Direct effect of regorafenib on tyrosine kinase activities in treatment-naive colorectal cancer (CRC) primary tumor tissue

Riet Hilhorst[1], Rosanne van den Oord[2], Hans Pruijt[2], Adriaan van den Brûle[2], Peet Nooijen[2], Rik de Wijn[1], Liesbeth Houkes[1], Rob Ruijtenbeek[1].
[1]PamGene International BV, 's-Hertogenbosch, Netherlands; [2]Jeroen Bosch Hospital, 's-Hertogenbosch, Netherlands

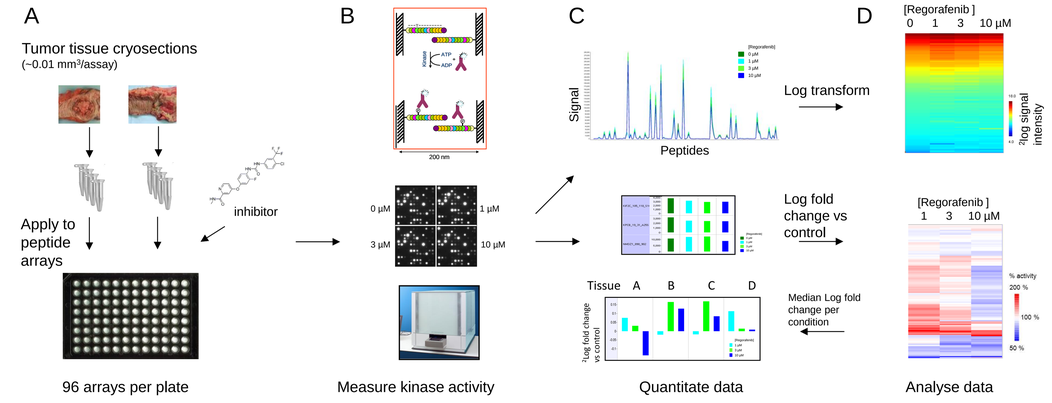
AACR 2014 # 5511

Background

Regorafenib (BAY 73-4506) is the first small molecule multi kinase inhibitor that has been approved for treatment of advanced CRC. In the Correct trial[1], 1% of participants had a partial response to regorafenib and 42.8 % showed stable disease versus 15% in the placebo group. KRAS wild type and mutants showed equal response rates.
Regorafenib is a multitarget kinase inhibitor with nM IC50's against CRAF, VEGFR2, KIT and RET and is about tenfold less potent for BRAF, BRAFV600E, PDGFRβ and VEGFR1 and 3. We tested the effect of regorafenib and other RAF targeting inhibitors on patient-derived kinases in treatment naive colon tumour samples for potency and selectivity of inhibition.

References
1. Grothey et al, Lancet 2013 381(9863):303-12

PamChip ® Cell-Based Kinase Assay



Discussion

Kinase activity profiling of tumor tissue in the presence and absence of inhibitor permits the analysis of the effect of an inhibitor on global kinase activity.
In fresh frozen, treatment-naive colon tumor tissue, 50 µM regorafenib is needed to obtain inhibition of serine/threonine kinase activity whereas with 3 µM regorafenib about 75% inhibition of tyrosine kinase activity is observed. Similar studies with Sorafenib confirm that the inhibitor is more potent on the tyrosine kinases than on serine/threonine kinase activity.

Although mutations in KRAS are assumed to increase activity of the RAS/RAF pathway, no differences in inhibition were observed between KRAS mutant and WT samples, which is in line with the absence of differences between such groups in the CORRECT Trial.
Therefore, this class of inhibitors most likely acts by inhibition of its tyrosine kinase targets.

Dabrafenib (and vemurafenib to a lesser extent) showed inhibition at 10 µM concentrations in a BRAF mutant colon tumor lysate.

Results

Profiles without inhibitor

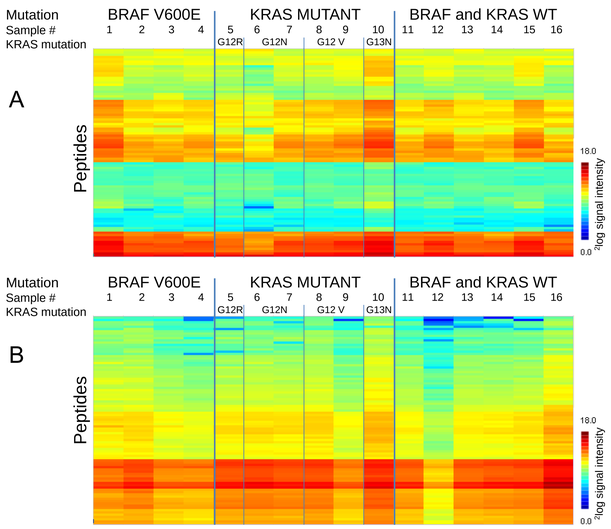


Fig. 2. Heatmap representation of serine/threonine (A) and tyrosine (B) kinase activity profiles of lysates of 16 colon tumor tissue for wild type, BRAF V600E and KRAS mutants in the absence of inhibitor.

