# *Ex vivo* kinome profiling of tumors from rectal cancer patients for possible identification of functional biomarkers of EGFR signaling pathway druggability

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## BACKGROUND AND OBJECTIVE

The tumor's individual profile of tyrosine kinase activity might serve as functional biomarker of druggability by tyrosine kinase inhibiting agents.

In metastatic colorectal cancer, resistance to anti-EGFR antibody therapeutics is correlated to tumor mutations within *KRAS*, *BRAF*, and *PIK3CA* genes, encoding effector proteins downstream of EGFR in the signaling cascade.

May prediction success in patient selection for anti-EGFR antibody treatment improve by applying tumor tyrosine kinase activity analysis?

## PATIENTS AND METHODS

Primary tumor samples
– from 63 patients with locally advanced rectal cancer

Mutation analysis of *KRAS*, *BRAF*, and *PIK3CA*
– by denaturant capillary electrophoresis

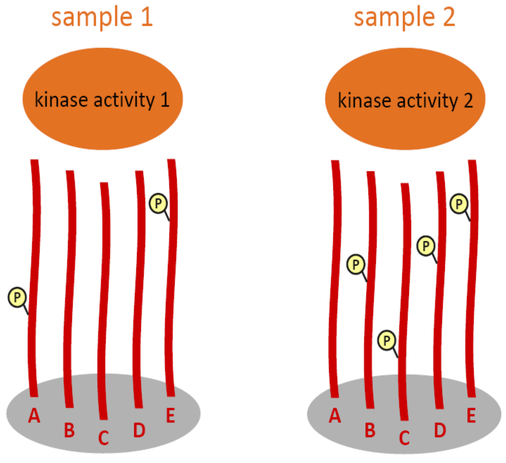
Profiling of tumor tyrosine kinase activity
– by peptide arrays with tyrosine kinase substrates

## CONCLUSION

Tumor tyrosine kinase activity may provide information on EGFR signaling pathway druggability.

## MULTIPLEX TECHNOLOGY

### TYROSINE KINASE SUBSTRATE ARRAYS




Each tyrosine kinase array consists of 144 peptide substrates with sites for phosphorylation (five examples, denoted A–E, are depicted), representing 100 different proteins.

Each sample lysate generates an individual phosphosubstrate signature (P; phosphate group).

## RESULTS

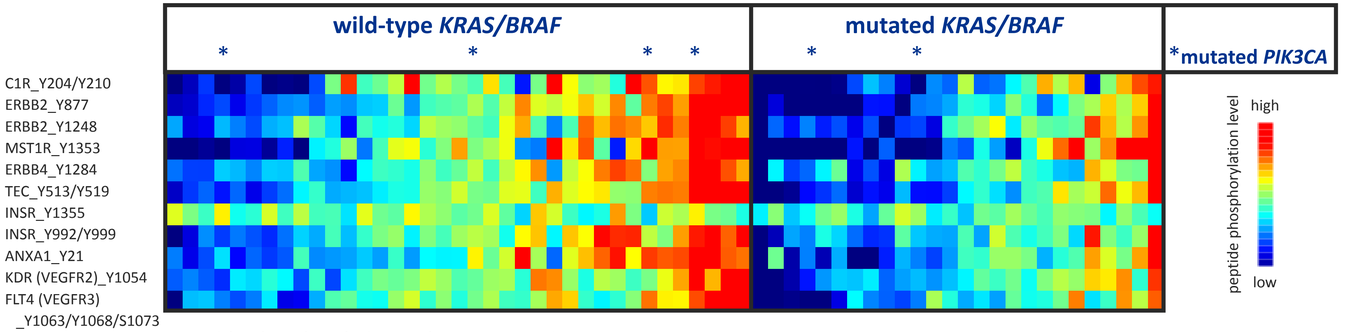
| | | *n* (%) |
|---|---|---|
| *KRAS* exon 2 | | 22 (35) |
| | p.G12D | 8 (13) |
| | p.G12V | 6 (9.5) |
| | p.G13D | 3 (4.8) |
| | p.G12C | 2 (3.2) |
| | p.G12S | 1 (1.6) |
| | p.G13S | 1 (1.6) |
| | unspecified | 1 (1.6) |
| *BRAF* exon 15 | | 4 (6.3) |
| | p.D594G | 2 (3.2) |
| | p.V600E | 2 (3.2) |
| *PIK3CA* | | 6 (9.5) |
| | exon 9 | 5 (7.9) |
| | exon 20 | 2 (3.2) |

Incidence of tumor *KRAS*, *BRAF*, and *PIK3CA* mutations in 63 patients with locally advanced rectal cancer.

Concordance between phosphopeptide profiles generated by tumors and their *KRAS/BRAF* mutation status was found in 67% of cases.

Phosphorylation of 11 peptide array substrates, mainly representing EGFR signaling factors, by tumor samples was significantly higher in *KRAS/BRAF* wild-type cases.

These findings may add to the ongoing debate on the current practice of patient selection for anti-EGFR antibody treatment based on tumor *KRAS/BRAF* mutation status.




Peptide phosphorylation and tumor mutation status. Tumor samples along horizontal axis, phosphosubstrates along vertical axis.

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