

signaling and other "targeted" drugs work

Trastuzumab, Imatinib, Erlotinib, PLX4032, Olaparib...

responses are not durable

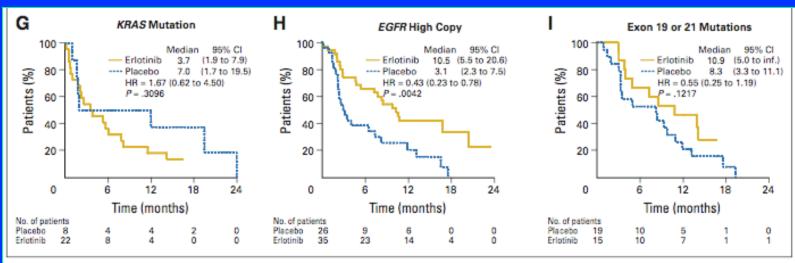
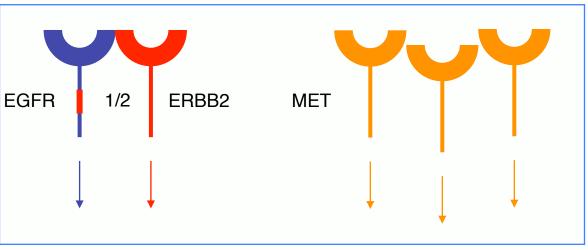
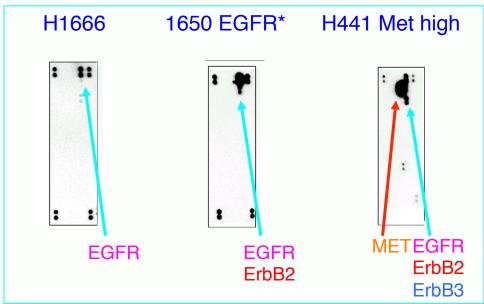


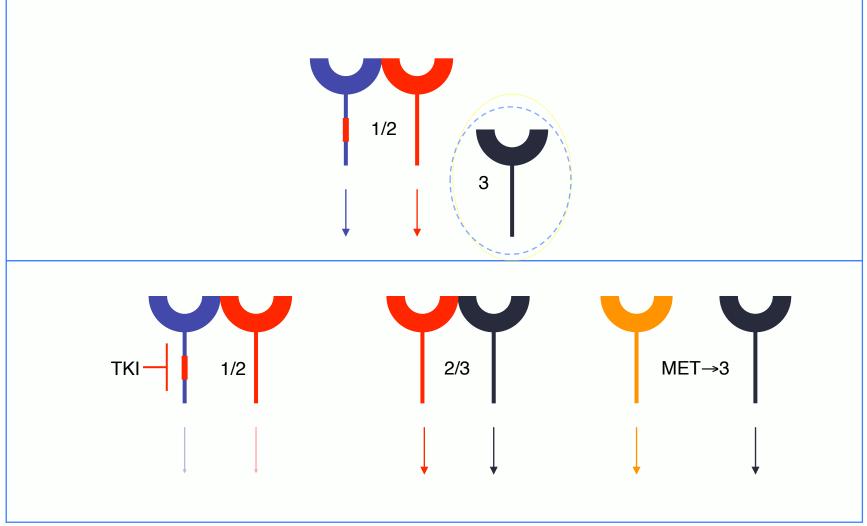
Fig 1. Kaplan-Meier survival curves of subgroup studied for various markers as well as impact of erlotinib according to marker status. HR, hazard ratio.

