

NCI-60 Response Profiles of >400 Investigational Oncology Agents: A Resource Enabling Drug and Biomarker Discovery

Joel Morris¹, Mark Kunkel¹, Eric Polley², Susan Holbeck¹, Kazimierz Wrzeszczynski² Anne Monks³, David Evans³, Annamaria Rapisarda³, Jerry Collins¹, and Beverly A. Teicher^{1,3} ¹Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Rockville, Maryland 20852; ²Biometric Research Branch, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Rockville, MD 20852; ³Molecular Pharmacology Branch, SAIC-Frederick, Frederick National Laboratory for Cancer Research, Frederick, Maryland 21702

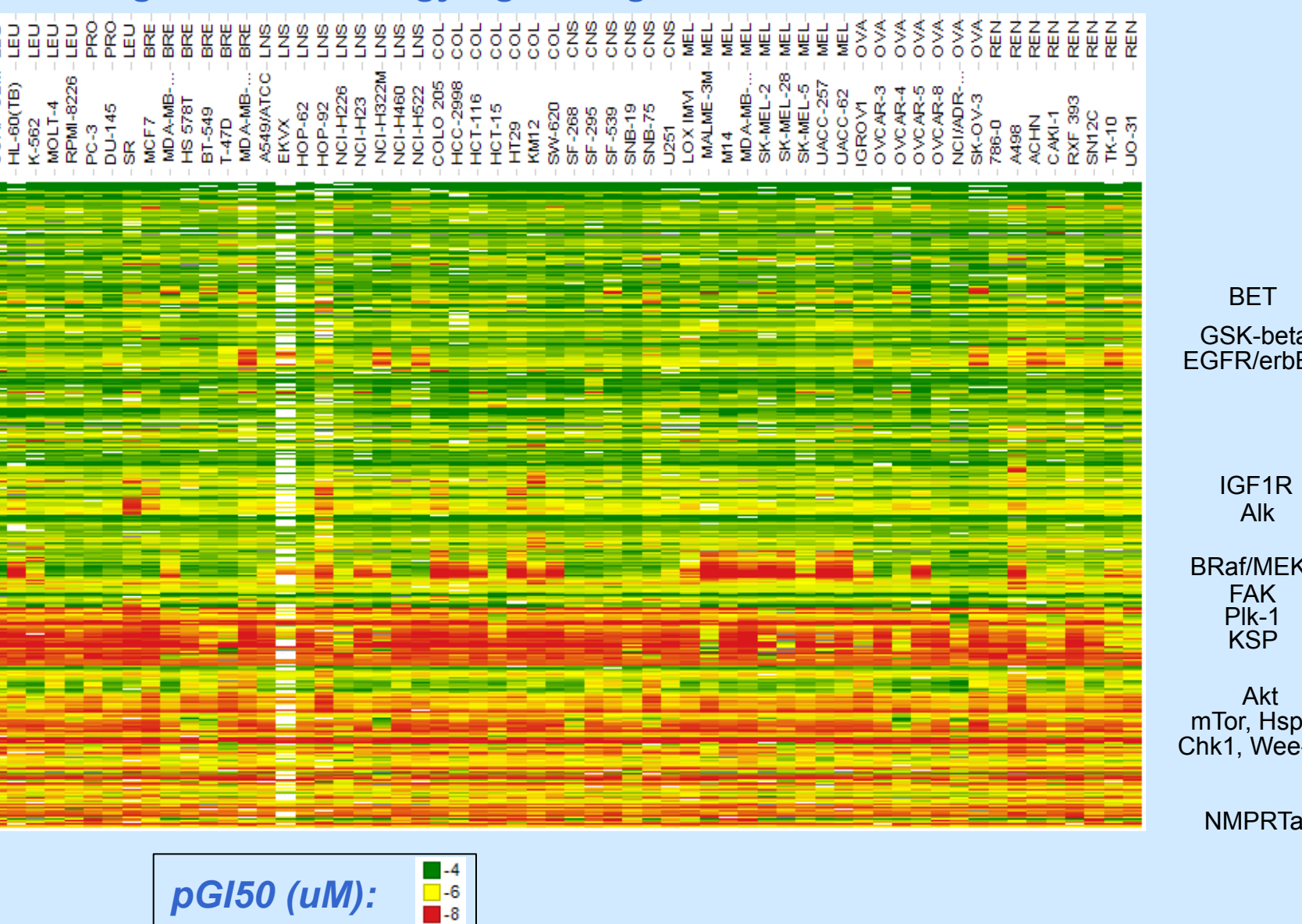
Abstract

We have acquired >400 investigational oncology agents, comprised primarily of targeted small molecules currently in clinical and/or preclinical anticancer studies. As oncology treatment moves toward personalized targeted therapeutic agents, the NCI-60 human tumor cell line panel is an ideal community-wide tool to further understanding of the disease targets of new agents. The panel includes cell lines from nine tumor types, and is extremely well characterized at the molecular level, with both in-house and crowd-sourced characterization, including exome sequence for mutations, SNPs, DNA methylation, metabolome, mRNA, microRNA, and protein expression. This molecular characterization dataset enables interrogation of patterns of growth inhibition by the investigational drug set looking for characteristics of the cell lines that determine sensitivity. More than 150,000 small molecules, including all (> 100) FDA-approved anticancer drugs¹ and now our acquired set of 400 investigational oncology agents have been screened against the panel for their effects on cell growth. We have used a number of online tools to enable data analysis for this set, including COMPARE (<http://ntp.nci.nih.gov/compare/>), which provided for the identification of compounds and/or genes that have highly correlated response patterns for any selected 'seed' compound.² This presentation provides the first public disclosure of the NCI-60 data for this set of novel, targeted, investigational oncology agents. We anticipate that these data will enable comparison between drug sensitivity profiles that could lead to the elucidation of common mechanistic targets or pathways, associations with potential response biomarkers, the confirmation of mechanism of action or identification of novel mechanisms, and the uncovering of unexpected "off-target" activities. For example, Akt, p13K, PDK, and mTOR inhibitors, multiple agents targeting one signaling pathway, display strong correlations with one another. Using the allosteric Akt inhibitor MK-2206 as the seed compound, response patterns for the ATP-competitive Akt inhibitors PF-4173640 (0.84), GDC-0068 (0.80), AZD-5363 (0.83), GSK-690693 (0.67), and CCT-128930 (0.69) are highly correlative. Moreover, the NCI-60 response pattern for the androgen receptor modulator AZD-3514 has a 0.77 correlation with the BET bromodomain inhibitor JQ-1, suggesting a commonality of target/pathway for these compounds.

