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

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Abstract
As oncology treatment moves toward personalized targeted therapeutic agents, the NCI-60 human tumor cell lines have become an invaluable tool for understanding 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 characteristic cell lines that determine sensitivity. We have used a number of online tools to enable data analysis, including COMPARE (Dr. Paull et al., 2001), which provided the identification of compounds resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells.

Methods and Materials
The NCI-60 human tumor line panel is grown in RPMI 1640 medium containing 5% FBS & 2 mM L-glutamine. For staining, cells are inoculated into 96 well plates in 100 μl/well. The plates are incubated for 48 h. The NCI-60 human tumor lines are grown in RPMI 1640 medium containing 5% FBS & 2 mM L-glutamine. As oncology treatment moves toward personalized targeted therapeutic agents, the NCI-60 human tumor cell lines have become an invaluable tool for understanding 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 characteristic cell lines that determine sensitivity. We have used a number of online tools to enable data analysis, including COMPARE (Dr. Paull et al., 2001), which provided the identification of compounds resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells.

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

Akt Inhibitors show high correlation by COMPARE analysis and GI50 pattern

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

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

NCl-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

Summary
Hierarchical clustering of growth inhibition response patterns (GI50s) for > 400 investigational oncology agents against the NCI-60 cell line panel elucidates similarities among agents with common mechanism of action (including EGFR/erbB2, AKT, BRaf, Max, bromodomain, sirtuin, ALK, TRKA, Chk1).

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

Comparison with other NCI-60 GI50s: 

- **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.

**KINASE profiling**

- **Sapatinib** and **SB-550535**
- **Vandetinib** and **AZD-0530**
- **Bcr-abl**, **ibrutinib**, and **batelrobib** have a mutant **BRaf** GI50 sensitivity pattern in addition to **K-562 sensitivity**.

**KINASE profiling**

- **KINASE profiling**
- **LogGI50 (μM)**
- **Avg % Enzyme Activity (relative to DMSO control)**
- **Δ**

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

- 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 between these multi-kinase inhibitors and the NCI-60 panel. This association was confirmed by evaluating the GI50 response patterns for **AZD-3514** and **HPI-1** in a bromodomain thermal shift assay (C-TD (OTX-015) and (++)-JQ-1, respectively).

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

- The NCI-60 cell line panel has evolved high sensitivity to Alk inhibitors, including **crizotinib**, **GDC-0068**, and **SB-590885**. High expression of this kinase is associated with the **SR** cell line. In addition, the cell line **SKM2** shows high sensitivity to **SB-590885** and **GDC-0068**, and correlations between these responses are observed in other NCI-60 cell lines. The **NCl-60** cell line panel is frequently used to assess the activity of **IBRITINIB** in a bromodomain shift assay in conjunction with **K-562** (a cell line associated with the **SR** line). In addition, the **SR** cell line panel is frequently used to assess the activity of **IBRITINIB** in a bromodomain shift assay in conjunction with **K-562** (a cell line associated with the **SR** line).