Multiple Drivers





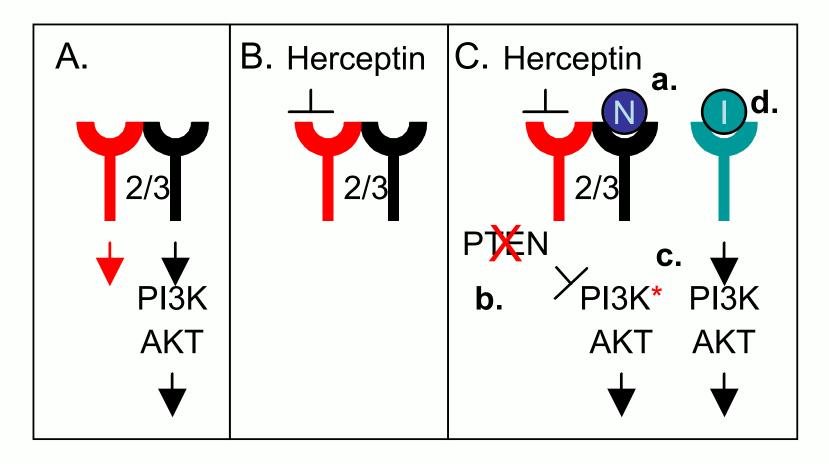
Receptor Bypass



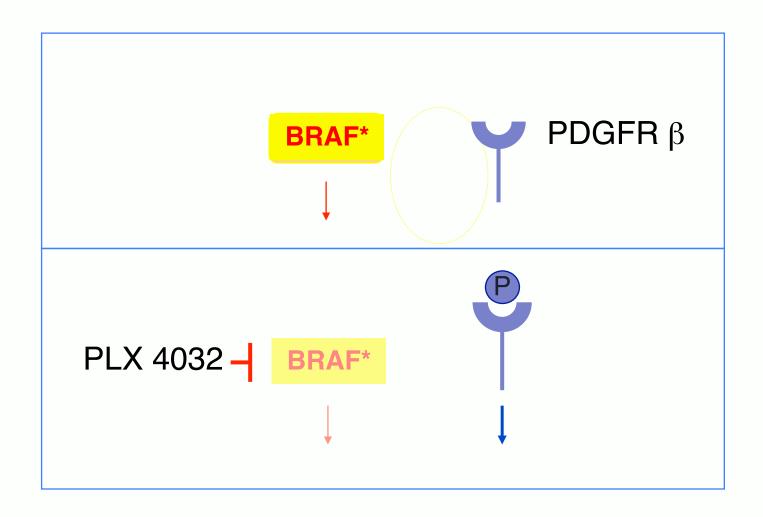
Ligand Bypass



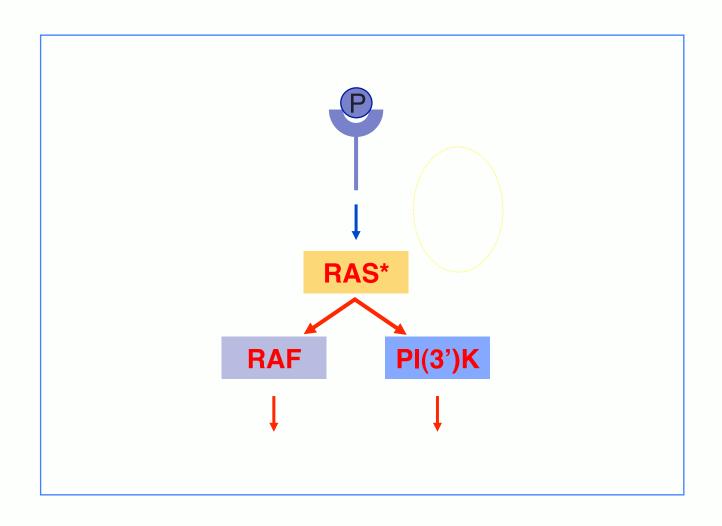
Pathway Resistance



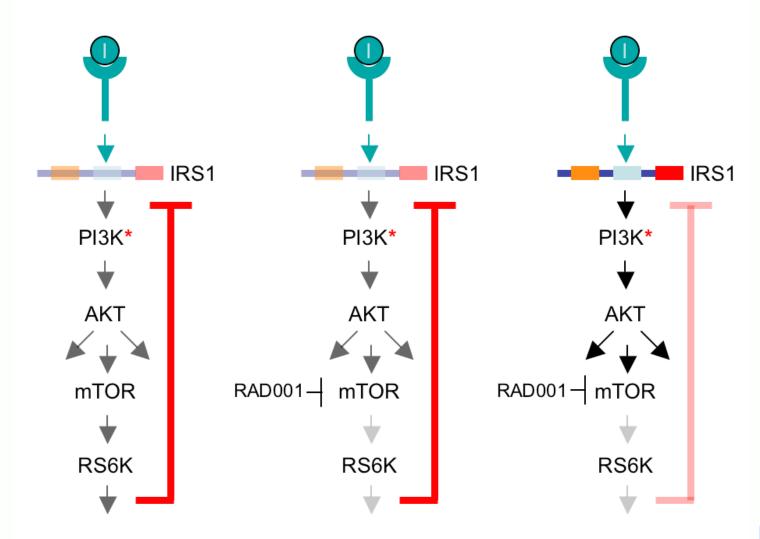
Autocrine Bypass



Pathway Bifurcation



Homeostatic Feedback Mechanisms



SURGERY

CHEMOTHERAPY

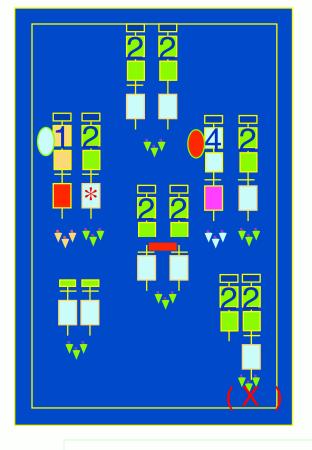
RADIOTHERAPY

