Interrogation of NCI-60 Patterns of Growth Inhibition in Conjunction with Investigational Oncology Agents Kinase Profiling for the Elucidation of Mechanistic Targets

Joel Morris[1], Mark Kunkel[1], Eric Polley[2], Susan Holbeck[1], Anne Monks[3], David Evans[3], Annamaria Rapisarda[3], Jerry Collins[1], and Beverly A. Teicher[1,3] [1]Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Rockville, Maryland 20852; [2]Biometric Research Branch, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Rockville, MD 20852; [3]Molecular Pharmacology Branch, Leidos-Frederick, Frederick National Laboratory for Cancer Research, Frederick, Maryland 21702

Abstract

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 and molecular targets of new agents. The panel includes cell lines from nine tumor types, and is extremely well characterized at the molecular level, enabling interrogation of patterns of growth inhibition by a set of targeted investigational oncology agents looking for characteristics of the cell lines that determine sensitivity. We have used a number of online tools to enable data analysis, including COMPARE (http://dtp.nci.nih.gov/compare/), which provided the identification of compounds and/or genes that have highly correlated response patterns for any selected 'seed' compound. These data enable comparisons between drug sensitivity profiles that 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, 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 correlated. In addition, examination of the response profile for vandetinib produced a set of highly correlative agents including the corresponding EGFR inhibitors sapatinib (0.81) and AEE-788 (0.83), as well as the recently FDA-approved BTK inhibitor, ibrutinib (0.72) and the SRC inhibitor, AZD-0530 (0.74). Not surprisingly, kinase profiling of these 5 agents (0.5 uM) showed >90% inhibition of EGFR in all cases. In a third example, the BRAF V600E mutated cell lines were found to be sensitive to the bcr-abl inhibitors bafetinib and rebastinib, similarly to vemurafenib. This association suggested BRAF inhibitory activity for the former agents, which was confirmed through kinase profiling. Moreover, the NCI-60 response pattern for the androgen receptor modulator AZD-3514 has a high correlation with the BET bromodomain inhibitors JQ-1 (0.77), I-Bet-151 (0.80), and I-Bet-762 (0.78), suggesting a commonality of target/pathway for these compounds. Further correlations, associations and hypotheses generated from interrogating the compound response patterns, gene expression profiles, mutations and other characteristics will be presented.

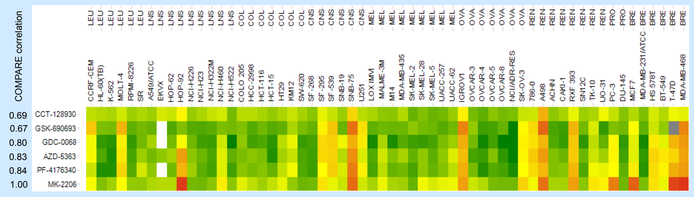
Methods and Materials

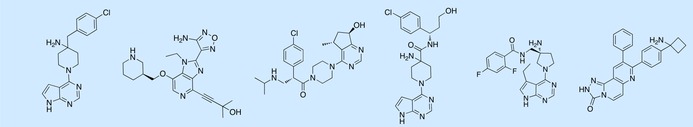
The NCI-60 human tumor lines are grown in RPMI 1640 medium containing 5% FBS & 2 mM L-glutamine. For experiments, cells are inoculated into 96 well plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual lines. The plates are incubated 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 (Ti)], the % growth is calculated.
Growth inhibition of 50 % (GI50) is calculated from [(Ti-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.

Kinase profiling was run against 342 kinases by Reaction Biology. Compounds were tested in a single dose duplicate mode at a concentration of 0.5 uM. Reactions were carried out at 10 uM ATP.

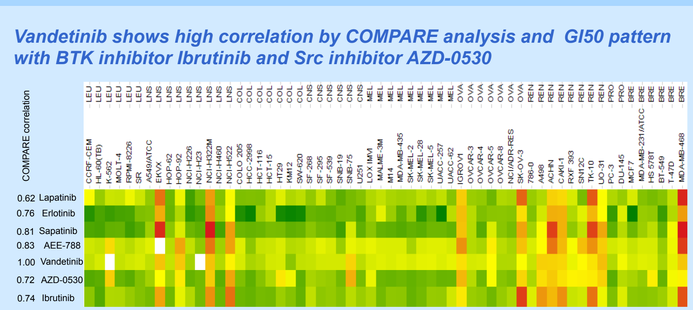
A set of >400 investigational oncology agents, comprised primarily of targeted small molecules currently in clinical and/or preclinical studies was acquired (synthesis and/or purchase) and screened (along with >100 FDA-approved anticancer drugs) against the NCI-60 cell line panel for their effects on cell growth.

