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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543 Poster

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.

544 Poster

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.

545 Poster

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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546 Poster

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