SIGNALING

IMMUNOTHERAPY

DNA DAMAGE

IMMUNOMODULATION

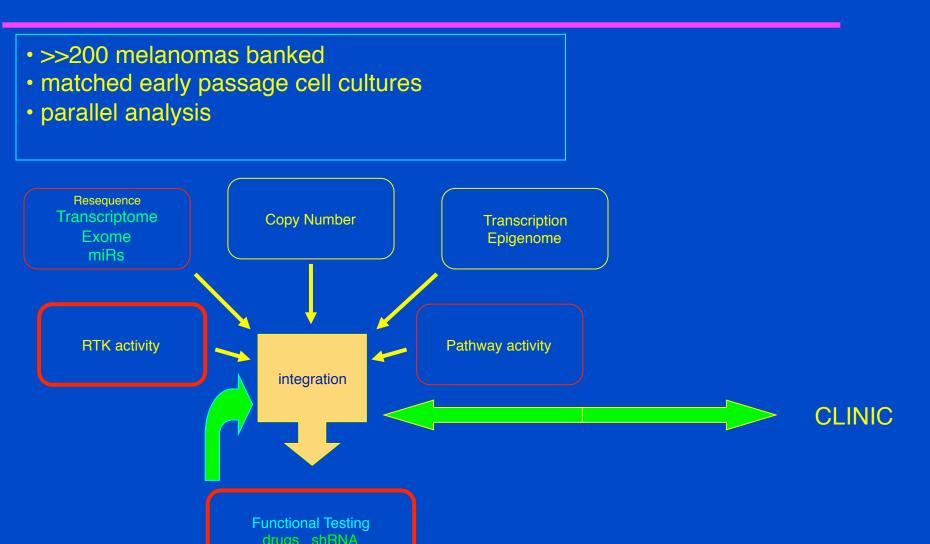


DNA/cDNA sequencing

Copy Number
Transcription
SNP
Substitutions, InDels
DNA Rearrangements
microRNA
Epigenetics
proteomics

identify driver(s)
identify patterns of sensitivity
identify drug sensitivity signatures
anticipate drug resistance
learn how to kill cancer cells
distill for clinical practice