Ratio Profiles with inhibitor

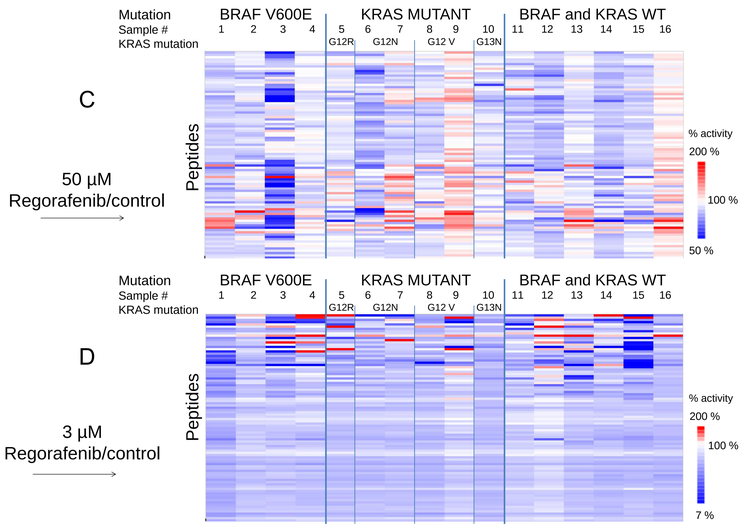


Fig. 3. Ratio heatmap representation of serine/threonine (C) and tyrosine (D) kinase activity profiles of lysates of colon tumor tissue with 50 or 3 µM regorafenib. [2]Log ratios of signal intensities with and without inhibitor are displayed.

Fig. 1. Kinase activity profiles of lysates of tumor tissue in the presence and absence of the inhibitor regorafenib were measured on PamChip® peptide microarrays (A, B). Signal intensities for the sample without inhibitor and 3 concentrations of regorafenib were converted to activity profiles (C) and to heatmaps of [2]log signal intensity or ratio profiles ([2]log (signal treatment/signal control)) per peptide (D) and the mean % inhibition for four samples with three inhibitor concentrations (C, bottom figure).

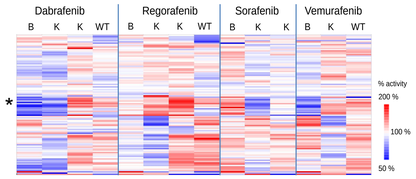


Fig. 4. Comparison of efficacy of 10 µM Raf inhibitors on serine/threonine kinase activity. B: BRAF V600E; K: KRAS mutant; WT: No BRAF or KRAS mutation.
* BRAF substrate peptides.

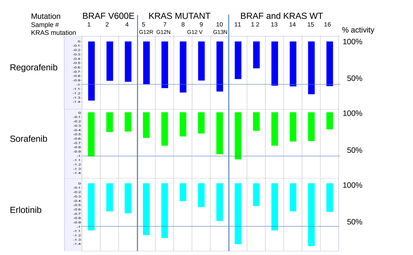


Fig. 5. Comparison of efficacy of 3 µM regorafenib, sorafenib and erlotinib on tyrosine kinase activity of 14 colon samples. Median % of inhibition for all peptides per sample.

Conclusions

For the first time the direct biochemical effect of regorafenib (and sorafenib) has been tested on CRC patient-derived tumour tissues. These data support previous findings suggesting that regorafenib, besides its angiogenic activity on vascular epithelial cells, can inhibit oncogenic tyrosine kinases in the tumour. These results will be a basis for biomarker analysis by correlating on-chip drug effects to clinical responses for existing drugs like regorafenib and others to come.

This project is part-financed by the European Regional Development Fund under OP-Zuid.

OP-Zuid
Europees Economisch Stimuleringsprogramma

Jeroen Bosch Ziekenhuis

PamGene

European Union
European Regional Development Fund
Investing in your future!