approaches. In this context, mass spectrometry based approaches were developed and applied towards the in vitro and in vivo evaluation of gonadotropin - releasing hormone (GnRH) and analogues in mice.

Materials and methods: A facile in vivo mouse model was developed for the pharmacokinetic evaluation of GnRH and analogues (such as leuprolide) and the subsequent quantification of testosterone (pharmacodynamic measurement) following intraperitoneal administration. Peptide stability and metabolism was determined in vitro by incubation of peptides with mouse kidney membrane preparations. High pressure liquid chromatography (HPLC) coupled to a platform that combines the benefits of triple quadrupole and Linear Ion Trap instruments (QqLIT) was employed for the study.

Results: Using the described methodology, GnRH and novel analogues were measured in mouse plasma with high sensitivity (e.g. limit of quantification for leuprolide: 0.1 ng/mL). In the same preclinical model, we demonstrated the versatility of our mass spectrometry based approach by the determination of plasma concentrations of testosterone, an established biomarker for the treatment of prostate cancer. Following dosing with agonists, circulating testosterone was increased significantly, compared to vehicle treated mice, providing the potential for biomarker based efficacy measurements. Peptide stability of GnRH and analogues was investigated at t = 0.5, 1 and 2h, followed by identification of major metabolites.

Conclusions: GnRH and novel peptide analogues with potential therapeutic advantages were evaluated in a novel and practical preclinical mouse model by mass spectrometry. A robust in vitro screen was also established for the determination of peptide stability and metabolism.

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Beer constituents inhibit prostate cancer cells proliferation

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Prostate cancer is one of the most frequent tumors in the developed countries and it has been reported that some antioxidants reduce cell proliferation and increase the efficiency of conventional treatments in prostate cancer cells. Epidemiological studies indicate that some diet components, with antioxidant properties, reduce prostate cancer occurrence. Beer is one of the most commonly consumed beverages in the world. The purpose of our study was to evaluate the anti-proliferative activity of beer components in prostate cancer cells. We have included in our study three types of beer: lager, stout and alcohol-free. We have analyzed the antioxidant capacity and the quantity of polyphenols present in these three types of beer. In all, there was a strong correlation between both parameters and the stout beer showed the higher antioxidant capacity and the biggest content in polyphenols. Additionally, we used LNCaP (androgen-dependent) and PC3 (androgen-independent) epithelial prostate cancer cell lines, cultured without or with the freeze-dried obtained from the three types of beer to study antiproliferative activity of its components. We observed that the higher concentration of beer liophilizate used, the most potent antiproliferative action observed in the three beers employed. Also, better results were obtained with stout beer (IC50: 3,83 mg/ml) followed by the larger beer (IC50: 10,47 mg/ml) and later the beer without alcohol (IC50: 36,37 mg/ml). These data confirm that a strong correlation between the total content in polyphenols and inhibition of tumor growth exits. Additionally, concentration above IC50 induces apoptosis in both prostate cancer cell lines. We have evaluated the antitumoral capacity of some specific polyphenols usually found as common beer constituents, including catechin, quercetin, caffeic acid, catechin galleate, epicatechin, p-coumaric acid, synaptic acid and gallic acid. Even at higher concentration than that found in the analyzed beer, these compounds don't show a significant antitumoral effect as in the freeze-dried beer. Antiproliferative activity of the beer comes from a synergic effect of the different compounds rather that being related with some specific compounds. Antiproliferative properties of beer seem to be related with a higher antioxidant capacity and a higher content at polyphenols found in this drink. This work was supported by "Centro de Informacion Cerveza y Salud (Ayuda-Paralela-07-FCS)".

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A paclitaxel-hyaluronan bioconjugate exerts a high in vivo therapeutic activity against ovarian cancer

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This study aimed at evaluating pharmacological and biological properties of a paclitaxel-hyaluronan bioconjugate (ONCOFID-P™) against IGROV-1 and OVCAR-3 human ovarian cancer xenografts following intraperitoneal administration. Paclitaxel is a potent anticancer agent, but its commercial formulation contains Cremophor that may lead to important adverse reactions. Drug conjugation with hyaluronic acid (HA) enables water solubilization. Moreover, HA-drug bioconjugates should present a markedly enhanced selectivity for cancerous cells, providing at the same time advantages in drug stabilization, localization, and controlled release.

