Co-treatment with simvastatin and cetuximab in KRAS mutant LoVo cells decreases PTK activity effectively and increases STK activity to overcome anti-EGFR resistance.

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Abstract #: 1846

Summary of Key Findings
In LoVo cells, the synergistic decrease of growth by simvastatin and cetuximab could be explained by the block of EGFR and downstream PTK signaling by cetuximab and simvastatin. STK activity is increased for all treatment conditions and this could be due to stress mechanisms or in case of the combination therapy due to initiation of STK controlled apoptosis pathways leading to reduced cell proliferation. Identification of the kinase involved in the observed phosphorylation profile in LoVo cells upon incubation with simvastatin and cetuximab, might reveal new treatment options for KRAS mutated tumors.

From these results, we conclude that in KRAS mutated cells, co-treatment with simvastatin and cetuximab decreases PTK activity effectively, and increases STK activity which provides a molecular explanation for overcoming the anti-EGFR resistance.

Preliminary pathway analysis indicates that the p21/p53 pathway may be one of the most prominent pathways affected by the PTK and STK activity.

Methodology
Cell survival assays were performed to study the effect of simvastatin, cetuximab or combination on proliferation.

The cell lines LoVo (KRAS mutant) and A431 (KRAS wildtype) were incubated for 48 hours with vehicle, simvastatin, cetuximab or the combinations followed by lysis.

Serine/threonine (STK) and tyrosine (PTK) kinase activity profiles were determined for all lysates in vitro using Pamgene’s PTK and STK peptide microarrays comprising each of 144 peptides which are substrates for human protein phosphorylation. Peptides significantly different with respect to the vehicle control are shown (Fig 3 A and B resp.).

For pathway analysis, peptides that were significantly different between the cetuximab and combination treatment were used as input (Fig 4).

Background
Statins inhibit the mevalonate pathway and thereby decreases formation of cholesterol and consequently release of prenylgroups, farnesyl-PP and geranylgeranyl-PP

Prenylgroups are used to prenylate proteins such as KRAS, which is a prerequisite to activate KRAS and consequently downstream signaling

The EGFR antibodies, cetuximab and panitumumab are registered for use in KRAS wildtype patients only, because in KRAS mutant patients, KRAS is permanently activated leading to constant cell signaling and proliferation independent of the EGFR.

We hypothesized that statins may inhibit the expression of the mutant KRAS phenotype by preventing the prenylation of the KRAS protein, and as a consequence preventing plasma membrane association and auto-activation of KRAS protein.

This study was aimed at further elucidating effects of these drugs on signal transduction and establishing kinase activity profiles using dynamic peptide microarrays, in order to identify new therapeutic targets to treat KRAS mutated tumors as well.