cultures to a select panel of agents with known targets.
2. Identify effective combinations and determine mechanisms.
3. Integrate with other analyses of same lines and linked tumor material including resequencing, transcription profiling, phosphoproteomics of signaling molecules to understand pathway interactions and improve drug sensitivity prediction.

ACKNOWLEDGMENTS

Jonathan Haskins for experimental work, Harriet Kluger, Mario Sznol, Karen Anderson, Yung-Chi Cheng, Gil Mor, Rick Bucala for helpful discussions and reagents. Screening conducted at the Yale Center for Molecular Discovery with special thanks to Janie Merkel and Mariya Kolesnikova. Supported by a grant from an Anonymous Foundation to MWB and DFS, the Harry J. Lloyd Charitable Trust (MAH, CGL, JTP, DFS), USPHS R01CA45708 (DFS), and the Yale SPORE in Skin Cancer, funded by the National Cancer Institute grant number 1 P50 CA121974 (R. Halaban, PI). The authors declare no conflicts of interest.

RESULTS



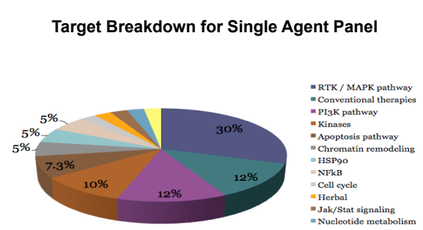


Figure 1. Workflow

- 150 compounds chosen for **single agent screening.**
- Signaling targets are active receptors in melanoma, other potential contributing pathways, also include genotoxic drugs and microtubule-targeted agents. Preference was given to compounds that are FDA-approved, or in clinical trials, or related to such agents.
- 16-point CellTiterGlo dose-effects.
- 27 early passage cell cultures with known *BRAF* and *NRAS* genotypes (Yale SPORE in Skin Cancer).
- 40 agents chosen for **combination screening**: three doses each all 7140 pairwise combinations against 8 *BRAF*, 7 *RAS*, and 5 *RAS*/*RAF* WT lines (Screening at Yale Center for Molecular Discovery).
- Evaluate best hits for cytotoxicity, Chou-Talalay synergy, mechanism.

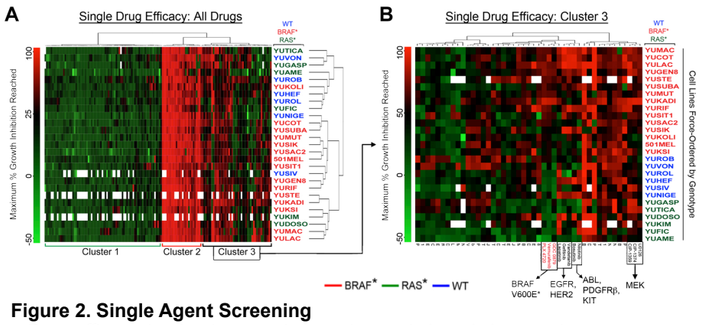


Figure 2. Single Agent Screening

- Drug efficacy (and potency not shown) assorts largely by genotype.
- *RAS* mutants and "WT" are more resistant (Cluster 3, panel B).
- *BRAF* mutants are sensitive to *BRAF* inhibitors as expected.
- Some *BRAF* lines are resistant to *BRAF* inhibitors.

(**A**) Unsupervised clustering heatmap of maximal growth inhibition for each single agent (columns) per melanoma cell line (rows) ranging from -50% growth inhibition (green) to 100% growth inhibition (red) relative to vehicle and high DMSO controls. Cell lines named in red have *BRAF* mutations; green *NRAS* or *HRAS* mutations; blue, wildtype *RAS* and *BRAF*. (**B**) Second round of unsupervised clustering on drugs from Cluster 3 in (A) to survey for genotype-associated biases. Cell lines are ordered by genotype.

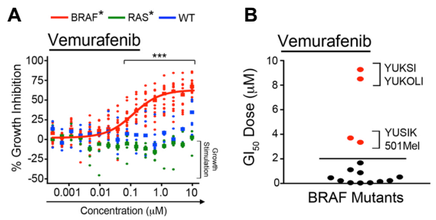
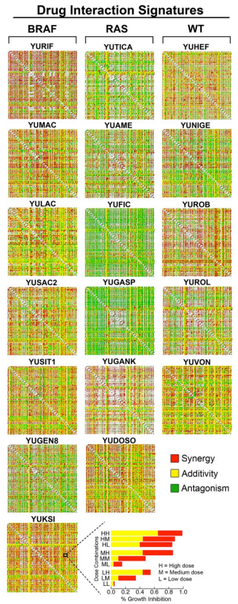


Figure 3. Single Agents: Vemurafenib

- Genotype-selective response to Vemurafenib, including growth stimulation with *wtBRAF*.
- Vemurafenib-resistant *BRAF* mutant lines (Panel B, Red).