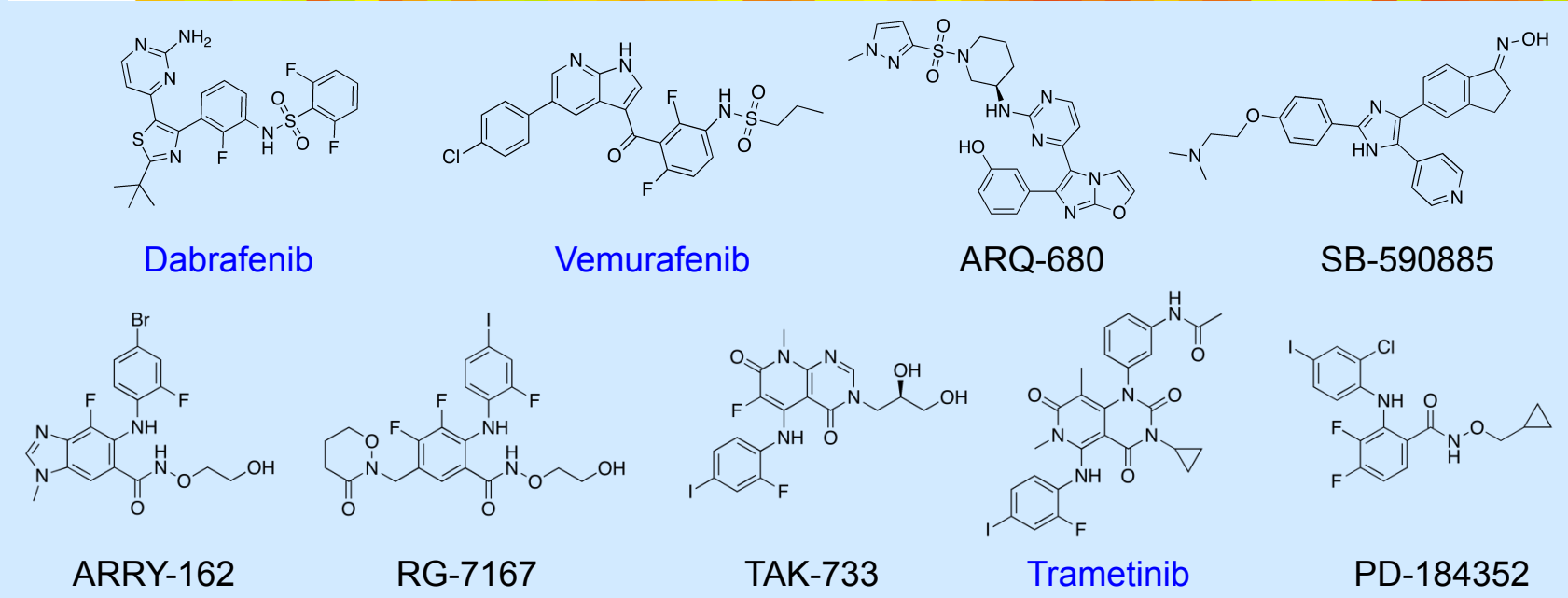
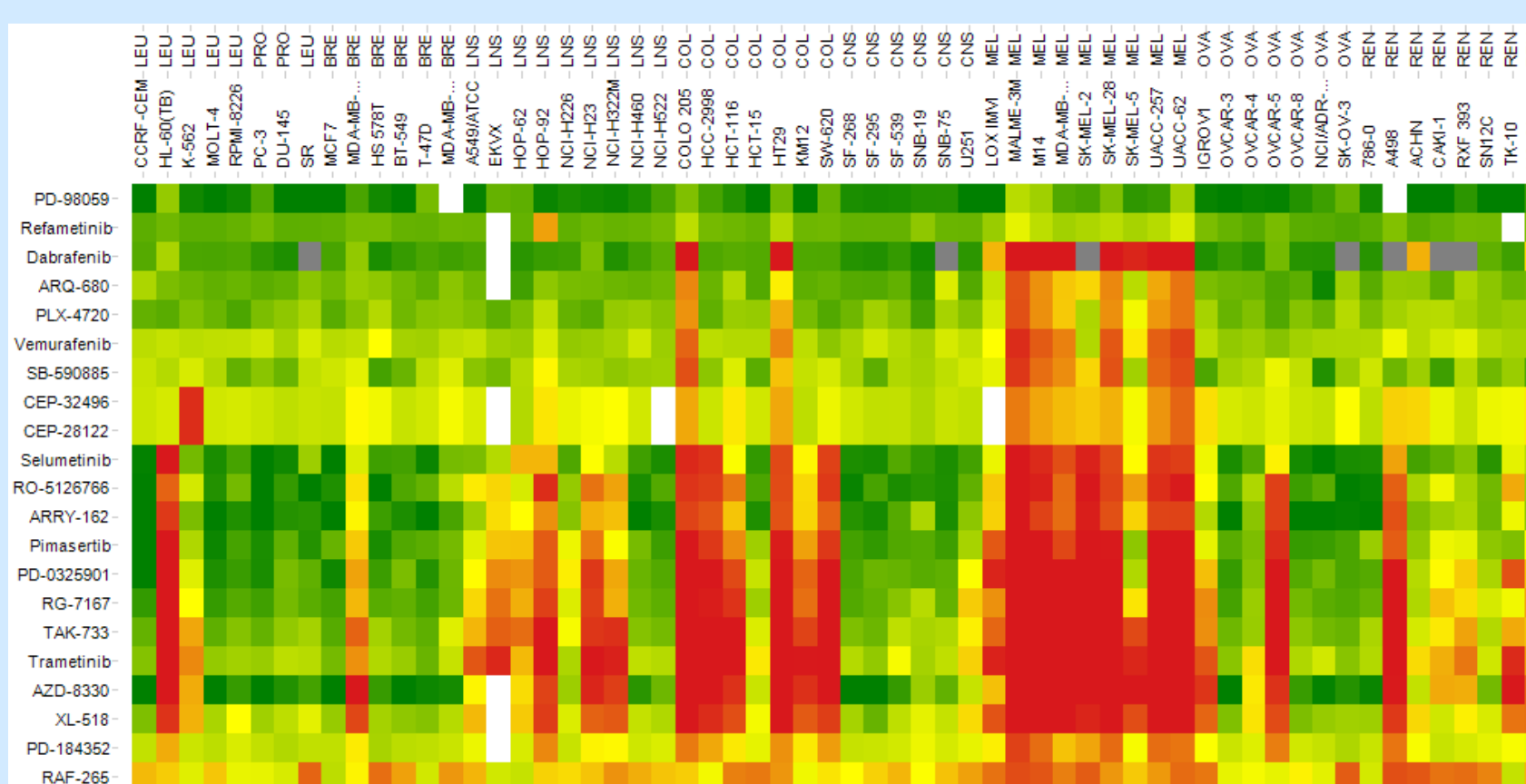
Methods and Materials

The NCI-60 human tumor lines are grown in RPMI 1640 medium containing 5% FBS & 2 mM L-glutamine. For experiments, cells are inoculated into 96 well plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual lines. The plates are incubated at 37° C, humidified 5% CO₂, 95% air for 24 h. After 24 h, two plates of each cell line are fixed with TCA, for the time zero read (Tz). Compounds are dissolved in DMSO. For compound addition, the solution is diluted to twice the desired final max test concentration with complete medium, 1 log dilutions are made & 100 µl are added to the wells. The plates are incubated for 48 h. For staining, sulforhodamine B (SRB) solution (100 µl) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at rt. The SRB is solubilized, & the absorbance at 515 nm is read. Using the absorbance measurements [time zero, (Tz), control growth, (C), & test growth (T)], the % growth is calculated. Growth inhibition of 50% (GI₅₀) is calculated from [(T-Tz)/(C-Tz)] x 100 = 50, which is the compound concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells.