Yale SPORE in Skin Cancer Ruth Halaban, PI



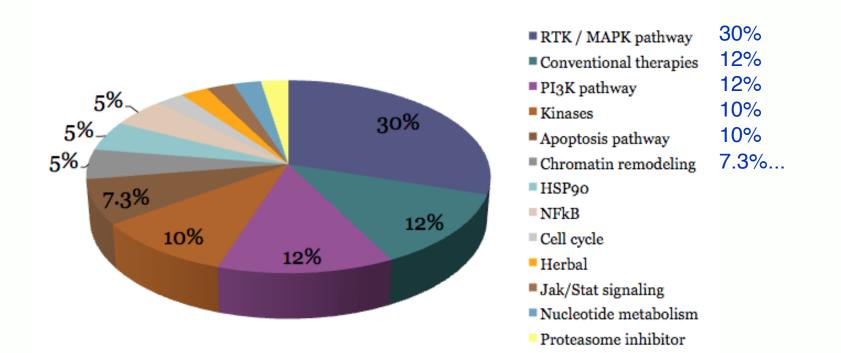
Single agent screening MARCUS BOSENBERG

- Single agent screening
 - 153 compounds
 - 16 point dose response curves
 - 26 human melanoma lines, two breast, mouse
- clustering for patterns of single agent dose responses
- integration with SPORE data to...
 - identify genotype-based sensitivity
 - identify biomarkers predictive of response or resistance

Single agent screening

Yale Center for Small Molecule Discovery

- 153 compounds
- 16 point dose response curves in triplicate
- 3 day assays: CellTiterGlo
- 26 human melanoma lines, two breast, mouse



2 EGFR 2 EGFR/HER2 3 FGFR 3 IGF1R 4 MET 5 FGFR, VEGF, KIT 5 AXL, Src.JAK, 5 Kit, P, Abl 5 PDGFR/VEGFR 5 Abl/Src 5 Abl/Src 5 VEGFR/EGFR 5 VEGFR/PDGFR/k 5 VEGFR, Kit, PDGF 6 JAK2 6 Stat3 6 Stat3 8 p38 9 Jnk 10Raf 10 Raf* 10 MEK 10 MEK 10 MEK 12 Chk 11 Akt 11 Akt 11 mTOR 11 mTOR NFKb, mTOR 11 PI3K,pentose 12 kinases 12 Rho kinase 13 HSP901 12 Aurora 12 GSK3beta 12 CDK4 12 CDK 12 CDK 14 TLR 14 NOTCH 14 ER 14 RXR 14 RAR 14 Hedge Hog 14 Hedge Hog 14 Cox2 14 MIF 14 Bol-2 inhibitor 14 Bcl-2 inhibitor 14 proteasome