In vitro tumor sensitivity to ONCOFID-P™ was analyzed by the MTT assay, while bioconjugate interaction with cells was studied cyto-fluorimetrically and by confocal microscopy using amino-BODIPY-labeled ONCOFID-P™. In vivo toxicity was assessed by single-dose Maximum Tolerated Dose (MTD) and peripheral blood cell count determination, and by histological analysis. Biodistribution of the compound was evaluated with a small animal-dedicated scintigraphy gamma-camera following injection of 99mTc-labeled ONCOFID-P™. Pharmacokinetics analysis was also carried out. Female SCID mice implanted with ovarian cancer cells underwent treatment with ONCOFID-P™ or free paclitaxel starting from day 7 or 14 after tumor injection, and survivals were compared.

ONCOFID-P™ interacted with CD44, entered cells through a receptor-mediated mechanism and exerted a concentration-dependent inhibitory effect against tumor cell growth. After intraperitoneal administration, the bioconjugate distributed quite uniformly within the peritoneal cavity, was well tolerated and not associated to local histological toxicity. Pharmacokinetic studies revealed that blood levels of bioconjugate-derived paclitaxel were much higher and persisted longer than those obtained with the unconjugated free drug. Intraperitoneal treatment of tumor-bearing mice with the bioconjugate disclosed that ONCOFID-P™ exerted a relevant increase in therapeutic activity in comparison to free drug.

Therefore, ONCOFID-P™ significantly improved results obtained with conventional paclitaxel, in terms of in vivo tolerability and therapeutic efficacy; these data strongly support its development for loco-regional treatment of ovarian cancer.

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Targeting of cancer-associated microRNAs using short LNA-antimiR oligonucleotides

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microRNAs play important roles in development and physiology. Recent data suggest that miRNAs are aberrantly expressed in many human cancers and that they may play significant roles as oncogenes or tumour suppressors. One such example is microRNA-155, which is required for normal function of T and B lymphocytes and dendritic cells, whereas overexpression of miR-155 has been reported in lymphomas as well as in breast and lung cancer, being associated with poor prognosis. On the other hand, microRNA-21 has been reported to be over-expressed in many solid tumours, including glioblastomas. Moreover, it has been shown that inhibition of miR-21 leads to apoptosis and reduced invasion/metastasis. Thus, miR-21 and miR-155 could represent novel targets for therapeutics, which, in turn, requires the development of efficient and safe approaches for sequence-specific microRNA silencing in vivo. Locked Nucleic Acid (LNA)–modified oligonucleotides show high binding affinity to complementary RNA molecules and high stability in blood and tissues in vivo. We report here that short LNA oligonucleotides can mediate potent and specific inhibition of microRNA function in vitro and in vivo.

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Synthesis and biological evaluation of a new series of imidazo[1,2-a]pyridines substituted as CDK inhibitors

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Because of the CDKs critical role in regulation of the cell cycle and the observed expression/activity pattern in most human cancers, considerable effort has been focused on the development of small molecule inhibitors that block CDK activity. Recently has been showed that imidazo[1,2-a]pyridine scaffold represents a new structural class of CDK inhibitors.

Here we shown a new series of imidazo[1,2-a]pyridines 2,6 disubstituted (Compounds 1-9) with cytotoxicity against five cancer cell lines U251 (colon), PC-3 (lung), K-562 (leukemia), HCT-15 (colon), MCF-7 (cervix) and SKLU-1 (prostate). The IC50 values will be reported. Moreover, we will report inhibitory activity against activated CDK2. Compounds 1-9 were prepared with several reaction conditions and typical transformations starting from 2-chloropyridine, 6-chloronicotinylchloride or 2-aminopyridine to obtain the imidazo[1,2-a]pyridine nucleus substituted at 2 or 6 positions.

