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.
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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.

Results: N-octanoyl-glucosylceramide (GC) was identified as a potent enhancer of drug uptake in vitro. Enhanced cellular uptake strongly correlated with amphiphilicity. Whereas the sphingolipid analogue itself was not toxic, incorporation of 10 mol% GC in doxorubicin-containing liposomes significantly enhanced their cytotoxicity in A431 cells resulting in an increase in EC50 values up to 10 fold, as compared to standard liposomal doxorubicin. In vivo studies confirmed the in vitro observations. Enhanced efficacy of GC-enriched doxorubicin liposomes over standard doxorubicin liposomes towards A431 human tumor xenografts in nude mice was demonstrated. With respect to tumor growth and toxicity the optimal concentration of GC-enriched and standard doxorubicin liposomes was set at 6 mg doxorubicin/kg bodyweight. The tumor growth delay for reaching 200% initial volume was 6 and 11.5 days (2-fold delay) for mice treated with standard liposomal doxorubicin and GC-enriched liposomes, respectively, as compared to untreated animals.
Conclusions: Short chain sphingolipids can be used as enhancers for delivery of amphiphilic compounds. GC-enriched doxorubicin liposomes displayed superior in vitro and in vivo anti-tumor activity, as compared to standard doxorubicin liposomes. Liposomal formulations enriched with short chain sphingolipids represent an advanced and versatile technology and provide opportunities for improving drug delivery of anti-cancer agents.

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In vitro and in vivo activation of the tumor suppressor Lats1 by the transcriptional regulator CDP/Cux

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The transcription regulator CDP/Cux binds to distinct promoter regions and up-regulates or down-regulates transcription of several genes involved in cell cycle and tumorogenesis. We tested distinct promoter regions of several genes involved in tumorogenesis and found the CDP/Cux regulates transcription of the tumor suppressor gene Lats1. We utilized scanning ChIP and identified the exact region bound by one of the short isoforms of CDP/Cux (p110) in the Lats1 gene region. We therefore tested regulation of Lats1 transcription in vitro by both short isoforms of CDP/Cux (p110 and p75) and demonstrated that the short isoforms up-regulates transciption of this tumor suppressor. Several tumor cell lines over-expressing distinct short isoforms of CDP/Cux were tested for Lats1 expression and likewise revealed an increase in Lats1 transcription. Additionally, transgenic mice over-expressing the short isoforms of CDP/Cux and developing mammary gland tumor, uterine tumors and myeloproliferative disease like myeloid leukemia show an enhance in transcription of Lats1. We postulate that CDP/Cux regulation of the tumor suppressor gene Lats1 in cell lines and tumor cells may express an altered Lats1 protein that has an altered role in tumorogenesis.

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−308G>A TNF-alpha polymorphism is a genetic susceptibility marker for nasopharyngeal carcinoma development

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Introduction: Nasopharyngeal carcinoma (NPC) is a viral associated neoplasia, extremely rare in western populations, on which genetic polymorphisms related to immune response have been associated to its development. The Tumor Necrosis Factor Alpha (TNFα) is a pro-inflammatory cytokine that has been associated to several cancers, especially to viral associated neoplasia. We have designed a study to analyse the role of a common Single Nucleotide Polymorphism (SNP) on the promoter region of the TNFα (−308G>A) on the development of NPC.
Material and Methods: We developed a cross-sectional study considering a total of 547 individuals from the Northern Region of Portugal, including 101 patients with the undifferentiated type of NPC (UNPC) and 446 healthy individuals without evidence of neoplastic disease. The genetic analysis was performed by Real-Time PCR with a TaqMan® SNP Genotyping Assay from Applied Biosystems (Assay C___7514879_10).
Results: This study revealed an increased frequency of the −308A TNFα allele in patients with UNPC rather than in healthy individuals, which represents almost a five-fold risk increase for −308A homozygous (p = 0.002; OR = 4.67; 95% CI 1.21–5.90). Moreover, logistic regression analysis revealed that having −308A homozygosis (p = 0.010; OR = 4.24; 95% CI 1.41–12.73), being male gender (p = 0.002; OR = 2.11; 95% CI 1.31–3.40) and having age >49 at diagnosis (p = 0.001; OR = 2.12; 95% CI 1.36–3.32) can represent predictive factors for the development of NPC.
Conclusions: These results confirm that in a Portuguese population −308A TNFα homozygosis can represent a risk factor for NPC development, and also corroborate data from previous studies where male gender and age >49 at diagnosis are known as specific markers for NPC, contributing for the knowledge of NPC aetiology.