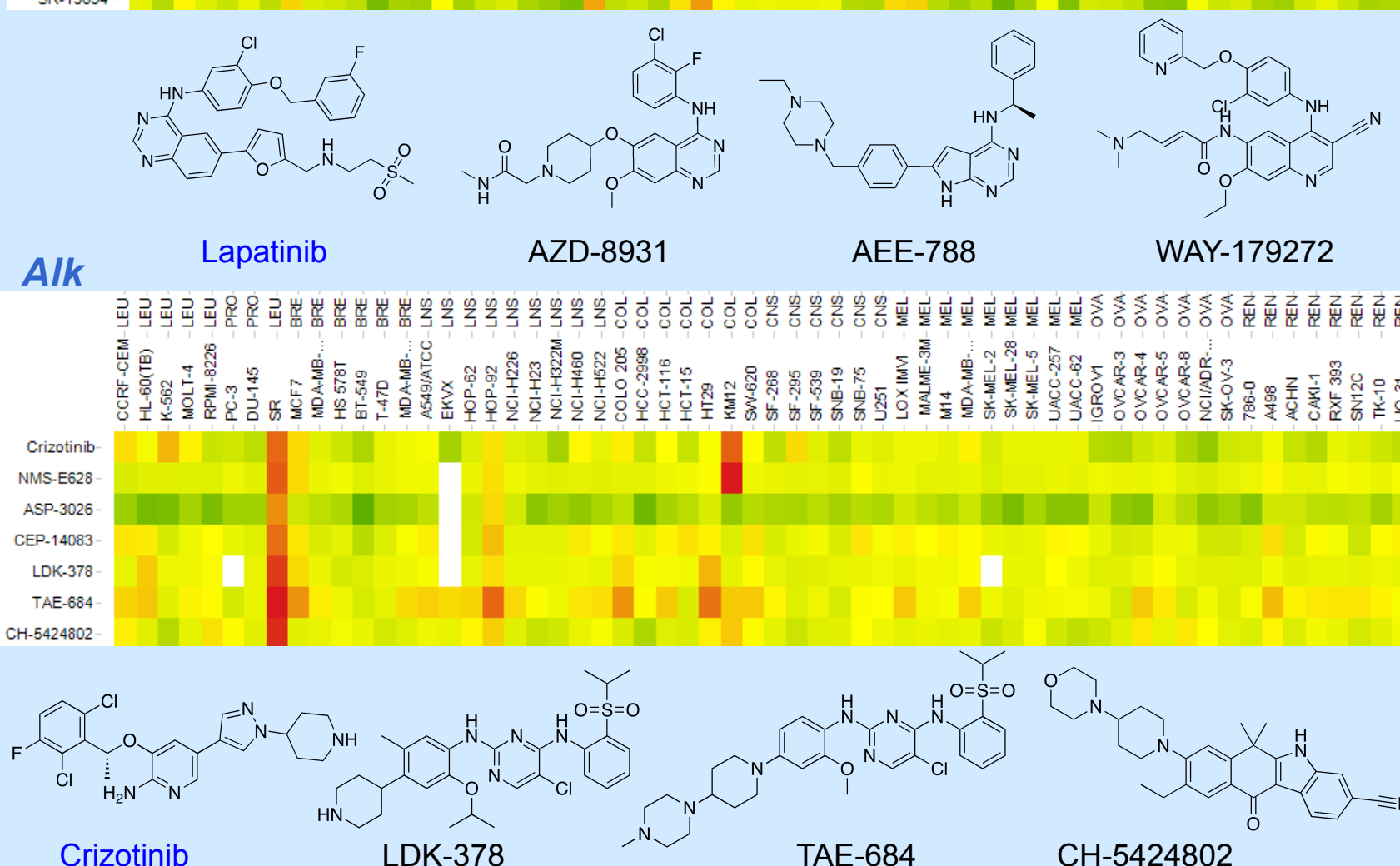
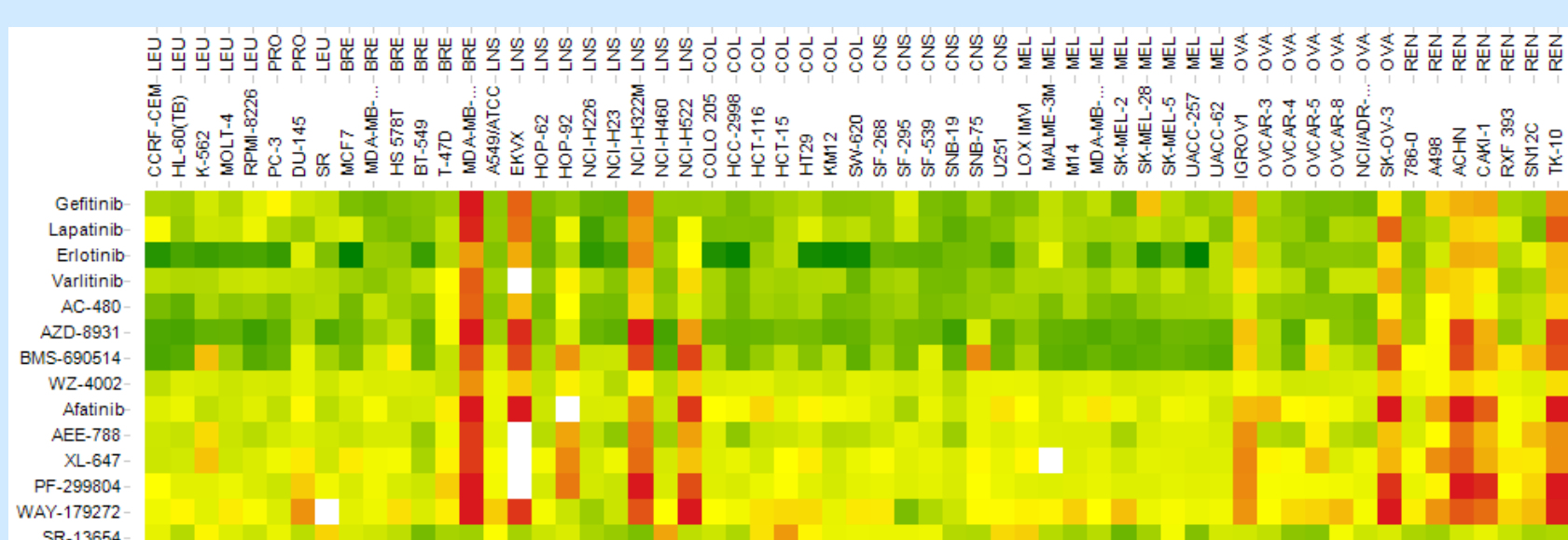
Hierarchical Clustering of Growth Response Patterns (GI₅₀s) for Investigational Oncology Agents against NCI-60 Cell Line Panel