21 microtubule

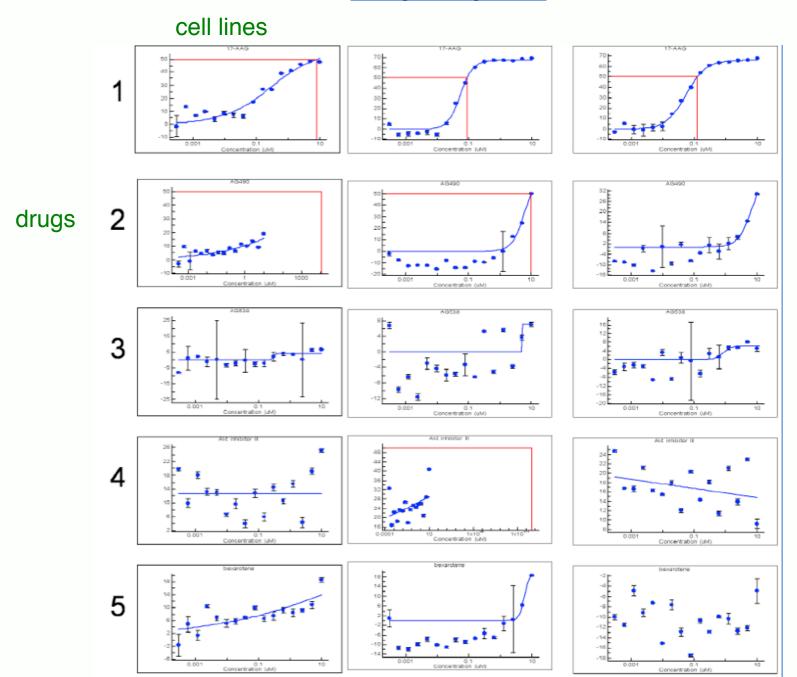
21 microtubule

21 microtubule 21 microtubule 22 HDAC

22 SAHA HDACI 22 5-AzaC

1 ALK

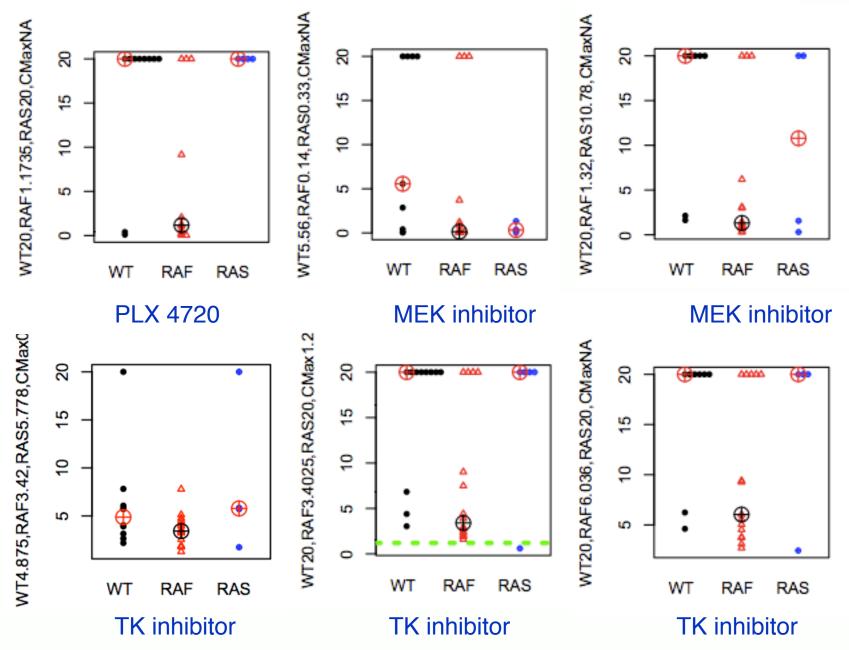
Single Agents



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Does drug sensitivity assort with Genotype? ${\tt uM\ conc.\ 50\%\ Growth\ Inhibition\ by\ Genotype}$



