Kinase activity profiling can visualize differential modulation of kinase-activity by therapeutic Ab’s against the same growth factor receptor.

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Study Design
Three therapeutic antibodies (Ab 1,2,3) against different epitopes of the same target, a growth-factor receptor (GFR), were compared. At t₀, GFR expressing cells received treatment, i.e. growth factor (GF) alone or with one of the Abs, or not (control). After 1, 3 and 24 hours, cells were harvested, lysed and lysates were subjected to protein tyrosine kinase activity profiling on the PamChip® microarray. The 144 kinase target substrates (peptides) on this microarray generate, a 144-point ‘response-signature’, for each treatment that can be color coded. (figure 2A) Per timepoint, targets with significantly modulated phosphorylation (vs. control, Students t-Test) by at least one of the Ab treatments were selected. Per time point the response similarity between treatments and targets was visualized by unsupervised clustering.

Key Findings
• GF-induced alteration of intracellular kinase activity is modulated by GFR Abs as shown on PamChip arrays.
• Abs to different epitopes of the same GFR differentially modulate the intracellular kinase response to GF.
• Ab-induced modulation was different from 1 to 3 to 24 hrs indicating an effect on pathway kinetics
• At each timepoint the same (dis)similarity-based treatment clustering was obtained.
• Ab profiles were distinct from GF alone. Ab 1 and 3 induced effects that were similar and distinct from Ab 2.
• Differentially affected kinase targets are now a lead for mechanism of action elucidation through pathway analysis software.

“Author Quote”
These arrays allow us to study Ab effects at a pathway level.

Background
Development of therapeutic antibodies (Ab) is a strongly expanding field and highly competitive. Novel Abs are often directed against the same target and are positioned as “biobetter” drugs. Differentiation of the Ab effects on signal transduction in comparison with other potentially competing Ab is demanding when using classical methods like Western blots (WB). PamChip® kinase activity profiling is a broad spectrum analysis and does not require an a priori hypothesis like WB. Besides this differentiation kinase profiling support the elucidation of the mechanism of action (or mechanism of resistance).

Conclusion
PamChip® kinase activity profiling differentiates competing therapeutic antibodies targeting the same growth factor receptor on signal transduction effects and provides information on mechanism of action. This differentiation helps to position these antibody therapeutics in the therapeutic areas.