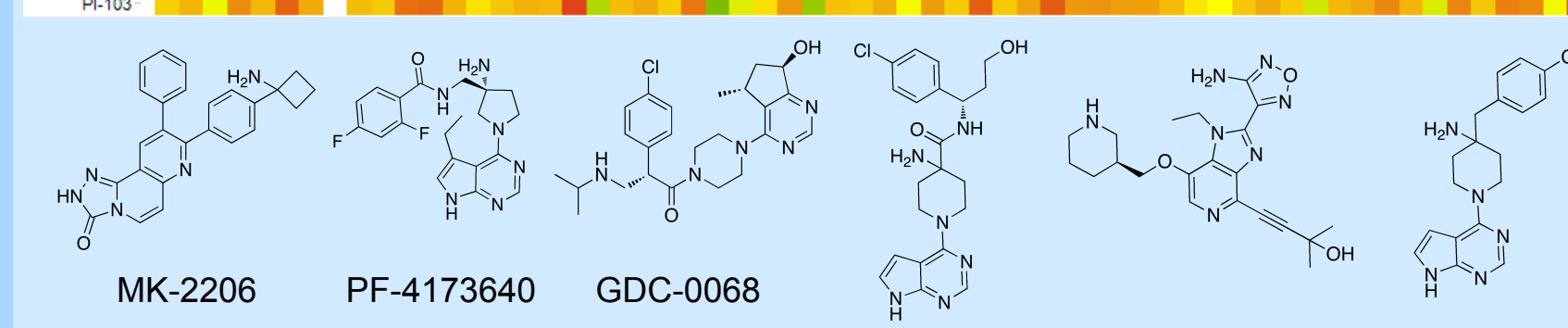
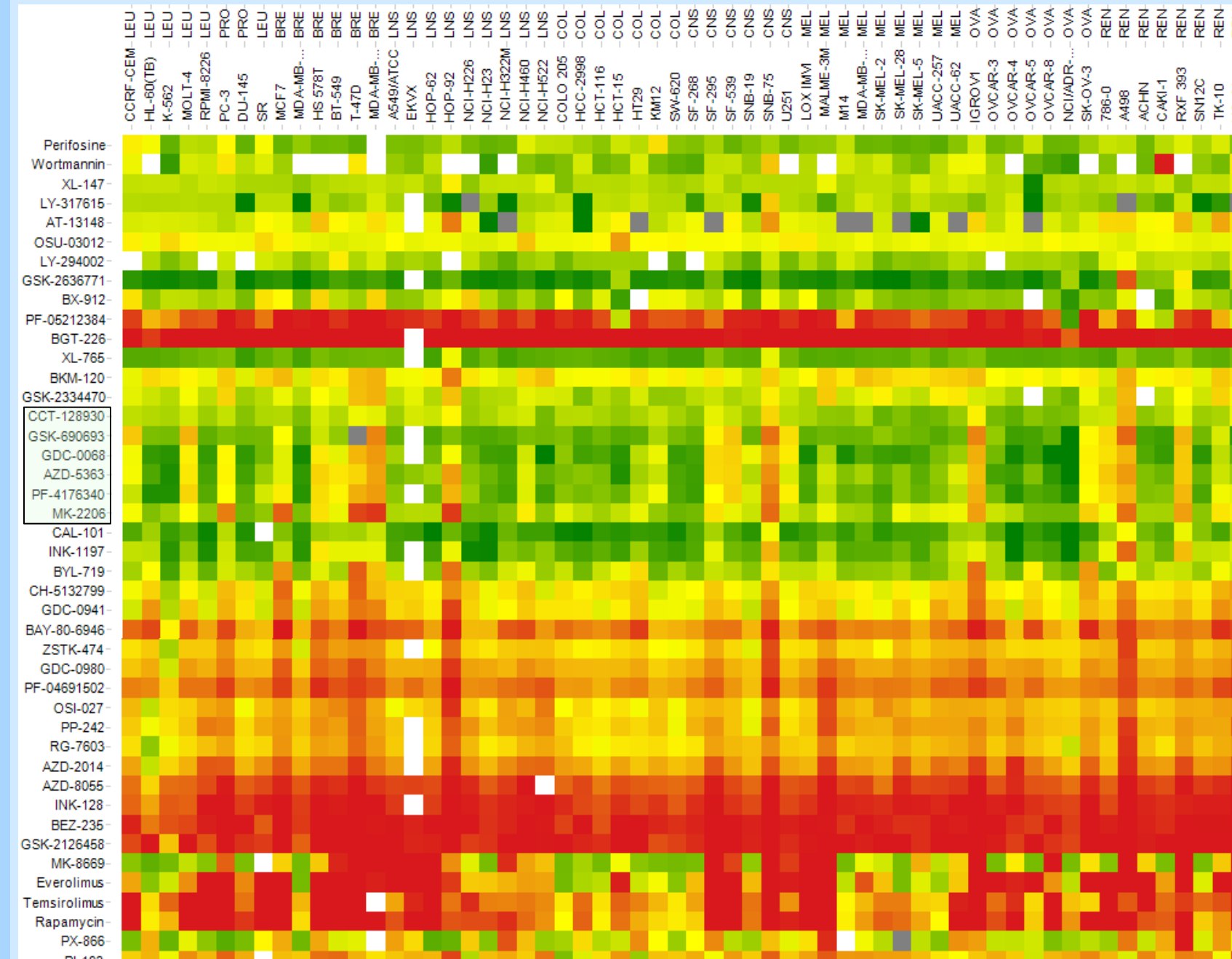
BRAF and MEK