Compound	Name
1	2,2,2-trifluoro-N-(6-(2-fluoro-5-methylbenzoyl)imidazo-[1,2-a]pyridin-2-yl)acetamide.

2	2,2,2-trifluoro-N-(6-(2-fluoro-4-(trifluoromethyl)benzoyl)imidazo-[1,2-a]pyridin-2- yl)acetamide
3	2,2,2-trifluoro-N-(6-(3-fluoro-4-methylbenzoyl)imidazo-[1,2-a]pyridin-2-yl)acetamide.
4	2,2,2-trifluoro-N-(6-(1-methyl-1H-indole-3-carbonyl)imidazo-[1,2-a]pyridin-2-yl)acetamide
5	2,2,2-trifluoro-N-(6-(thiazole-2-carbonyl)imidazo-[1,2-a]pyridin-2-yl)acetamide.
6	2,2,2-trifluoro-N-(6-(1-methyl-1H-imidazole-2-carbonyl)imidazo-[1,2-a]pyridin-2-yl)acetamide
7	diethyl 4-(imidazo[1,2-a]pyridin-2-ylamino)benzylphosphonate
8	6-(imidazo[1,2-a]pyridin-2-ylamino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione.
9	N-(4-(trifluoromethyl)phenyl)imidazo[1,2-a]pyridin-2-amine.

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Effect of some bis-mannich bases and corresponding piperidinols on DNA topoisomerase I as a possible mechanism of their cytotoxic actions

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Some acetophenone-derived bis-Mannich bases, bis-(3-aryl-3-oxo-propyl)-methylamine hydrochlorides (B1 through B5) and their structural isomers, piperidinols 4-aryl-3-arylcarbonyl-1-methl-4-piperidinol hydrochlorides (C1, C2 and C5) were synthesized and their effects on mammalian DNA topoisomerase I was tested. Chemical structures of the compounds were confirmed by UV, IR, 1H NMR, 13C NMR, ESI-MS spectra and elemental analysis. Among the compounds, all bis-Mannich bases, and 4-(2-thienyl)-3-(2-thienylcarbonyl)-1-methyl-4-piperidinol hydrochloride were found to inhibit DNA topo isomerase I at varying degrees. The compounds B1-B5 and C5 manifested an average of 46%, 20%, 40%, 22%, 24% and 22%, inhibition on topoisomerase I, respectively, which might suggest the cytotoxic actions of these compounds, previously reported by our laboratory, might be linked to DNA topoisomerase I inhibition. These compounds can be considered as the potential candidates for further studies in developing new cytotoxic and anticancer agents.

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Selection of high-affinity human monoclonal antibodies specific to the constant domain of versican as tools for tumor targeting

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Conventional cytotoxic therapies of cancer often suffer from a lack of specificity, leading to a poor therapeutic index and considerable toxicities to normal organs.One of the most promising new avenues for the development of more specific and efficacious cancer therapies relies on the selective delivery of therapeutics to the tumor site by conjugation with specific antibodies against tumor-associated markers. Markers expressed on the tumor's vasculature represent particularly attractive targets for site-specific drug delivery due to their accessibility for blood-borne agents and the various therapeutic options that they allow.In a study recently published in collaboration with our group1 a pool of tumor associated antigens that could be suitable targets for antibody-based anticancer therapy was identified. Among these potential tumor markers, versican was chosen for further studies. Versican is a member of the large aggregating chondroitin sulphate proteoglycan (CSPG) family. Structurally, versican is composed of a N-terminal G1 domain, two glycosaminoglycan (GAG) attachment regions and a C-terminal G3 domain. Alternative splicing generates at least four isoforms of versican, named V0, V1, V2 and V3. Versican is highly expressed in the early stages of tissue developments, during wound repair and tumor growth, this expression pattern of versican was proven to be a reliable prognostic factor and a good tumor marker in a number of publications.The aim of this work is to express recombinant forms of a constant domain of versican in different mammalian cell expression systems and use these recombinant proteins as target for selection of human monoclonal antibody in the scFv format from a large synthetic human antibody phage display library cloned in our lab2. The versican specific antibodies will be validated in vitro and in vivo before being used as building block for the development of antibody-based targeted anti-cancer therapeutics. (1) Castronovo, V.; Waltregny, D.; Kischel, P.; Roesli, C.; Elia, G.; Rybak, J. N.; Neri, D. Mol Cell Proteomics 2006, 5, 2083-91. (2) Silacci, M.; Brack, S.; Schirru, G.; Marlind, J.; Ettorre, A.; Merlo, A.; Viti, F.; Neri, D. Proteomics 2005, 5, 2340-50.