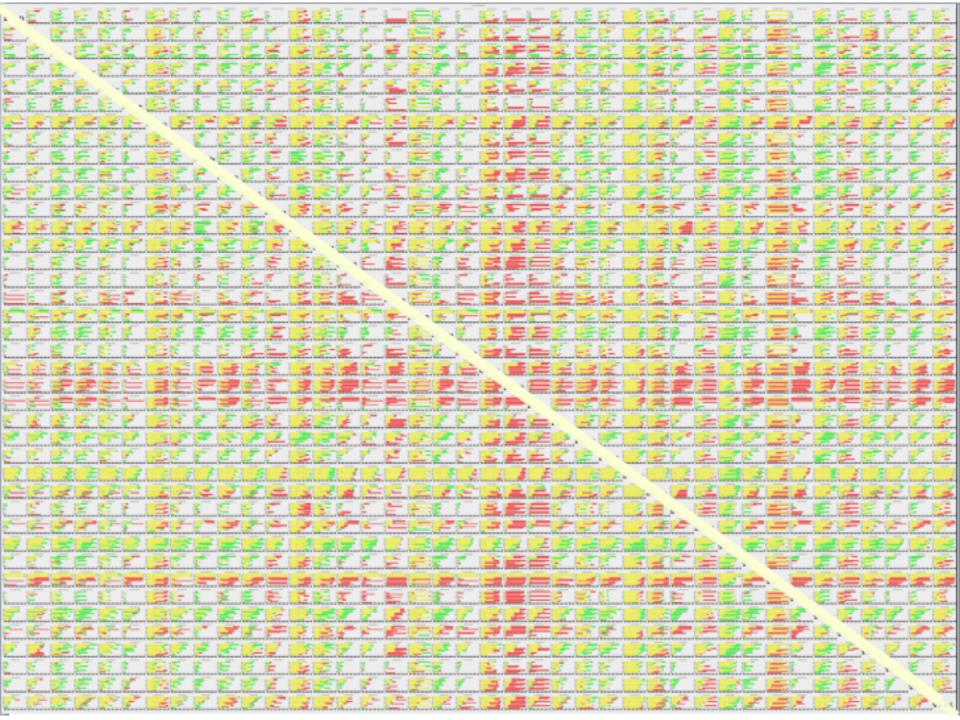
combination screening

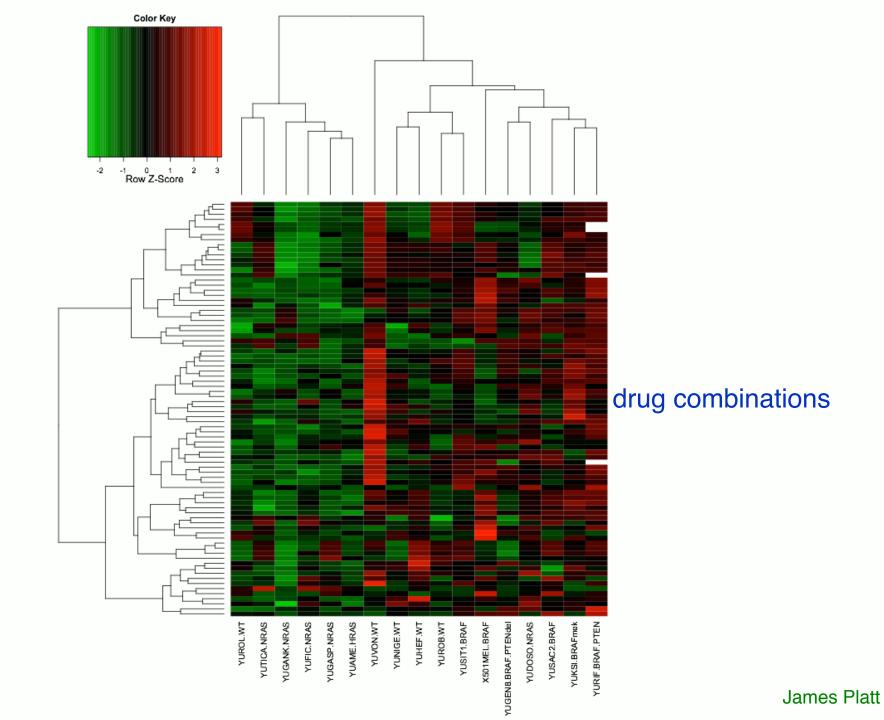
Yale Center for Small Molecule Discovery

- Identify potentiators of...
 - » maximal effect
 - » dose-dependence
 - » cell killing
- drugs
- interacting pathways

Combination testing

- 40 compounds at 3 concentrations
- BRAF, RAS, WT
- filter data to detect:
 - Synergy
 - Selective effect (genotype based)
 - Completeness of effect
 - Antagonistic effects
 - Enrichment for one agent in combos



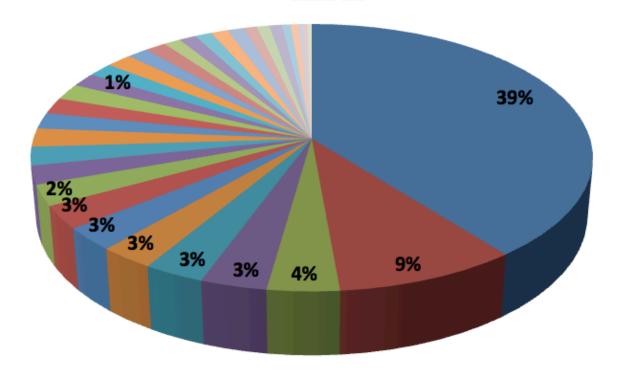


Drug	#times in a combo
A	141
В	33
B C	13
D	12
E	11
F	10
D E F G	10
H	10
1	9
J	8
K	8
L	8 7 7
M	7
N	
0	7
Р	6
Q R S	6
R	6
S	5
Т	5
U	4
V	4
W	4
T U V W X Y Z	4
Υ	4
Z	3
AA	3
BB	3
CC	2
DD	3 3 2 2 1 1 1
EE	1
FF	1
GG	1

