II inhibit phosphorylations of the retinoblastoma protein, with a direct impact on cell cycle progression and proliferation. However, increased cellular effectivity of olomoucine II over roscovitine that probably stems from its higher affinity to CDK9, predestines this pair of compounds for comparative studies. One of the processes affected by inhibition of CDK9 is p53-dependent transcription. We therefore compared effect of both inhibitors in two multiple myeloma cell lines that differ in p53 status; RPMI-8226 bears temperature sensitive p53 (E285K), while U266 expresses completely inactive protein (A161T). When kept at 37°C, the inhibitors reduced phosphorylation of pRB and induced apoptosis in both cell lines in a dose-dependent manner, but did not influence level of p53. Conversely, p53 and Mcl-1 protein levels, as well as fragmentation of PARP were significantly changed in RMPI-8226 cultivated at 32°C. Although it was previously shown that CDK inhibitors trigger apoptosis in cell lines regardless of p53 status, we demonstrated that active p53 contributes to induction of apoptosis in multiple myeloma cells by roscovitine. The obtained data are in line with the findings that roscovitine targets not only cell cycle machinery, but also transcriptional CDKs, and that this combination is advantageous for the therapy. Currently, combination therapies to increase the potency of individual agents are often used, but with roscovitine multiple processes are targeted simultaneously.

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Updates in diagnosis and treatment of chronic myeloid leukemia

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Background: Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder, which accounts for about 15% of all leukemia cases in adults. Imatinib mesylate (STI 571) is a recently developed promising targeted treatment option for CML, but data on its efficacy and safety are still scanty. The aim of the study was to evaluate the diagnosis assertion, the short-term results and the safety of STI 571 therapy in patients with different phases of CML.

Materials and methods: Fourteen GIPAP qualified CML patients (males – 6, females – 8) at the age of 14 – 55 years have been treated with imatinib mesylate, and followed up between 2006 – 2008 at the Hematology Division of the Institute of Oncology. Cytogenetic examination of the bone marrow cells revealed Ph-chromosome and BCR-ABL p210 oncogene in all cases. However, the rate range of t(9; 22)-positive myeloid cells was 55 – 100%. Seven (50.0%) patients had been diagnosed in the chronic phase, 3 (21.4%) – in the accelerated, and 4 (28.6%) – in the acute phase of CML. Leukocyte count ranged between 6.6 - 205.0 x 10^9/l, thrombocyte count - 226,8 - 2340.0 x 10^9/l. The initial dosage of STI 571 varied between 400 – 800 mg daily, depending on CML phase. All the patients had previously failed to respond, or relapsed after the treatment with conventional chemotherapy regimens and interferon.

Results: The period diagnosis date – STI 571 starting date ranged from 1.5 to 58 months (median – 22.7 months). Complete hematologic response had been achieved in 10 (71.4%) patients within 1 – 3 months of the therapy with STI 571 ($p < 0.05$). A trend to the earlier complete hematologic response was observed in cases with chronic phase, shorter duration of CML, and lower leukocyte count ($p < 0.05$). Two (14.3%) patients with the acute CML phase have experienced clinical and hematologic improvement on the date of inclusion in the current study. The cytogenetic examination of the bone marrow cellular elements performed within 4 – 8 months of the treatment with imatinib mesylate established the decrease of Ph-positive myeloid cells up to 20 – 33%. Only 2 (14.3%) patients with acute phase failed to respond to imatinib mesylate ($p < 0.05$). Frequently registered side effects were dry mouth, angioedema, nausea, dyspepsia, abdominal pain, neutropenia, and thrombocytopenia, occurred in different combinations in 5 (35.7%) cases. Marked neutropenia developed in 3 (21.4%) patients, that required temporary cessation of treatment.

Conclusions: The combined screening for Ph-chromosome and BCR-ABL p210 oncogene is highly useful for diagnosis assertion in patients fairly suspected for CML. Imatinib mesylate may be considered as an effective and tolerable targeted medication for CML patients, even in those initially managed with conventional chemotherapy and interferon. A shorter duration of CML is associated with better response to imatinib mesylate.

