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Cyclin-dependent kinase (CDK) inhibitor CYC202 (R-roscovitine) induces clock gene mPer2 mRNA expression rhythm in tumor: relevance for antitumor efficacy

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Background: G2/M and G1/S gating are negatively controlled by the molecular circadian clock (Fu et al., Cell 2002; Matsuo et al., Science 2003). Common regulatory pathways are shared by the circadian clock and the cell cycle (Canaple et al., Cancer Res 2003).
Methods: We examined the relation between CYC202 antitumor efficacy and the circadian rhythm in clock gene *mPer2* mRNA expression in liver and tumor. Glasgow osteosarcoma was inoculated to 132 male $B6D2F_1$ mice synchronized with 12 hours (h) of light (L) and 12 h of darkness (D). Nine days (d) later, CYC202 (300 mg/kg/d) or vehicle (50 mM HCl) were given daily $\times$ 5d to 96 treated and 36 controls, with tumor weight of 100 to 300mg. CYC202 or vehicle was administered via oral gavage at one of three circadian times (CT) 8 h apart, during L (3 or 11 Hours After L Onset-HALO) or D (19 HALO). Liver and tumor were sampled at four CT, 6 h apart (5, 11, 17 or 23 HALO) in order to determine mRNA expression of *mPer2* and *36B4* as a reference invariant gene using quantitative PCR with Light Cycler.
Results: Tumor growth was slowed down in the treated animals as compared to controls in a dosing time-dependent fashion. CYC202 was more effective if it was given at 3 or 11 HALO as compared to 19 HALO Over the 5-day treatment span, the average tumor weight increased by 711 ± 53 mg in controls and by 331 ± 33 mg, 317 ± 47 mg and 461 ± 38 mg in the mice treated at 3, 11 or 19 HALO respectively (ANOVA $p < 0.001$)). In control mice, mean *mPer2* expression was 10 times as low in tumor as compared to liver. Mean *mPer2* expression increased ~3.5-fold from a nadir at 5 HALO to a peak at 15 HALO in liver (p from ANOVA <0.0001) whereas significant rhythm was found in tumor. CYC202 administration markedly altered the 24-h pattern of *mPer2* in liver, as the 24 h-rhythm was ablated in the mice treated at 19 HALO. Conversely a highly significant rhythm in *mPer2* expression was induced in tumor, with a peak during L for the most active schedules (3 and 11 HALO) and during D for the least effective one (19 HALO).
Conclusions: The anti-tumor efficacy of CYC202 may relate to the ability of this drug to induce a circadian rhythm in clock gene *mPer2* expression. Optimal efficacy was associated with peak *mPer2* expression during light (rest span) in tumor, raising the possibility that such timing could be important to best down regulate cell cycle progression in malignant cells.
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In vivo and in vitro anticancer activity of the lanthanum compound KP772 (FFC24)

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Background: The aim of this study was to determine the in vivo and in vitro anticancer activity and in vivo toxicity of the 1,10-phenanthroline-derived lanthanum compound KP772 (FFC24) as well as molecular mechanisms which determine cellular KP772 sensitivity/resistance.
Materials and Methods: In vivo toxicity was examined in Sprague Dawley rats and outbred albino mice. In vivo efficacy was assessed in DLD-1 xenografts (4, 8, or 12 mg/kg i.v. qdx5). In vitro cytotoxicity exerted against 6 tumor cell lines and their chemoresistant sublines (over-expressing either P-gp, LRP, MRP1, or BCRP) was determined by MTT-assay. Induction of apoptosis was investigated by DAPI staining. Mitochondrial membrane depolarisation and cell cycle analyses were performed by FACS. DNA damage was determined by comet assay.
Results: The LD_{50} and no-observed-adverse-effect level (NOAEL) of KP772 in rats were 21.6 mg/kg (11.5 mg/kg for females alone) and 7.5 mg/kg, respectively. In mice, the LD_{50} and NOAEL were 62 mg/kg (26.6 mg/kg for males alone) and 10 mg/kg, respectively. In DLD-1 xenografts the activity in terms of relative tumor volume doubling time was comparable to cisplatin and methotrexate without significant adverse effects.
In vitro IC_{50} of KP772 at 72 hrs exposure were in low μM range. None of the multidrug resistant cell models displayed reduced KP772 sensitivity. In contrast, all ABC-transporter-over-expressing (P-gp, MRP1 and BCRP) cells were KP772-hypersensitive. Apoptosis, which was already induced at 5 and 10 μM KP772 at 12 hours treatment, was demonstrated by PARP and caspase 7 cleavage. Morphologically, cells showed classical signs of apoptosis (cell shrinkage, condensed chromatin). Almost no mitochondrial membrane depolarisation was observed after 24 hours drug incubation. Treatment with KP772 led to massive G_0/G_1 block within 12 hours, paralleled by a decrease of cyclin B and E but not cyclin A. Comet assay indicated no DNA damage after exposure to KP772. Correspondingly, N-acetylcysteine had not protective activity indicating that cytotoxicity was not based on oxygen radical formation.
Conclusions: KP772 has promising anticancer activity in vitro and in vivo and comparably mild adverse effects. The drug exerts its anticancer activity via a potent cell cycle arrest and induction of apoptosis. Cell death is not based on DNA damage or radical formation but a more complex mechanism, which seems especially active against ABC-transporter overexpressing cells.