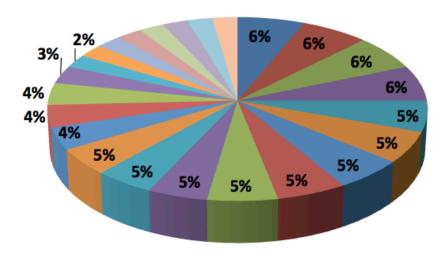
filters for.. threshold combination effect threshold maximum effect internal consistency of combination data points < CMax

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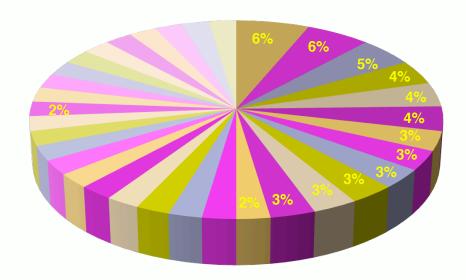
Frequency drug appears in a combination selective for NRas mt lines



Frequency of combinations selective for <u>NRas mt</u> lines (at least 3 combinations as cutoff)

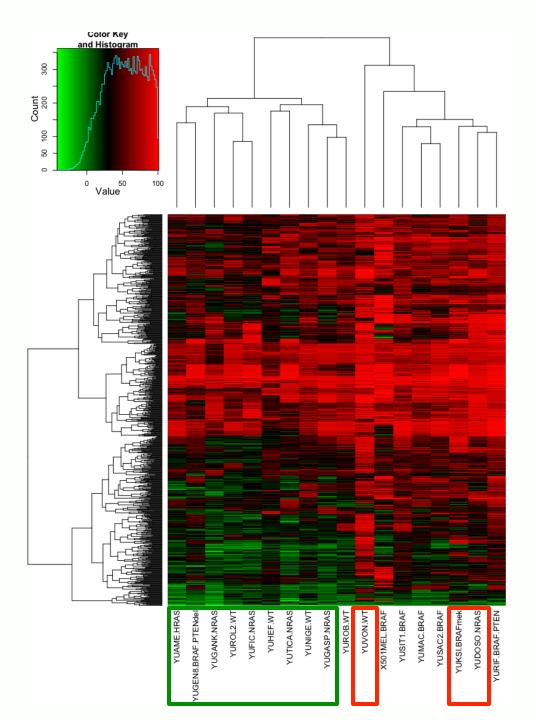


Frequency of combinations selective for BRaf mt lines (at least 3 combinations as cutoff)



What accounts for variable sensitivity?

uM conc. 50% Growth Inhibition by Genotype WT20,RAF1.1735,RAS20,CMaxNA WT20,RAF1.32,RAS10.78,CMaxN WT5.56,RAF0.14,RAS0.33,CMaxN 20 8 8 5 5 5 \oplus 10 9 9 5 S 2 0 RAS WT RAF RAS WT RAF RAS WT PLX 4720 **MEK** inhibitor **MEK** inhibitor WT4.875,RAF3.42,RAS5.778,CMax0 WT20, RAF3.4025, RAS20, CMax 1.2 WT20,RAF6.036,RAS20,CMaxNA 20 20 20 15 15 15 9 9 10 **(1)** 2 2 2 RAF RAS WT **RAS** WT RAF RAS **RAF** WT TK inhibitor TK inhibitor TK inhibitor



Resistance Signature: Supervised clustering transcription profiles resistant vs Sensitive WT/NRAS

Summary

ongoing:

- cells from defined mouse models (Marcus Bosenberg)
- connect to phosphoproteomic data
- link to exome sequencing, CN, ...
- animals

for...

