over-expression. Irofulven has demonstrated efficacy including partial and complete responses against the MV522 lung carcinoma xenograft model, an aggressive metastatic tumor that is refractory to treatment with standard chemotherapeutic agents. In this study, irofulven and gemcitabine (a pyrimidine antimetabolite) were combined to elucidate the efficacy of these two agents against MV522 lung carcinoma *in vitro* and *in vivo*. As determined by median-effect principle analyses, additive and synergistic antitumor activity was observed *in vitro* when MV522 cells were treated for 48 hours concurrently with combinations of irofulven (ranging from 25 to 250 ng/ml) and gemcitabine (ranging from 0.5 to 8 ng/ml). Female athymic nude mice implanted subcutaneously with MV522 tumors were treated with irofulven and/or gemcitabine on an intermittent dosing schedule (q3dx4). Mice administered 20 mg/kg/d of gemcitabine demonstrated 2 partial responses (PR) (mean tumor shrinkage of 69%), whereas 40 mg/kg/d gemcitabine produced 2 PR (mean tumor shrinkage of 42%). Irofulven given at 3 mg/kg/d demonstrated no PR or CR (mean tumor growth inhibition (TGI) of 43 %) and at 4.5 mg/kg/d doses displayed a mean TGI of 48% with 1 CR. In contrast to the limited activity produced by this sub-MTD dosing as monotherapy, marked antitumor activity was observed when the two agents were combined. Irofulven administered at doses of 3 mg/kg/d combined with 40 mg/kg/d gemcitabine produced 6 PR (mean tumor shrinkage 57%) and 1 CR. Furthermore, administration of 4.5 mg/kg doses of irofulven plus either 20 mg/kg or 40 mg/kg gemcitabine demonstrated 3 PR (mean tumor shrinkage 67%) and 6 CR, or 2 PR (mean tumor shrinkage 79%) and 8 CR, respectively. Minimal body weight loss (maximum -6.3%) demonstrated the limited toxicity of this combination. In summary, the combination of irofulven and gemcitabine produces greater than additive antitumor activity. This combination is currently being evaluated in a Phase I clinical trial.

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Characterisation of the roles of Topoisomerase I and II in the mechanism of action of novel anti-tumour agents XR11576 (MLN576) and XR5944 (MLN944)

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Topoisomerase I and II (Topo I and Topo II) are the targets of many antitumour agents currently used in the clinic. Their main mechanism of cytotoxicity involves stabilising an otherwise reversible DNA-topoisomerase covalent complex (cleavable complex). Collisions of DNA tracking proteins convert these complexes into permanent single or double strand breaks thus triggering cell death. XR11576 (MLN576) and XR5944 (MLN944) are two novel DNA targeting agents with potent activity against a panel of human tumour cell lines and human tumour xenografts in mice. Both compounds retain good activity in cell lines expressing either P-gp or MRP multidrug resistance *In vitro* cleavage data shows XR11576 and XR5944 stabilise cleavable complexes for both Topo I and Topo II in a dose dependent fashion. The agents are also able to overcome 'atypical' resistance due to down-regulation of Topo II alpha. These data therefore suggest that XR11576 and XR5944 can act as 'dual' inhibitors of both Topo I and Topo II. Cleavable complex formation by XR11576 and XR5944 has been analysed in human leukaemic K562 cells using the TARDIS assay. Data obtained showed that both drugs induced cleavable complex formation for both Topo I and Topo II (alpha and beta) in a dose and time dependent manner. The levels of XR11576 and XR5944 induced cleavable complexes were significantly higher than untreated controls. Interestingly, the cleavable complex formation was detectable only after a minimum of 24 hours of drug exposure and, at present, the reason for the marked time dependency remains unclear. XTT growth inhibition assays were performed at comparable exposure times to the TARDIS assay to correlate cytotoxicity with cleavable complex formation. Future studies will further investigate the role of Topo I and Topo II in XR11576 and XR5944 induced cell death.