(**A**) Dose-effect curves fitted to median growth inhibition values for Vemurafenib in the mutant *BRAF*, mutant *NRAS*, and *wtBRAF*/*wtRAS* genotypic groups. Bracket indicates the dose range of significant differences in drug potency; p<0.0001, Kruskal-Wallis test. (**B**) Comparison of Vemurafenib doses required to induce 50% growth inhibition (GI50) in mutant *BRAF* melanoma lines. Red points indicate cell lines with intrinsic resistance to Vemurafenib.

Figure 4. Combination Signatures



- Identification of candidate synergistic interactions

Drug interaction signatures for each cell line representing all combinatorial data compiled in a 40 drug by 40 drug matrix. **Inset at bottom**: Magnified view of the nine dose combinations for a representative drug pair within its drug interaction matrix, to indicate scale. Yellow bar is the Bliss additive sum of growth inhibition for single agents; red indicates synergy, and green, antagonism. Note common antagonism (sub-additive drug impact) for *RAS*-mutant lines.

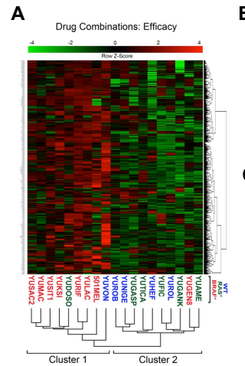


Figure 5 Combination Efficacy

- *BRAF* mutants are sensitive to many more combinations (Cluster 1).
- Some combinations are effective on mutant *RAS* and/or WT.
- One partner predominates for *RAS* mutants.

(**A**) Unsupervised clustering of maximal growth inhibition out of nine combinations tested for each cell line (columns) for each unique drug pair (rows). (**B,C**) Frequency of agents that in pairwise combinations elicit GI25 or higher effect level and >15% growth inhibition in mutant BRAF group (B) or RAS group (C) relative to others.

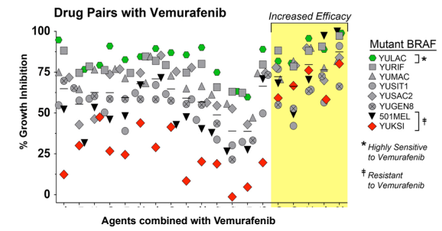


Figure 6. Vemurafenib Combinations

- Several drugs paired with Vemurafenib are effective on intrinsically Vemurafenib-resistant *BRAF* mutants (red, black).

Maximal percent growth inhibition of mutant BRAF lines for vemurafenib combined with other agents. YUKSI (red) and 501Mel (black) are the most intrinsically resistant to Vemurafenib, and are also less sensitive to combinations. YULAC is most sensitive to Vemurafenib combinations. Yellow highlight marks Vemurafenib combinations that inhibited resistant lines YUKSI and 501MEL

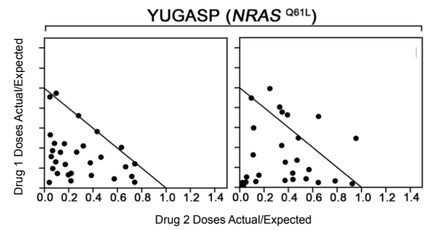
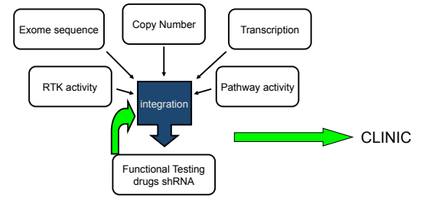


Figure 7. Synergy

- Chou-Talalay isobologram analysis confirms synergy for subset of combinations.
- Flow analysis (not shown) verifies cytotoxicity.

Two different drug combinations shown for *NRAS* mutant. Chou-Talalay isobologram analysis for testing drug synergy. Data are normalized, with connecting line at X = 1 and Y = 1 corresponding to the line of additivity. Datapoints falling below line are synergistic, along or near the line are additive, and above the line are antagonistic. Data represent averages for three separate experiments.

SUMMARY



- 150 single agents (16 dose points) 27 early passage melanoma cultures.
- 40 agents (3 dose points) 7140 pairwise combinations 18 early passage melanoma cultures.
- Effective genotype-selective combinations identified, subset verified for cytotoxicity/synergy.
- Impact for Vemurafenib-resistant *BRAF* mutants, and treatment-resistant *NRAS*

ONGOING

- Mechanisms effective combinations.
- Xenografts best combinations.
- Integration with extensive sequencing, transcriptional, phosphoproteomic data for these cell lines and associated tumors for...
  - Target/pathway interactions
  - Predicting sensitivity and resistance