**439** POSTER

**MKC-1 a novel cell cycle inhibitor: preclinical studies to support Phase 2 clinical trial evaluations in pancreatic and non-small cell lung cancers (NSCLC)**

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MKC-1 (previously Ro 31–7453) was identified in a screen for cyclin dependent kinase (cdk) inhibitors. While it has weak affinity for cdks, treatment of cells with MKC-1 results in G2/M cell cycle arrest and broad antiproliferative activity in tumor cell lines. To determine if MKC-1 inhibits other kinases, we evaluated MKC-1 against a panel of 100 recombinant kinases. GSK-3beta was revealed as the only kinase significantly inhibited (IC50 7 nM). In vitro screening indicates pancreatic cell lines are particularly sensitive to MKC-1 activity, which may be due in part to the contribution of GSK-3beta to proliferation and survival in these lines (Ougolkov, et al). Several non-enzymatic targets for MKC-1 have been identified, including members of the importin β family, and the colchicine binding site of tubulin. These binding partners are consistent with effects observed in cells treated with MKC-1, such as aberrant mitotic spindle formation and cell cycle arrest. Functional consequences of the binding of MKC-1 to importin β are currently under investigation. A decrease in levels of HIF-1α, activation of pNF-κB and pStat3 and transcriptional activity of the three transcription factors is observed after 24 h treatment.
MKC-1 has demonstrated significant antitumor activity in several preclinical tumor models. Additionally, in Phase 1 and 2 clinical studies, this orally active agent has shown signs of efficacy with partial responses in NSCLC and breast cancer, and minor responses and tumor marker reductions in NSCLC, breast, pancreatic and ovarian cancer patients. The DLT was neutropenia, which is typical for this class of compounds. Importantly, no neurotoxicity, cardiotoxicity or secretory diarrhea was observed. Clinical studies are underway to further evaluate the recommended Phase 2 dose of 125 mg/m$^2$ bid × 14 days × q4 weeks. In vitro antiproliferative studies using H2122, a NSCLC cell line, have demonstrated the combination of MKC-1 and Tarceva results in enhanced activity (CI < 1). In a H2122 human tumor xenograft on day 42, tumor growth inhibition was observed with both MKC-1 and Tarceva, and the combination resulted in additive antitumor activity (100 mg/kg MKC-1 33% TGI, 50 mg/kg Tarceva 53% TGI, and the combination 72% TGI). Our preclinical mechanism of action studies and combination studies are being used to support clinical trials in several new indications.

**440** POSTER

**The proto-oncoprotein SYT activates transcription by interacting with the SWI/SNF chromatin remodelling protein BAF250, whilst the synovial sarcoma oncoprotein SYT-SSX acts as a repressor of transcription**

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Synovial sarcoma is a highly malignant soft tissue sarcoma that accounts for about 7–10% of all human soft-tissue sarcomas and it is primarily seen in patients between the age of 15 and 40 years old. It can occur in any part of the body and nearly all synovial sarcomas have a specific t(X;18)(p11.2;q11.2) chromosomal abnormality that appears to be independent of tumour location. This translocation results in the fusion between the *SYT* gene on chromosome 18 and *SSX1*, *SSX2* or *SSX4* genes on chromosome X; the chimeric genes resulting from the translocation are *SYT-SSX1*, *SYT-SSX2* or *SYT-SSX4*. These gene products together with the fusion proteins are localised in the nucleus but excluded from the nucleoli. However, no DNA-binding domain has been identified in SYT or SSX. We have previously reported that both SYT and SYT-SSX proteins interact with the two DNA-dependent ATPases subunits of the chromatin remodelling complexes SWI/SNF named hBRM and BRG1. In this work we show that the transcriptional regulatory functions of SYT and SYT-SSX are exerted also through interaction with another chromatin remodelling protein called BAF250. BAF250 is the human homologue of the *Drosophila* Osa protein and BAF250 has been shown to mediate the interaction between the glucocorticoid receptor and the SWI/SNF remodelling complex. Here we identify the domains of interaction as the C-terminus domain of SYT, named QPGY, and the related repeat sequences present at the N-terminal end of BAF250. In both proteins the repeat sequences constitute very strong transcriptional activating domains. To further investigate the functionality of this interaction we have used a luciferase reporter construct containing glucocorticoid response elements transfected in T47D cell line (a breast carcinoma cell line which does not express BAF250). The SYT protein activates transcription only in presence of exogenous BAF250 and interestingly SYT-SSX acts as a repressor in the same reporter assay. We have also discovered that the transcriptional regulation involving SYT and SYT-SSX is hormone dependent. The interaction between BAF250 and SYT-SSX could be a potential new target area for the development of new therapeutic options for synovial sarcoma.