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Toxicological evaluation in non human primates of the mAb h-R3, used in the treatment of the cancer

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The humanized monoclonal antibody (mAb h-R3) is a product that will be dedicated to the treatment in the human, for intravenous route, of the neoplasic of transformed cells that they on-express the receiver of the Factor of Epidermal Growth in head and neck. The objective was to evaluate the toxicity for intravenous route of the mAb h-R3 in two studies to dose repeated respectively in monkeys Cercopithecus aethiops of 14 days and 26 weeks, three experimental groups conformed by group control, treated low and high dose of 2.85 and 28.57 (mg/Kg) respectively. Deaths were not observed, the body weight had a significant increase for weeks, toxic effects were not observed in the hematological and sanguine chemistry parameters. In the electrocardiography registrations, it was observed a I increase fast of the heart frequency in animals treaties, There were neither neurotoxic effects on the studied variables nor macro and microscopic lesions in the skin.

POSTER SESSION

Signalling pathways 3

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Butyrate simultaneously activates extrinsic and intrinsic apoptosis in colon adenoma andcarcinoma cell lines

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It is an accepted fact that butyrate, a product of bacterial fermentation of dietary fibre in the colon, induces apoptosis in colon cancer cells and may therefore be important in secondary chemoprevention of colorectal cancer. Since controversial results are available concerning the molecular mechanisms of butyrate-induced apoptosis, we analyzed how butyrate influenced molecular parameters of extrinsic and intrinsic apoptosis, respectively, and compared the effects in a human colon adenoma (LT97) and a colon carcinoma cell line (HT29). Effects of butyrate on caspase (-2, -3, -8 and -9) activity was analysed using ApoAlert® caspase activity profiling plates (Clontech). Protein activation of Bid was investigated using Western Blotting and mRNA expression of Bid, TRAIL, DR4 and DR5 was examined by real-time RT-PCR.Butyrate increased activity of caspase-2, -3, -8 and -9 in both HT29 and LT97 cells, with LT97 cells being more susceptible to the treatment. Simultaneous activation of caspase -8 and -9 is a hint that extrinsic as well as intrinsic apoptosis signaling was turned on by butyrate. Consequently, the BH3-only protein Bid, a member of the Bcl-2 protein family which connects extrinsic and intrinsic apoptosis, was activated by butyrate treatment in both cell lines as shown by Western Blotting using specific antibodies against Bid. On the mRNA level, however, Bid was not modulated by butyrate in either cell line, indicating the involvement of post-translational mechanisms. Activation of Bid was more pronounced in LT97 cells, demonstrating again an increased sensitivity of LT97 adenoma cells towards butyrate treatment. Gene expression of TRAIL receptors DR4 and DR5 was induced by butyrate in both cell lines, with LT97 cells showing a greater induction than HT29 cells. Gene expression of the ligand TRAIL, on the other hand was only increased in HT29 cells. Thus, different mechanisms may be involved in activation of apoptosis in HT29 and LT97 cells. In conclusion, extrinsic and intrinsic apoptosis is simultaneously activated by butyrate in colon cancer cells and this activation is mediated by increased Bid protein activity and increased mRNA levels of death receptors. The importance of butyrate in secondary chemoprevention of colorectal cancers is highlighted by the fact that LT97 adenoma cells, were more sensitive towards butyrate than HT29 carcinoma cells. Therefore butyrate may inhibit the formation of malignant tumors by killing early stage adenoma cells.

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ILEI, an essential cytokine for tumor progression - how does it act?

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ILEI (Interleukin-like EMT Inducer) is essential for tumor formation and progression in a murine mammary epithelial cell model. Stable expression