- mechanism
- apoptosis
- pathway/target interactions
- predict sensitivity

Rationale:

Scientific challenges for single signaling therapies

Resilience through transcriptional routes (autocrine circuits)

Resilience through intra-pathway feedback and inter-pathway cross-talk

Pathways have multiple efferents

Redundant drivers (EGFR + MET amplification)

Bypass mutations (PTEN)

Rapid routes to resistance

Genetic and epigenetic plasticity

On-target mutations

Bypass mutations

Tumor cell population heterogeneity

Ignorance about...

Inducing tumor cell kill

Rationale:

Scientific **opportunities** for signaling therapies

Feedback/network responses to signaling perturbations

Crosstalk of signaling, DNA damage responses, cell cycle regulation

Best combinations of pathway inhibition

In vitro and in vivo assay development

Epigenetic regulation, esp. for reactivation TS genes

Combinations with other modalities...

DNA damage agents/Radiotherapy with repair inhibitors Immunomodulation to complement other approaches Immunomodulation in partnership with Ab therapies

Practical Challenges/opportunities for investigation

Academic role

- Cancer biology/genetics
- Target identification (molecules, pathways, interactions)
- Pharmacology, medicinal chemistry

Access to compounds

- IP
- Federal: NCI DTP, Experimental Therapeutics Program (NExT)
- Pharma: on-target but shelved compounds
- si/shRNA for target selection

Practical Challenges/opportunities for investigation

Scale: exponential nature of combination screens

- Focus on targets/pathways rather than drugs
- Hypothesis: know the target and relevant pathways
 - e.g. Trastuzumab to dual and pan-ErbB
 - · e.g. immunomodulation
 - e.g. PARP inhibitors X BRCA1
- How do effective therapies work?
 - Trastuzumab, Lapatinib
- Sensitization screens vs. single challengers
- Genetic and functional information about co-activation

7. What lessons have been learned, and what would you recommend to improve?

Still much basic and applied science remains

interactions of target pathways

apoptosis

patterns of drug response

patterns of drug resistance

interpreting transcriptional phenotypes

feedback pathways

Toxicology

Patient selection

- Mapping t.c. and animal models onto clinical predictions
- Clinical: rebiopsies, neoadjuvant models
- Monitoring: biopsy, CTC for tumor burden

9. Is there a role for precompetitive collaboration for development of combination Cancer therapies?

- Overall alignment of Academic, Pharma, and NIH goals
- But...

IP, proprietary information, credit, priorities

- Government: economy of scale, drug and shRNA libraries
- Bidirectional interaction Pharma and Academia

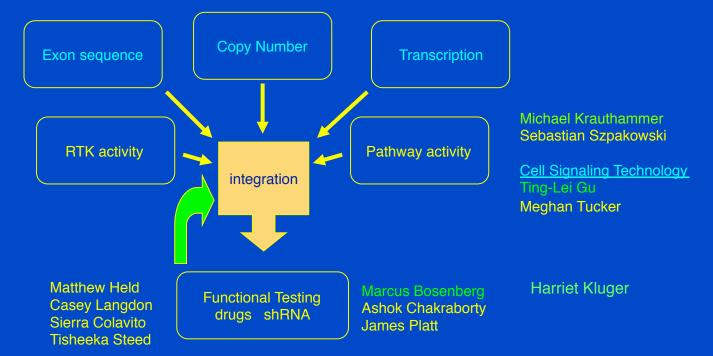
Yale SPORE in Skin Cancer Ruth Halaban, Pl

M. Sznol, R.Tigelaar co-PI

Antonella Bachiocchi

Kat Tworkoski Garima Singhal Tina Zito

Rahul Dalal Betsy Cowell



Roslyn and Jeremy Meyer Fund Harold J. Lloyd Trust Anonymous

Yale Small Molecule Discovery Center
Janie Merkel

Michael Salcius Mariya Kolesnikova