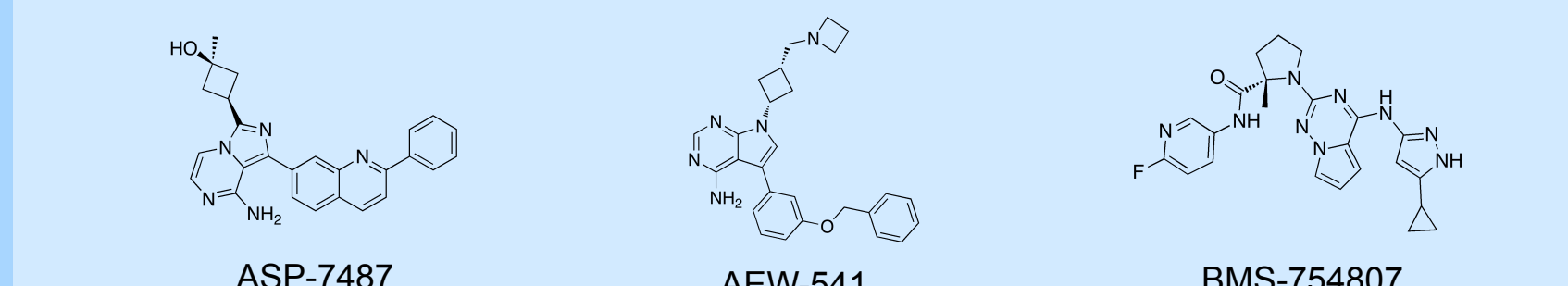
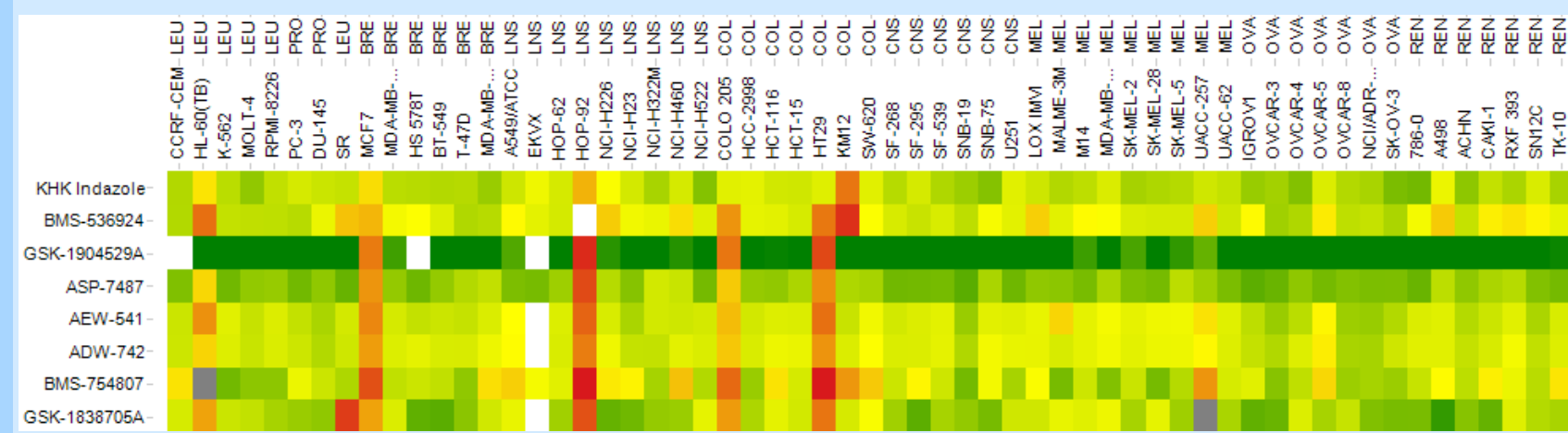
EGFR/erbB2