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The induction of orphan nuclear receptor Nur77 expression by n-butylenephthalide aspharmaceuticals on hepatocellular carcinoma (HCC) cells therapy

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N-butylidenephthalide, isolated from the chloroform extract of Angelica sinensis, has been examined for its antitumor effects on glioblastoma multiforme brain tumors; however, little is known about its antitumor effects on hepatocellular carcinoma cells. Two hepatocellular carcinoma cell lines, HepG2 and J5, were treated with either nbutylidenephthalide or a vehicle, and cell viability and apoptosis were evaluated. Apoptosis-related mRNA and proteins expressed, including orphan receptor family Nurr1, NOR-1, and Nur77 were evaluated as well as the effect of n-butylidenephthalide in an in vivo xenograft model. N-butylidenephthalide caused growth inhibition of both the cell lines at 25 μg/ml. Further, n-butylidenephthalide-induced apoptosis appears to be related to Nur77 translocation from nucleus to cytosol, which lead to cytochrome c release and caspase-3-dependent apoptosis. N-butylidenephthalide-related tumor apoptosis was associated with PI3K/AKT/GSK3β rather than the MAPK or PKC pathway. Blockade of AKT activation enhanced proliferation inhibition and the induction of phospho-Bcl-2 and Nur77 proteins. Nur77 short interfering RNA (siRNA) blocked n-butylidenephthalideinduced apoptosis in J5 cells, and n-butylidenephthalide treatment increased luciferase activity of Nur77 in J5 cells. Administration of n-butylidenephthalide showed similar antitumoral effects in both HepG2 and J5 xenograft tumors. N-butylidenephthalide-induced apoptosis in hepatocellular carcinoma cells, both in vitro and in vivo, suggesting a potential clinical use of this compound for improving the prognosis of HCCs.

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Design, characterization and in vitro applications of novel chemotactic peptide-based drug delivery systems against cancer

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In the field of targeted drug delivery, numerous bioconjugates have been developed to enhance the efficiency and specificity of novel antitumor therapeutics. These kind of drug delivery system usually consist of a carrier component, a drug or drugs and targeting moieties. During the past decade, several carrier systems (e.g. liposomes, niosomes, nanoparticles, microparticles, cyclodextrins, polymers etc) have been envolved depended on the target organ. Forasmuch the receptor mediated endocytosis may provide the appropriate pathway for cellular uptake, targeting moieties have modulated the palette of drug delivery systems.

The goal of this project was to develop a targeted peptide-based drug delivery system for the treatment of cancer. Drug-conjugates consist of methotrexate as drug, GFLGC pentapeptide as enzyme-degradable spacer sequence, Tp20 (H-$[TKPPR]_4$-NH_2) as oligopeptide carrier and TKPR, For-TKPR, TKPPR, For-TKPPR as targeting peptide moieties were designed, synthesized, characterized and applied in several biological system.

Carriers with targeting moieties in branches were synthesized by solid phase synthesis using mixed Boc and Fmoc strategies. Drug molecules with enzyme-degradable spacer were attached to the carrier system in solution. The bioconjugates were characterized by analytical HPLC and ESI-MS.

In vitro biological assays such as chemotaxis, internalization and citotoxicity were investigated. The bioconjugates and their components (carrier, targeting moieties and drug-spacer) were studied on Tetrahymena pyriformis, THP-1 human tumor cell line. Cellular uptake of the fluorescent-labeled analogues was studied by flow cytometry. Most of the conjugates had advatageous chemotactic properties, they can be internalized rapidly and could trigger toxic effect on the cells.

Our results confirmed the feasibility of this novel drug targeting strategy for increasing the efficacy and specificity of chemotherapy.

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Evaluation of gonadotropin-releasing hormone analogues in mice - pharmacokinetic studies and biomarker based efficacy by mass spetrometry

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Background: The study of pharmacologically active peptides is central for the understanding of diseases and the development of novel therapeutic

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.