and T315I mutant BCR-ABL (Ki's of 30 and 40 nM, respectively) and Jak2 (Ki 123 nM). Clinical investigation of MK-0457 in patients with solid and haematologic malignancies is ongoing. Aurora kinase activity is essential for microtubule spindle assembly and cytokinesis. The effects of MK-0457 and the microtubule stabilizer Dtx alone or in combination were evaluated in cancer cell lines.
Methods: A panel of eighteen NSCLC cell lines (wild-type, mutant, or p53 null) and a pair of genetically engineered p53 wild-type or p53 null alveolar epithelial cell lines were exposed to MK-0457 and/or Dtx. Cell Titer Glo (Promega) was used to measure cell viability. Cell cycle profiles were evaluated by FACS analysis. Colony formation assays were also performed (in soft agar and on plastic). The Bliss Independence method was used to assess the combinatorial effects of MK-0457 and Dtx.
Results: NSCLC cell lines showed variable single agent sensitivity to MK-0457 (IC50 range: 50 nM to >5 mM) and Dtx (0.4 to 10 nM). By FACS analysis, MK-0457 induced G2/M arrest and polyploidy, characteristic of aurora kinase inhibition. The combinatorial effects of MK-0457 and Dtx ranged from antagonism to synergy and were sequence, concentration, and cell context dependent. In viability assays, simultaneous exposure to MK-0457 and Dtx did not reveal synergy, however sequential exposure yielded synergy at low concentrations of both agents. Simultaneous exposure to MK-0457 and Dtx in long-term colony formation survival assays enhanced cell death compared to either single agent.
Conclusions: MK-0457 in combination with Dtx may result in synergistic anticancer activity, particularly in long-term CFU survival assays. Evaluation of MK-0457 and Dtx combination regimens in xenograft models is warranted.

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Preliminary microscopic evaluation of 64CuATSM as a PET radiotracer for tumour hypoxia

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Background: Tumour hypoxia results in a more aggressive phenotype together with resistance to treatment. 64CuATSM is being investigated as a positron emitting tomography (PET) radiotracer for hypoxia. Validation would provide clinicians with a non-invasive technique for tumour hypoxia assessment. This information could be used as a tool for determining most appropriate cancer therapy, prognostic information and subvolume delineation for radiotherapy dose escalation to radioresistant hypoxic regions. We looked for a correlation between 64CuATSM uptake and immunohistochemical marker of hypoxia pimonidazole in a tumour model.
Methods: Five BD-9 rats had syngeneic P22 carcinosarcoma allografts implanted subcutaneously in the left flank. After 14 days when the tumours had reached a size of 1.5 cm (approx) in diameter the rats were selected for study. Pimonidazole (60 mg/kg) i.p. was given at time = 0 hours. Anaesthetisia was administered i.p. and venous and arterial access was obtained. At time = 3 h the rats received a bolus i.v. injection of 64CuATSM (mean dose 37.17 MBq). At time = 4.25 h the animals were sacrificed and tumours resected. The tumours underwent rapid formalin fixation, wax embedding and 5 μm sections were taken. These were placed in a cassette and exposed to a phosphor screen for detection of 64CuATSM distribution. A StormTM phosphor imager obtained images at 10 days using ImageQuantTM software. The same slides were then stained for pimonidazole with HypoxyprobeTM-1 (Chemicon International). The distribution of 64CuATSM from autoradiographic detection was compared with pimonidazole distribution using linear unmixing tool TRI2 (Gray Cancer Institute in-house software) after microscopic image capture by an in-house spectral imager. Paint Shop Pro 7TM was used to orient and co-register the two distributions and ImageJ software (National Institutes of Health) was used to compare autoradiographic and pimonidazole intensity levels on a pixel by pixel basis.
Results: There was no statistically significant correlation (range −0.108 to 0.0382) between pimonidazole and 64CuATSM distribution.
Conclusion: 64CuATSM uptake in P22 carcinosarcoma in this animal model is not representative of hypoxia in the time scale indicated. It has been correlated with immunohistochemical markers of hypoxia on a microscopic level in some, but not all, rodent tumour models however in this model it is likely that other factors that determine tumour distribution of 64CuATSM dominate.