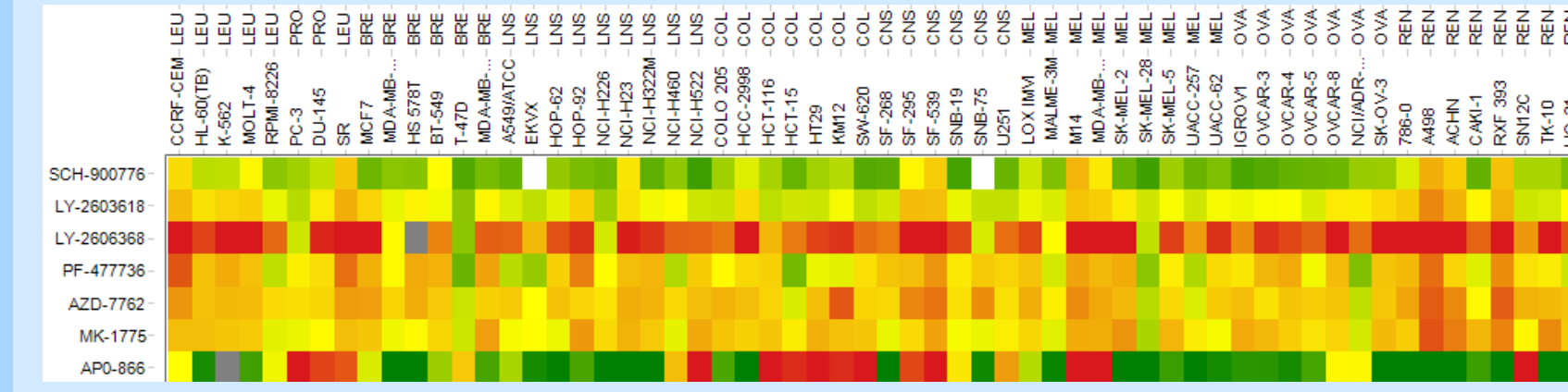
p13K Pathway



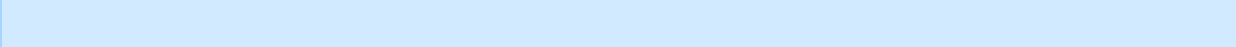
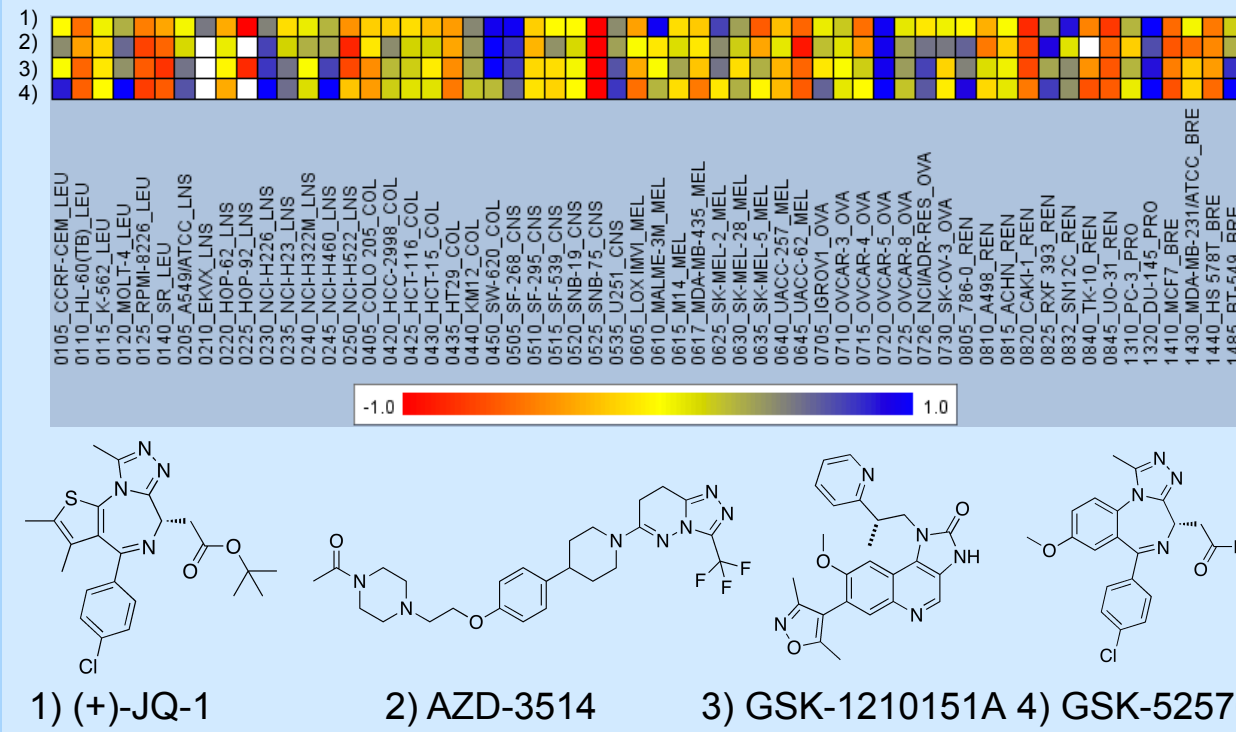
IGF-1R



Chk-1, Wee-1



Normalized NCI-60 GI₅₀ Response for AZD-3514 (androgen receptor modulator) and Bromodomain Inhibitors



Summary

- Hierarchical clustering of growth inhibition response patterns (GI₅₀s) for > 400 investigational oncology agents against the NCI-60 cell line panel show similarities among agents with common mechanism of action (including EGFR/erbB2, IGF-1R, Akt, BRAf, MEK, Akt, mTOR, Chk-1).
- Using the allosteric Akt inhibitor MK-2206 as the seed compound, response patterns for the ATP-competitive Akt inhibitors PF-4173640 (0.84), GDC-0068 (0.80), AZD-5363 (0.83), GSK-690693 (0.67), and CCT-128930 (0.69) are highly correlative by COMPARE algorithm.
- Patterns for investigational agents targeting one pathway (eg. BRAf/MEK and p13K/PDK-1/Akt/mTOR) show significantly strong correlations toward one another and are found to be subsets of each within the class.
