Using pathway analysis with PamChip® measurements to discover potential on/off target effects of a compound.

Rene Houtman, Dirk Pijnenburg, Rob Ruijtenbeek; PamGene International B.V., The Netherlands

Study Design
The phosphorylation profile of A549 lysated cell line (derived from lung adenocarcinoma tissue), is measured under five conditions, one control and four treatments, using a 144 peptide tyrosine PamChip. EGF stimulation of A549 cells is performed prior to testing efficacy of Iressa (Gefitinib) at three different concentrations as shown in figure 1. As a control we used the A549 cell line without EGF stimulation. A t-test of treatment vs. control is employed to select significantly different peptides for each of four treatments. A sub-selection of the common peptides are chosen and corresponding ratio values (treatment vs control) were uploaded to MetaCore (GeneGo) for pathway analysis.

Key Findings:
The most significant pathway to score is the EGFR signaling pathway. A drill down on this pathway is indicated in figure 2. Data is superimposed onto the pathway and is represented by thermometers. Red/Blue indicates high/low phosphorylation respectively. The EGF stimulation condition (1) clearly increases the phosphorylation activity (i.e. red thermometers). Iressa inhibition condition (2,3,4) clearly decrease phosphorylation (i.e. blue thermometer). Nodes which significant peptide substrates participating in both stimulation and inhibition (i.e. red circles) represent off-target effects. Nodes with inhibition only i.e c-RAF (i.e. blue circle) can be due to constitutively active KRAS or potential off target effects of Iressa.

“Author Quote”
Being able to observe phosphorylation profile changes in a cell line while varying its stimulation and its inhibition parameters is an exciting discovery tool.

Background:
Elucidating how a drugs behaves in the context of a cell is essential for the development of a more efficacious and potent drug. Specifically, the ability to measure potential off-target effects of a drug (such as Iressa) is critical to developing drugs with less side effects.

Conclusion
PamGene’s phosphorylation measurement profiles are a powerful tool to observe signaling pathways under different perturbation/treatment effects of biological models.