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Release of GST-pi promoter hypermethylation and activity of brostallicin in human prostate cancer cells

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The a-bromoacrylic DNA minor groove binder brostallicin (PNU-166196), is a new promising anticancer agent which has shown an outstanding preclinical activity profile and is currently in Phase II clinical evaluation. Differently from several antineoplastic drugs, the activity of brostallicin is increased, either *in vitro* or *in vivo*, in cells expressing high levels of glutathione (GSH) and glutathione S-transferases (GST). Among the isoenzymes, GST-pi is the stronger activator of brostallicin efficacy. It has been reported that in a high percentage of human prostate cancers, the levels of GST-pi are negligible because of hypermethylation of the promoter region of GST-pi gene. Evidences have been provided that the treatment of prostate cancer cells with DNA methyltransferase inhibitors resulted in a demethylation and activation of the GST-pi gene and, consequently, the intracellular level and activity of the GST-pi protein increases. The cytotoxic activity of brostallicin has been tested against the non-GST-pi-expressing human prostate cancer cell line (LNCaP) where the GST-pi promoter is completely methylated. Brostallicin is five times less cytotoxic on LNCaP cells compared with the GST-pi-expressing (with methylated promoter) Du145 human prostate cancer cells (IC_{50} 200 and 38 ng/ml, respectively). Aim of this work was to verify *in vitro* whether treatment with agents releasing the expression of GST-pi such as 5'aza-2'deoxy cytidine, procainamide or HDAC inhibitors could activate the expression of GST-pi in LNCaP cells and, consequently, increase the antitumor activity of brostallicin. Our results indicate that pre-treatment with procanaimide (7 days pretreatment, a scheme previously reported to induce hypomethylation of GST-pi promoter) results in an increased cytotoxicity of brostallicin compared to untreated LNCaP cells. These data indicate that the association of brostallicin with hypomethylating agents could be synergistic in prostate cancer cells

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Analysis of *in vitro* and *in vivo* activity of a newly synthesized psorospermin analog

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Psorospermin (Ps) is a natural product isolated from the African plant Psorospermum febrifugum. This compound was previously shown to be active against drug-resistant leukemia lines and AIDS-related lymphoma (Cassady, J.M., et al. JNCI, Vol #53, 23-41). Previous studies have also shown that Ps alkylates the N7 position of guanine weakly, but in the presence of Topo II, alkylation is greatly enhanced at specific sequences determined by Topo II (Kwok, Y., et al., PNAS, Vol #95, 13531-13536). Because of this distinctive mechanism of action and activity profile in the NCI Compare program, we synthesized the diastereomeric pairs of O5-methyl-(±)-(2'R,3'R)-Ps (PsMeO). The diastereomeric pair enriched in the active compound showed *in vitro* activity in AML (IC_{50} = 0.3 μM), CML (0.4 μM), multiple myeloma (0.3 μM), pancreatic (0.6μM), breast (2 μM), and ovarian cancers (0.7 μM). Cytotoxicity studies using matched pairs of multidrug resistant and wild-type tumor cell lines showed the resistant cells were considerably more sensitive to PsMeO than the selecting agent (doxorubicin, mitoxantrone). Cell cycle studies show a reduced number of cells in G1 and G2 and a concurrent increase of cells in S phase, suggesting that PsMeO accelerates G1/S entry and/or delays cell exit from S to G2/M stages. Apoptosis was also increased 6-12 hours post treatment. To determine whether the drug effects we and others have observed *in vitro* can be achieved following the systemic administration *in vivo*, we examined the antitumor activity of PsMeO in severe combined immune deficient (SCID) mice bearing established human pancreatic cancer MiaPaCa-2 xenografts. There was a considerable decrease in tumor volume in mice treated with PsMeO compared to untreated controls. These results, together with the Topo II alkylation specificity and activity in several tumor types, demonstrate PsMeO to have a unique mechanism of action that distinguishes it from current chemotherapeutic agents.