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P53 codon 72 PRO/PRO genotype is a genetic susceptibility maker for gastric adenocarcinoma development

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Introduction: Gastric adenocarcinoma (GC), a major public health problem worldwide, has been associated with several gene deregulations. TP53 gene encodes for the p53 protein, one of the key genes on cancer development. Moreover, p53 has been suggested as altered in over 50% of all human cancer cases. Genetic polymorphisms have been analyzed by several studies as genetic markers for cancer development. A common polymorphism on p53 codon 72, which causes a replacement of an Arginine by a Proline aminoacid, has been suggested as a susceptibility factor for the development of several cancers. We have attempted to evaluate the role of the p53 codon 72 polymorphism in the development of GC in a population from the Northern region of Portugal.
Materials and Methods: A cross-sectional study was performed in 427 individuals, considering both patients with histological confirmed GC (n = 126) and healthy blood donors without evidence of neoplastic disease (n = 281). DNA was extracted from peripheral blood leucocytes and genetic analysis was performed by Real-Time PCR with a TaqMan® SNP Genotyping Assay from Applied Biosystems (Assay C___2403545_10).
Results: Our results revealed and increased frequency of the Pro allele in patients with GC than in healthy donors (46.0% vs 36.8%). We also found an almost three-fold increase risk of GC development among Pro/Pro genotype (p = 0.015; OR = 2.58; 95% CI 1.18–5.66), which is also confirmed by logistic regression adjusted for age (p = 0.015; OR = 2.72; 95% CI 1.21–6.07). Moreover, Pro/Pro homozygous seem to have s shorter median time-to-onset of GC (68.0 months vs 75.0 months; p = 0.030).
Conclusions: TP53 is an import gene in cell regulation and has a major role on cancer development. Previous studies have revealed that the p53 codon 72 polymorphism seems to influence the risk for cancer development since the two polymorphic variants of p53 might different regulations. Our study reveals that p53 codon 72 Pro allele represents a susceptibility marker for GC development and might contribute to the understanding of GC etiology.

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ERK activity in B-1 cells is important for increasing the metastatic potential of murine melanoma cells

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Introduction and Objectives: B-1 cells are the most prevalent linage of B cells in the peritoneal and pleural cavities of mice. Previous studies in our group demonstrated that co-cultivation of B-1 cells with B16F10 murine melanoma cells increases the metastatic potential of the latter. However, the mechanisms underlying how B1 cells influence the intracellular signaling pathways involved in this effect have not yet been addressed. Among the signaling pathways, activation of the extra-cellular signal-regulated kinase (ERK) and protein kinase C (PKC) have been correlated with metastatic spread in several cancer types, including melanoma. Therefore, the aim of this work was to investigate whether B1 cells increases the metastatic potential of B16F10 melanoma cells by modulating the activation of ERK and/or PKC in these cells.
Methods and Results: Protein expression and phosphorylation status of PKC and ERK were evaluated in lysates of melanoma cells co-cultured or not with B-1 cells in the presence or absence of pharmacological inhibitors of either PKC (Gö6976) or ERK (PD98059) by western blotting. The biological effects of these inhibitors were studied by experimental metastases assays in vivo. We showed that (1) ERK is constitutively