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Loss of IFN gamma sensitivity is accompanied by constitutive expression of SOCS3 and attenuation of SOCS genes induction in melanoma

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Background: The resistance to interferons (IFNs) limits their anticancer therapeutic efficacy but no attempts were made to correlate expression levels with IFN sensitivity.
Purpose: We aimed to investigate the relationship between IFN sensitivity and expression of STAT and SOCS genes.
Material and Methods: We used two subclones of human malignant melanoma WM1158 line that differ in IFNγ sensitivity. Repeated cloning of the parental cells resulted in the isolation of resistant WM1158R and sensitive WM1158S sublines.
Results: We studied the evolution of an IFN-resistant state in vitro using melanoma sublines. We found that the cells became less sensitive to antiproliferative effect of IFNγ after prolonged cultivation. While IFNγ retarded the growth of WM1158S by 80−90%, the growth of WM1158R was significantly less inhibited. The growth properties and cell morphology of both subclones in the absence of IFNγ were similar. The antiproliferative effect of IFNγ was further studied in several additional melanoma lines. These cells can be categorized into high (WM1158S, WM39, WM1552C), medium (WM1158R) and low (WM9, 1205Lu) sensitive ones.
We investigated transcription of STAT1−6 and SOCS1−3 genes as well as phosphorylation of STAT1 protein. WM1158R differed from WM1158S by a constitutive expression of SOCS3, weak SOCS1−3 induction after IFNγ, and short duration of cytokine activatory signal. Similar correlations were observed in additional melanoma lines differing in IFN sensitivities. At the protein level, IFNγ induced strong and prolonged STAT1 activation at S727 in WM1158R while this phosphorylation was less pronounced in WM1158S. On the other hand, phosphorylation of Y701 was stimulated regardless of the sensitivity phenotype.
Conclusions: Prolonged maintenance of melanoma cells in cell culture may lead to reduction of their sensitivity to IFNγ. At the molecular level, this process is associated with increased constitutive expression of SOCS3 whose levels are no longer or marginally influenced by IFN signals. Our data suggest that changes in the SOCS3 expression are tightly bound with the progression of melanoma cells from IFN sensitive to IFN resistant phenotype and may account for a growth advantage of melanoma in vivo at its advanced stages.
Supported by the Internal Grant Agency of the Czech Ministry of Health (NR/8341−3), the Grant Agency of the Czech Republic (301/06/0912) and Academy of Sciences of the Czech Republic (A50040507).

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Improved in vitro and in vivo anti-tumor efficacy of glucosylceramide-enriched liposomal doxorubicin

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Introduction: The continued evolution of liposomal therapeutics has resulted in new agents with remarkable antitumor efficacy and relatively mild toxicity profiles. Anti-cancer drugs generally have intracellular targets, implicating transport over the plasma membrane. For amphiphilic agents, such as the anthracycline doxorubicin, this occurs by passive diffusion. We investigated whether exogenous short-chain sphingolipid analogues improve doxorubicin influx in vitro as such and when co-administered in a liposomal formulation. Furthermore, the efficacy and toxicity of sphingolipid-modified liposomal doxorubicin on tumor growth in vivo were studied.
Material and Methods: Combinations of drugs and lipid analogues were co-administered to various (tumor) cell lines, and subsequent drug accumulation in cells was quantified. For in vivo studies, BALB/c nude mice were subcutaneously inoculated with A431 squamous carcinoma cells. The anti-tumor efficacy of sphingolipid-modified liposomal doxorubicin was compared to standard liposomal doxorubicin in a dose-escalation study. Tumor growth and regression, as well as changes in bodyweight were measured for a period of 2 weeks.