- A normalized growth inhibition pattern presentation of the androgen receptor modulator AZD-3514 shows a high COMPARE correlation (0.77) to the bromodomain inhibitor (+)-JQ-1. Experiments are underway to determine whether AZD-3514 binds to the BRD4 protein.
- The Wee-1 inhibitor MK-1775 shows a COMPARE correlation to the Chk-1 inhibitors AZD-7762 (0.62), LY-2606368 (0.63), and PF-477736 (0.62).
- The leukemia cell line SR displays high sensitivity to all Akt inhibitors, including crizotinib, NMS-E628, LDK-378, TAE-684 and CH-5424802. High expression of Akt is known to be associated with the SR line. In addition, the colon cell line KM12 shows exclusively high sensitivity to the Akt inhibitors crizotinib and NMS-E628 suggesting the involvement of an unrelated mechanism.

References

- Holbeck, Susan L.; Collins, Jerry M.; Doroshov, James H. Analysis of Food and Drug Administration-Approved Anticancer Agents in the NCI60 Panel of Human Tumor Cell Lines. *Mol Cancer Ther* 2010; 9:1451-1460.
- Paull K. D.; Shoemaker R. H.; Hodes L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. Display and analysis of patterns of differential activity of drugs against human tumor cell lines: development of mean graph and COMPARE algorithm. *J Natl Cancer Inst* 1989; 81:1088-92.