# Topoisomerase I inhibitors

**441** POSTER

**Imatinib mesylate potentiates topotecan antitumor activity in rhabdomyosarcoma preclinical models**

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**Background:** Imatinib mesylate (IM) is a protein tyrosine kinase inhibitor selective for bcr-abl, PDGFRα/β, c-kit, c-abl, ARG and c-fms. Recently, IM has also been shown to inhibit ABCG2 (breast cancer resistance protein, BCRP), a member of the ATP binding cassette (ABC) transporter superfamily that, extruding a variety of therapeutic compounds from tumor cells, can reverse drug resistance. In primary rhabdomyosarcoma (RMS), high levels of PDGFRα/β expression have been associated with disease progression. This study was aimed at investigating in RMS preclinical models the activity of IM as a single agent and in combination with topotecan (TPT), an ABCG2 substrate highly effective in advanced RMS.
**Materials and Methods:** Five human RMS cell lines of embryonal (RD, RD/18, CCA) and alveolar (RH30, RMZ-RC2) type were used. PDGFRα/β, c-kit, ABCG2 mRNA (real-time RT-PCR) and proteins (flow cytometry); PDGFRα/β and c-kit phosphorylation status (western blot); *in vitro* antitumor activity of IM and TPT alone and in combination (MTT assay); type of *in vitro* interaction of combined IM and TPT (Chou-Talalay combination index [CI] method); ABCG2-mediated Hoechst 33342 extrusion were evaluated. *In vivo* activity was assessed in RD and RH30 xenografts: IM (100 mg/kg/day), TPT (0.5 mg/kg/day), and the combination at the same dosage were given orally, 5 days a week, 3 consecutive weeks.
**Results:** PDGFRβ was expressed in all cell lines, with the highest levels in RD; PDGFRα and ABCG2 were detected at higher levels in RH30 and RMZ-RC2; c-kit was not expressed. Incubation with recombinant PDGF-BB showed an increase of PDGFRβ phosphorylation that was inhibited by co-incubation with IM. ABCG2-mediated Hoechst 33342 extrusion was effectively inhibited by IM in RH30 cells but only marginally in RD, and reflected the ABCG2 expression levels. *In vitro*, IM was active at concentrations not therapeutically achievable ($IC_{50}$ = 22.6–29.0 μM) but showed significant synergism with TPT in a wide range of concentrations (CI: 0.47−0.79). Significant synergism between IM and TPT was confirmed in RD and RH30 xenografts. No obvious toxicity was observed.
**Conclusions:** IM as single agent is marginally active in RMS preclinical models, but significantly potentiates TPT activity *in vitro* and *in vivo* through at least two mechanisms, inhibition of ABCG2 and/or PDGFRβ. In RMS, the combination of TPT and IM warrants further study in the clinical setting, especially in ABCG2-expressing tumors.

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**Pharmacokinetic of the novel oral camptothecin gimatecan in women with pre-treated advanced breast cancer**

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**Background:** Gimatecan (ST1481;7-[(E)-tert-butyloxyminomethyl]-camptothecin) is a new topoisomerase I inhibitor with a lipophilic substitution in position 7 of the camptothecin molecule to stabilize the lactone ring, thus improving the pharmacological properties. It showed striking activity against human tumor xenografts after oral administration.
**Aims:** To determine the pharmacokinetics of gimatecan given orally to patients participating to the phase II study performed in women with pre-treated breast cancer.