Conclusion: GSH modulation reverses the growth-promoting effect of GFs and thereby significantly enhances the anti-tumour response of WiDr cells to SN-38 (this work has been supported by grants of the University of the Basque Country/EHU, Basque Government and the Jesús Gangoiti Barrera Foundation).

231 Application of non-steroidal anti-inflammatory drugs to enhance 5-fluorouracil efficacy on experimental systems

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Background: The elevated cyclooxygenase-2 (COX-2) expression has been shown to affect the carcinogenesis and tumour progression. COX-2 is overexpressed in approximately 80% of sporadic colorectal carcinomas and the best defined target of non-steroidal anti-inflammatrory drugs (NSAIDs). In the chemotherapy of colorectal carcinomas 5-fluorouracil (5-FU) has been the most important of the basic drugs for more than 40 years. The rate-limiting enzyme of 5-FU catabolism is dihydropyrimidine dehydrogenase (DPD) since more than 80% of the administered 5-FU is catabolised by DPD. Tumoural DPD has become of clinical interest because elevated intratumoural DPD can decrease the tumour response to 5-FU therapy.
The main purpose of our experiments was to investigate the effect of COX inhibitors on the efficacy of 5-FU on high and low COX-2 expressing HCA-7 and HT-29 human colon adenocarcinoma cell lines, respectively and also on xenografts derived from HT-29 cells. The cytotoxic and antitumour effects of 5-FU in the presence of low doses of NSAIDs (indomethacin and NS-398) on the HT-29 and HCA-7 cells and also on the HT-29 xenograft were investigated. In addition our intention was to understand the mechanism(s) by which NSAIDs could enhance the cytotoxic effect of 5-FU.
Materials and Methods: The antiproliferative effect of 5-fluorouracil (5-FU)±NSAIDs was examined by sulphorhodamine B assay. The COX-2 and DPD expressions were visualized by immunofluorescent staining, and prostaglandin E_2 levels were measured by ELISA kit. The HT-29 xenograft was established in SCID mice and treated with 5-FU±NSAIDs for 5 days and with NSAIDs for 3 weeks. The tumour volume, DPD mRNA expression and enzyme activity were investigated by calliper, radioenzymatic method and real-time RT-PCR, respectively. The drug interaction was calculated for both combinations (5-FU+indomethacin and 5-FU+NS-398).
Results: Our data indicated that, the elevated COX-2 expressions of the HCA-7, the collagen-induced HT-29-C cells and of the HT-29 xenograft were associated with reduced 5-FU sensitivity. Based on the fact that at the same time the DPD activity was also increased it might be conceivable that a possible explanation for the decrease of 5-FU sensitivity is the co-existence of high COX-2 and DPD activity. Indomethacin or NS-398 enhanced in a simultaneous and significant manner the sensitiviy and cytotoxic effect of 5-FU on high COX-2 expressing cells and xenografts through the modulation of DPD – decrease of its mRNA expression and/or enzyme activity.
Conclusions: Based on our results it could be presumable that 5-FU efficacy is limited by the COX-2 associated high DPD expression and activity in patients with colorectal cancers as well, therefore further clinical studies are warranted to decide if NSAIDs in the therapeutic protocol might improve the antitumour potency of 5-FU.

232 Fluorine-18 and iodine-124 labeled cyclin-dependent kinase 4 and 6 inhibitors as radiotracers for tumour imaging by positron emission tomography (PET)

