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.