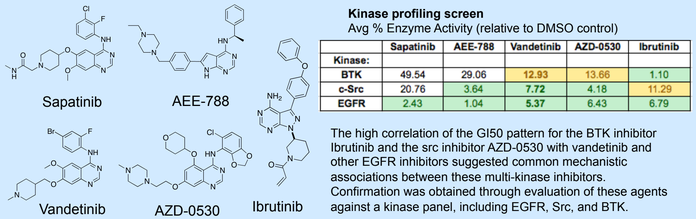
Akt Inhibitors show high correlation by COMPARE analysis and GI50 pattern





Vandetinib shows high correlation by COMPARE analysis and GI50 pattern with BTK inhibitor Ibrutinib and Src inhibitor AZD-0530



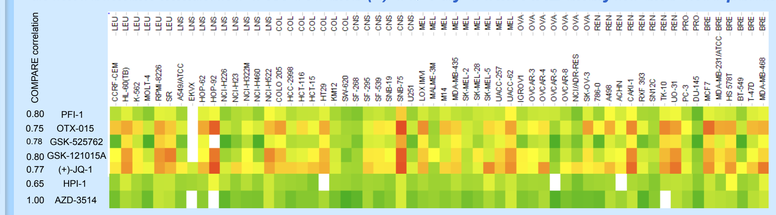


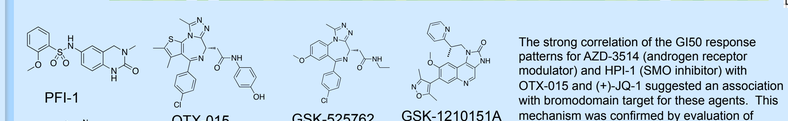
Kinase profiling screen
Avg % Enzyme Activity (relative to DMSO control)

Kinase:	Sapatinib	AEE-788	Vandetinib	AZD-0530	Ibrutinib
BTK	49.54	29.06	12.93	13.66	1.10
c-Src	20.76	3.64	7.72	4.18	11.29
EGFR	2.43	1.04	5.37	6.43	6.79

The high correlation of the GI50 pattern for the BTK inhibitor Ibrutinib and the src inhibitor AZD-0530 with vandetinib and other EGFR inhibitors suggested common mechanistic associations between these multi-kinase inhibitors. Confirmation was obtained through evaluation of these agents against a kinase panel, including EGFR, Src, and BTK.

NCI-60 GI50 response pattern for AZD-3514 (androgen receptor modulator) and HPI-1 (SMO Inhibitor) strongly correlate with bromodomain inhibitors OTX-015 and (+)-JQ-1 by COMPARE analysis and GI50 pattern



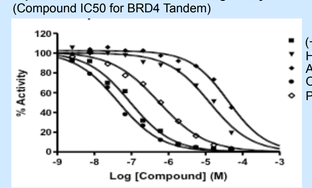


The strong correlation of the GI50 response patterns for AZD-3514 (androgen receptor modulator) and HPI-1 (SMO inhibitor) with OTX-015 and (+)-JQ-1 suggested an association with bromodomain target for these agents. This mechanism was confirmed by evaluation of AZD-3514 and HPI-1 in a bromodomain thermal shift assay (ΔTm of 3.0 and 5.8 °C, respectively at 33 uM) and in an alpha screen bromodomain binding assay (IC50s of 41 and 12 uM, respectively).

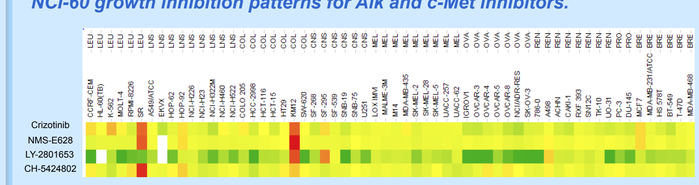
Bromodomain Thermal Shift Assay

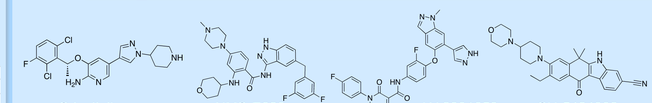
BRD4-T				
		ΔTm		
	DMSO	100 uM	33 uM	11.1 uM
PFI-1	0.0	7.5	6.3	4.5
OTX-015	0.0	13.0	11.3	9.0
GSK-525762	-0.5	11.3	8.8	6.8
GSK-121015A	0.0	12.0	10.3	7.8
(+)-JQ-1	0.0	12.0	11.0	9.3
HPI-1	0.0	8.3	5.8	3.5
AZD-3514	-0.3	4.5	3.0	2.0

Alpha Screen Bromodomain Binding Assay
(Compound IC50 for BRD4 Tandem)



NCI-60 growth inhibition patterns for Alk and c-Met inhibitors.