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Background: Cyclin-dependent kinases 4 and 6 (Cdk4/6) function as critical activators of cell cycle progression in human tumours. Pyrido[2,3-*d*]pyrimidine derivatives CKIA and CKIE are selective Cdk4/6 inhibitors with high potency for the inhibition of G_1 phase progression and tumour cell proliferation. The aim of this study was the evaluation of radiolabeled compounds [^{124}I]CKIA and [^{18}F]CKIE as radiotracers for PET imaging of Cdk4/6 in tumours *in vivo*.
Materials and Methods: Cellular uptake of radiotracers [^{124}I]CKIA and [^{18}F]CKIE was studied in human colorectal (HT-29) and squamous cell (FaDu) carcinoma cells. Small animal PET studies of both radiotracers were performed in FaDu xenograft-bearing nude mice.
Results: Radiotracer uptake studies showed fast and high uptake (up to 800%ID/mg protein) of [^{124}I]CKIA in both cell lines after 1 h at 37ºC. Cellular uptake of [^{18}F]CKIE was lower (HT-29, 46.3±11.2%ID/mg protein; FaDu, 46.2±13.8%ID/mg protein). Radiotracer uptake was significantly lower at 4ºC for [^{124}I]CKIA (150%ID/mg protein) and [^{18}F]CKIE (15%ID/mg protein) after 1 h in both cell lines. Cellular uptake of [^{18}F]CKIE was reduced to 18.0±4.9%ID/mg protein in the presence of 10 μM of nonradioactive CKIE at 37ºC. Dynamic small animal PET studies showed rapid clearance of [^{124}I]CKIA and [^{18}F]CKIE from the blood and fast hepatobiliary excretion. The half-life of radiotracer elimination from the blood was calculated to be 7.2 min for [^{124}I]CKIA and 7.9 min for [^{18}F]CKIE, respectively. Radiotracers were rapidly metabolized in blood *in vivo,* yielding >90% (1 min p.i.), 20% (30 min), and <5% (1 h) of the original compounds. Small animal PET studies with [^{124}I]CKIA only showed marginal uptake of the radiotracer in the FaDu tumour. In the case of [^{18}F]CKIE a higher uptake was detected in the peripheral proliferative region of the tumour after 1 h p.i. However, the constant tumour-to-muscle ratio of 1.5 suggests a non-Cdk4/6-mediated uptake of [^{18}F]CKIE in human tumour xenografts in mice.
Conclusions: Synthesis of the pyrido[2,3-*d*]pyrimidine-based radiotracers [^{124}I]CKIA and [^{18}F]CKIE allowed for the first time the quantification of cellular uptake *in vitro* and imaging of tissue-specific distribution of Cdk4/6 inhibitors *in vivo*. However, the short biological half-life in the blood and low tumour uptake of [^{124}I]CKIA and [^{18}F]CKIE limit the use of both radiotracers for the characterization of Cdk4/6 expression in tumours by means of PET. Further development of suitable radiolabeled Cdk4/6 inhibitors for functional characterization of Cdk4/6 in tumours continues to be of great interest in current translational cancer research.

233 Inhibition of PI3K as a potential treatment for cancer

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Background: Phosphoinositide-3-kinase (PI3K) is an enzyme that induces phosphorylation of phosphoinositides in position 3 of the inositol ring, producing compounds that act as intracellular second messengers. The PI3K class IA is formed by a regulatory (p85) and a catalytic subunit (p110). There are many p85 isoforms, and three for p110 (p110α, β, and δ), all of which are regulated by tyrosine kinases. As several reports showed the importance of PI3K in the development of human tumours, we tested specific inhibitors of p110α and β for potential therapeutic application in cancer.
Material and Methods: We used a group of luciferase-transfected murine tumour cell lines (colon carcinoma, breast cancer and small cell lung cancer cell lines), which allowed us to follow their growth through increases in light intensity. The cellular apoptosis was measured by flow cytometry. Levels of different proteins of the PI3K/Akt pathway were determined by Western-Blot. We used SCID mice for the in vivo studies, generating xenograft models.
Results: In each cell line, we measured the activation state of the PI3K/Akt pathway and the levels of the proteins implicated in this route. Subsequently, we analyzed the consequences of blocking p110β activity in the replication and apoptosis of these cells, and demonstrated that a colon carcinoma cell line was the most sensitive to the p110β inhibitor. In an in vivo mouse model, we are now studying the effect of the inhibitor in the growth of tumours derived from this carcinoma cell line.
Conclusions: All of the tumour cell lines tested were sensitive to p110β inhibition in vitro, but only the colon carcinoma cell line showed promising results in vivo.

234 Drugs with known pharmacological profiles, such as monensin, disulfiram and salinomycin, show cancer-selective inhibition of prostate cancer cell growth by increasing oxidative stress

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To identify novel therapeutic opportunities for patients with prostate cancer, we carried out a high-throughput cell-based screening (HTS) of 4,910 most currently marketed drugs and drug-like molecules to systematically explore for their efficacy in four prostate cancer (VCaP, LNCaP, DU 145, and PC-3) and two non-malignant prostate epithelial cell lines (RWPE-1 and EP156T). The EC50 values were determined for each cell type. Gene microarray studies, measurements of oxidative stress induction and cancer stem cell activity were used to explore the mechanism of selected compounds.
Monensin and disulfiram (DSF) were identified as nanomolar inhibitors of VCaP and LNCaP growth. In addition, two compounds structurally similar to monensin, salinomycin and nigericin, were included in studies and found to inhibit prostate cancer cell growth. Interestingly, all these compounds induced the expression of metal binding and oxidative stress responsive genes. DSF, monensin and salinomycin increased the level of oxidative stress and decreased the aldehyde dehydrogenase activity, suggesting deactivation of pathways linked to stem cell processes.
Our results indicate that several drugs with known pharmacological and toxicological profile showed unsuspected cancer-selective growth inhibitory potential in human prostate cancer cells. Analysis of the molecular mechanisms of action for these drugs indicated increased sensitization to