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Design and biological evaluation of new fluoroquinolones with a dual mechanism of action against topoisomerase II and G-quadruplex

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We have designed and synthesized four new fluoroquinoanthroxazines (FQAs) in order to investigate the combined effect of single compounds having dual mechanisms of action; i.e., G-quadruplex-interactive compounds with topoisomerase II poisons. We have extended the aromatic system of A62176 by introduced a naphthyl group at the cis(C) or trans (T) depending on the orientation of the extension. Also the chirality at the 3-C aminopyrrolidine carbon in these compounds yields two enantiomers, S- and R-, and therefore four novel stereoisomeric FQAs were prepared: FQA-CS, FQA-

CR, FQA-TS, FQA-TR. Several assays were performed to determine the biological profiles of these compounds with respect to G-quadruplex interactions and topoisomerase II inhibition. Among the four FQAs, FQA-CS and FQA-CR fulfilled the required mix of the different types of activity: FQA-CR showed a higher G-quadruplex interaction with less topoisomerase II poisoning activity while FQA-CS showed a stronger topoisomerase II poisoning activity with weak G-quadruplex interaction. These activities were then correlated with cytotoxicity in topoisomerase II-mediated drug resistant and drug sensitive cell lines and telomerase (+) and ALT (+) cell lines. The cytotoxicity assays showed the order of activity as follows: FQA-CR > FQA-CS > FQA-TR > FQA-TS. FQA-CS showed a significantly decreased activity in topoisomerase II-mediated resistant cells as compared to the drug sensitive parent, which is consistent with the cytotoxicity of FQA-CS being attributed to topoisomerase II poisoning activity. With FQA-CR, the best G-quadruplex-interactive compound, there was much less difference between the two cell lines; therefore the overall cytotoxicity of FQA-CR is not due to topoisomerase II poisoning activity but presumably due to its G-quadruplex interaction. The G-quadruplex interaction was corroborated in both telomerase-positive and ALT-positive cells when exposed to non-cytotoxic doses of FQA-CR and FQA-CS compounds over 6 weeks. In both tel (+) and ALT (+) cells, suppression of cell proliferation was observed within 3-4 weeks with FQA-CR, while FQA-CS-treated cells showed a growth curve similar to control cells. These compounds are under further evaluation to determine the consequences of a dual mechanism of action against topoisomerase II and G-quadruplexes, and to determine the optimum combination of these modes of action for activities *in vivo*.

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The effect of MDR1 on the ex vivo activity of XR5944 (MLN944) and XR11576 (MLN576), two novel DNA targeting agents

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Introduction: XR5944 (MLN944) and XR11576 (MLN576) are novel DNA targeting agents that act through a mechanism which includes the dual inhibition of topoisomerase I and II. These compounds have demonstrated antitumour activity, both *in vitro* and *in vivo*, against a number of murine and human tumour models. We have previously demonstrated that XR5944 and XR11576, have a 20-fold increased ex-vivo activity against human ovarian adenocarcinoma and skin melanoma when compared to the first generation compound XR5000. XR11576 has been shown to be unaffected by MDR mediated resistance, while the high potency of XR5944 is somewhat attenuated in cell lines overexpressing P-gp or MRP. The activity of both compounds has been reported to be unaffected by down-regulation of topoisomerase II.
Method: We have used an ATP-Tumour Chemosensitivity Assay (ATP-TCA) to assess the ex-vivo sensitivity of a variety of solid tumours (n=97). Immunohistochemistry was performed on paraffin embedded blocks for those cases for which samples were available (n=29). The relationship between chemosensitivity and the immunohistochemical expression of Topoisomerase I, Toposiomerase IIalpha and MDR1 protein was investigated using univariate linear regression analysis.
Results: The median IC90 values of XR5000, XR11576 and XR5944 were 5114 nM, 215 nM and 65 nM, respectively. The IC90 values of all three drugs tested on a variety of tumour types did not differ significantly from those previously reported in ovarian adenocarcinoma or skin melanoma samples. The new generation compounds, XR5944 and XR11576, still demonstrated at least a 20-fold greater activity than XR5000. No correlation was found between the chemosensitivity of XR5000 or XR11576 and the immunohistochemistry indices. A moderate positive correlation ($R=0.55$, $p<0.05$) was found between the IC_{50} value of XR5944 and P-gp staining, but not with either the topoisomerase I or IIalpha immunohistochemistry indices. These data are consistent with a dual topoisomerase mechanism of action for these agents.
Conclusion: These data confirm that XR5944 and XR11576 are much more active than earlier compound XR5000, with XR5944 being slightly more active than XR11576. MDR1 may be a mechanism of resistance to very low concentrations of XR5944, however these concentrations are likely to be exceeded in clinical practice. This work was funded by Xenova Ltd.