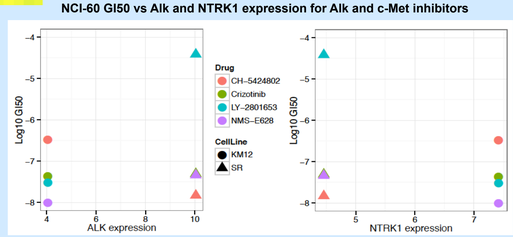


The leukemia cell line SR has marked high sensitivity to all Alk inhibitors, including crizotinib, NMS-E628 and CH-5424802. High expression of Alk is associated with the SR line. In addition, the colon cell line KM12 shows exclusively high sensitivity to crizotinib and NMS-E628 but does not express Alk, suggesting the involvement of an unrelated mechanism. One possibility is TrKA kinase – this is supported by the overexpression of the NTRK1 gene (that codes for TrkA) in KM12 as well as the kinase profiles for these agents as well as for the c-Met inhibitor LY-2801653.

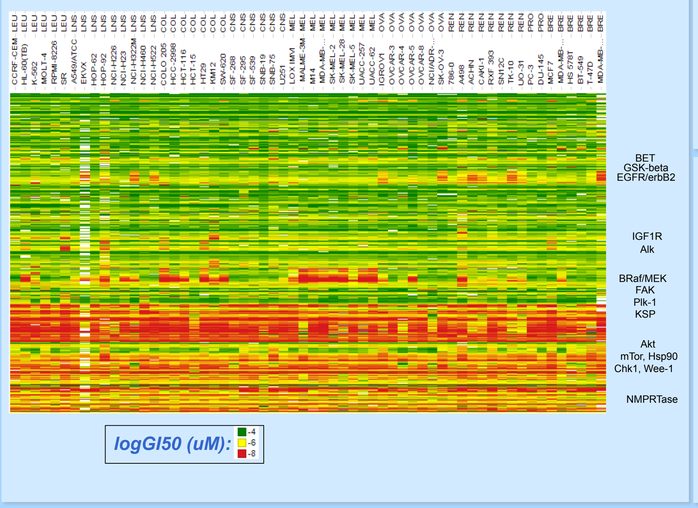
Kinase profiling screen

	Crizotinib	NMS-E628	CH-5424802	LY-2801653
Kinase:	% enzyme activity (relative to DMSO control)			
ALK	1.09	1.36	0.63	110.36
c-MET	-0.04	90.78	101.63	2.53
TRKA	6.13	3.02	43.22	5.05

NCI-60 GI50 vs Alk and NTRK1 expression for Alk and c-Met inhibitors



Hierarchical Clustering of NCI-60 Growth Response Patterns (GI50s) for Clinical and Preclinical Investigational Oncology Agents

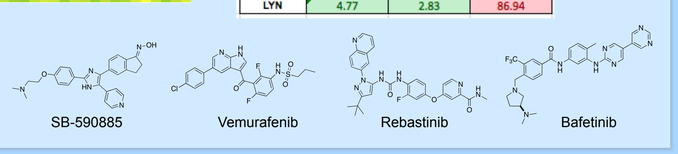


Bcr-abl inhibitors, rebastinib and bafetinib, have a mutant BRaf GI50 sensitivity pattern in addition to K-562 sensitivity

NCI-60 growth inhibition pattern for bcr-abl inhibitors rebastinib and bafetinib, suggested the presence of BRaf kinase inhibiton. Confirmation of this activity was obtained through kinase panel screening.

Kinase profiling screen

	bafetinib	rebastinib	vemurafenib
Kinase:	% enzyme activity (relative to DMSO control)		
ABL1	1.83	1.28	89.15
ABL2	2.92	4.98	90.40
BRAF	1.88	15.82	0.49
LYN	4.77	2.83	86.94



Summary

- Hierarchical clustering of growth inhibition response patterns (GI50s) for > 400 investigational oncology against the NCI-60 cell line panel elucidates similarities among agents with common mechanism of action (including EGFR/erbB2, Alk, BRaf, Mek, bromodomain, bcr-abl, Akt, TrKA, Chk-1).
- These data enable comparisons between drug sensitivity profiles that lead to the elucidation of common mechanistic targets or pathways, the confirmation of mechanism of action, the identification of novel mechanisms, and the uncovering of unexpected "off-target" activities.

References

1 Holbeck, Susan L.; Collins, Jerry M.; Doroshow, 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.

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