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The role of p27 in efficacy of chemo- and radiosensitivity of primary mouse squamous carcinoma cells

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The cyclin-dependent kinase inhibitor and tumor suppressor $p27_{Kip1}$ mediates G1-arrest induced by several stimuli. Interestingly, it was found that p27 expression levels positively correlate with patient survival prognosis in a wide range of human tumors. That can be partially explained by the growth inhibitory role of p27. However, patient survival also critically depends on responsiveness towards chemotherapy. Therein the role of p27 is unclear.
While p27 over-expression seems to inhibit tumor cell growth *in vitro*, high p27 levels can even reduce the sensitivity of cells towards many cell cycle-dependent cancer therapeutic agents or radiotherapy. In contrast, in some cell lines (such as squamous head and neck cancer cells) other agents such as the newly discovered drug UCN-01 up-regulate p27 protein. Therefore it has been hypothesized that the efficacy of UCN-01 critically depends upon p27 expression and thereby induces G1 arrest.
Here we will present data about the effect of p27 expression on efficacy of short term tumor therapy. We have analyzed the response of mouse squamous skin carcinoma cells derived from carcinogen-treated p27 deficient, p27 heterozygous and p27 wt mice towards UCN-01, and the cell cycle-dependent therapy by 5-FU and gamma irradiation. Interestingly, the expression levels of p27 do not seem to have a significant effect on toxicity of the therapeutic agents, as determined by MTT survival assays, apoptosis assays and colony assays.
These results demonstrate that in our primary squamous carcinoma cells p27 is not necessary for efficient tumor therapy with UCN-01. In addition, we show that down-regulation of p27 by gene targeting in mice, which leads to physiological relevant p27 protein levels similarly as observed in human tumors, is not indicative of a better therapy response (5-FU and radiotherapy) *in vitro*.

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PTEN inactivation is a common event in childhood leukemia

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Background: The candidate tumor suppressor gene PTEN, or MMAC1, located at chromosome 10q23.3 encodes a 403 amino acid dual-specificity phosphatase and is frequently altered in a number of solid tumors. Loss of PTEN expression is not consistently correlated with genetic or epigenetic alterations. Little is known about the status of PTEN in hematological malignancies. To evaluate the role of the PTEN/MMAC1 gene in leukemia, especially in childhood leukemia, we analyzed 11 leukemia cell lines and 17 primary pediatric leukemia patients for promoter methylation and expression in PTEN and pseudo PTEN (ψ PTEN) loci.
Material and Methods: We used methylation specific PCR to assess PTEN and ψ PTEN methylation in both leukemia cell lines and bone marrow mononuclear cell (BMMC) samples from primary pediatric leukemia, and RT-PCR technique and western blotting to analyze gene transcript and protein expression respectively.
Results: 10% (1/10) of cell lines was methylated at PTEN promoter region and 70% (7/10)of the cell lines were methylated at ψ PTEN locus. 18% (3/17) of BMMC samples were found methylation positive at PTEN and ψ PTEN loci. 27% (3/11) of the leukemia cell lines were found no protein expression, and 18% (2/11) dramatically reduced. Analysis of 17 BMMC samples from primary pediatric leukemia, and revealed that PTEN protein was absent in 41% (7/17), and reduced in 24% (4/17).
Conclusion: PTEN protein loss is a common event in leukemia cell lines and childhood leukemias, and it may play an important role in the development of this disease. Since only 18% of BMMC samples