Hormonal agents

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TGF-beta activated Smad and p38 signaling pathways are important mediators of antiestrogen action in breast cancer cells

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The antiestrogen Tamoxifen turned out to be very effective in the endocrine therapy of breast cancer. So far the mechanisms of antiestrogen action are only partially understood. One effect observed upon antiestrogen treatment of breast cancer cells is the activation of transforming growth factor beta (TGFb)system. TGFb is an important growth inhibitor of breast cancer cells. In order to investigate the role of TGFb as a mediator of antihormonal effects we specifically inhibited different components of the TGFb signal transduction pathway in hormone responsive MCF-7 breast cancer cells and analyzed the impact on antiestrogen action. Both the nonsteroidal partial antiestrogen 4-hydroxytamoxifen and the steroidal antiestrogen ICI 182.780 were used. In transient transfection assays the TGFb sensitive reporter plasmid p3TP-lux could be activated by both types of antiestrogens. Coexpression of dominant negative TGFb receptors strongly reduced the activation of p3TP-lux, indicating that TGFb signal transduction pathways are important mediators of antiestrogen action. TGFb signaling is very complex and many different pathways are activated by the TGFb receptors. One major pathway consists of the Smad proteins. Coexpression of dominant negative Smad4 also led to a reduction of the antiestrogen activation of p3TP-lux. As it has previously been shown that TGFb exerts its action not only by the Smad signal transduction pathway but also by MAP kinase cascades, we were interested in whether these cascades were involved in TGFb mediated antiestrogen action as well. We first analyzed the role of MEK, p38 and JNK in antiestrogen growth inhibition using specific pharmacological inhibitors. Inhibition of p38-kinase led to a strong reduction of antiestrogen growth inhibiton. No effects were observed for the other kinases. In transient transfection assays simultaneous treatment with antiestrogen and p38-inhibitor nearly completely abolished the induction of p3TP-lux observed under antiestrogen treatment alone. Our results show that TGFb is an important mediator of antiestrogen action and that at least two TGFb activated signal transduction pathways are involved: the Smad pathway and the p38 MAP kinase cascade. Furthermore our results suggest that Smad and p38 act in a synergistic manner and represent new targets for treatment of breast cancer.

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Pharmacologic characteristics of D-63153, a new potent GnRH antagonist

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We describe the pharmacological profile of the new GnRH antagonist D-63153. By introducing a N-Meth-Tyr at position 5 the solubility could be clearly increased as compared to other GnRH antagonists (Cetrorelix, Ganirelix). In the radioligand displacement binding assay D-63153 bound to human and rat GnRH receptor with high affinity (K_D=0,19 nM human / K_D=0,045 nM rat). The compound behaves as a full receptor antagonist as shown by complete inhibition of Triptorelin induced signaling via rat or human GnRH receptor in a luciferase reporter gene assay. *In vivo* administration of D-63153 resulted in dose-dependent LH and testosterone suppression in rats and dogs. *In vivo* action was reflected by corresponding plasma levels of D-63153. In castrated rats LH plasma levels were reduced to almost undetectable levels after s.c. application. Duration of suppression was dose-dependent and the antagonist induced LH suppression was inhibited by agonist Decapeptyl showing that binding of D-63153 to pituitary GnRH receptors is competitive and reversible. S.c. administration of D-63153 evoked a long lasting complete abrogation of testosterone production in rats. In addition, a loading dose / maintenance dose schedule resulted in castration testosterone levels over the complete treatment period. Atrophy of gonadal organs was nearly completely reversible 40 days after treatment termination. Anti-tumor effects of D-63153 were investigated in different animal models and showed a dose-dependent suppression of tumor growth. The duration of tumor inhibition was dose dependent. Our results demonstrate that the unique and favorable pharmacological properties of D-63153 make it an ideal candidate for the management of sex steroid-dependent diseases requiring inhibition of gonadal hormones.