111 ng/mL persisting for 96 hours following the fifth dose at 0.32 mg/kg. Plasma PD data will be analyzed in relation to PK data.
Conclusions: XL184 appears to have evidence of antitumor activity, even at doses not associated with toxicity. Its long terminal half-life may permit evaluation of alternative schedules.

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Establishment and in vivo evaluation of two human sarcoma xenograft models: results of tumor growth and chemotherapy sensitivity in models of mesenchymal chondrosarcoma (MCS) and leiomyosarcoma (LMS)

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Soft tissue sarcomas are malignant tumors which originate from fat, muscle, nerve, blood vessel, and fibrous or deep skin tissues and occur in most parts of the body. Mesenchymal chondrosarcoma is a rare, chondrogenic neoplasm often developing in the spine, ribs and jaw and metastasizing to lungs, lymph nodes and other bones. Leiomyosarcoma is a malignant tumor originating from smooth muscle tissue, most often the retroperitoneum, internal organs and blood vessels. While occurrence of either tumor is quite rare, current therapies are limited and treatment potential for newly approved agents and novel combination regimens are largely unknown, primarily due to lack of evaluable models for each disease.
The goal of this project was to develop and establish transplantable models of disease for MCS and LMS and evaluate tumor growth inhibition (TGI) or delay (TGD) potential of newly approved agents and novel combination therapies. For model development, MCS and LMS tumors were removed from patients and fragments xenografted into male CD-1 nude mice; tumors were propagated and amplified until growth was stable and take rate $\geqslant$80%; molecular profiling was then performed on each tumor to establish baseline levels of relevant proteins and signaling molecules. Following establishment, these models were evaluated for sensitivity towards a panel of single agent and combination therapies, many including bevacizumab (Avastin®) with significant TGI ($p < 0.05$) and partial/complete responses reported in several regimens. Further, some treatments resulted in selective activity for MCS or LMS tumors, suggesting model specificity. From this project, we report two established sarcoma models and demonstrate significant, directed antitumor activity of novel single agent and combination treatment regimens. Further experiments are underway comparing molecular profiles of treated versus control tumors to determine mechanism in sensitive models and to identify additional useful combination therapies for treatment of MCS and LMS.

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New derivative of betulinic acid induces apoptosis by mitochondrial disruption and direct interaction with cytochrome c

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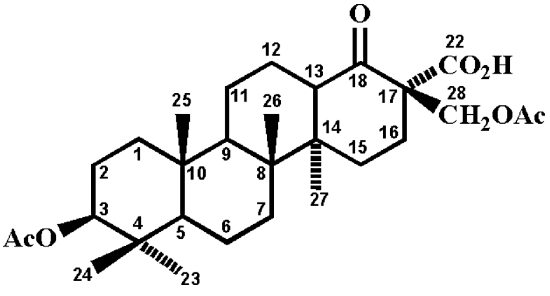
Background: The anti-tumour properties of lupane-derived triterpenoid compound were first discovered over 30 years ago when extract from the stem bark of various plants were tested for cytostatic ativity using different in vivo cancer model systems. We have recently developed a new synthetic betulinic acid derivative 3β,28-diacetoxy-18-oxo-19,20,21,29,30-pentanorlupan-22-oxic acid (JS8), that induces rapid apoptotic response in various tumor cells of different origin under in vitro conditions. The compound was effective in a concentration range IC_{50} = 0.19−4.66 μM.
Material and Methods: For further study of the apoptotic machinery, we used the CEM-lymphoblastic leukemia cell line and various molecular biology methods as western blotting, flow cytometry techniques, mass spectrometry and UV/VIS and resonance Raman spectroscopy.
Results: Several lines of evidence indicate that JS8 induced apoptosis depends on mitochondria. We found that JS8 promotes elevation of intracellular reactive oxygen species which may affect the mitochondrial transmembrane potential and mitochondrial membrane permeability profoundly. After disruption of the mitochondrial transmembrane potential, cytochrome c is released from mitochondria into the cytosol and this leads to activation of the caspase cascade. Release of cytochrome c and apoptosis was completely prevented by the antioxidant N-acetyl-L-cysteine and KCN, an inhibitor of oxidative phosphorylation complex IV. These data suggest that the primary target of JS8 is in between complexes III and IV, where cytochrome c is located. Direct coincubation of JS8 with purified cytochrome c in vitro resulted in the formation of noncovalent complexes which were characterized using UV/VIS and resonance Raman spectroscopy and mass spectrometry techniques. Selectivity of JS8-cytochrome c interaction was further confirmed by low or absent capacity of the compound to bind other (un)related proteins: cytochrome b_5, cytochrome P450, myoglobin and lysozyme.



3β,28-Diacetoxy-18-oxo-19,20,21,29,30-pentanorlupan-22-oxic acid (code name JS8).

Conclusions: The results show that cytochrome c is the primary target for betulinic acid derivative JS8 and potentially for other cytotoxic triterpenoid compounds.
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Human RNase 1 variants are effective anti-cancer agents

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The ability to destroy RNA in a cell-selective manner would provide a new pathway for attacking cancer cells: inhibiting the cancer cell from making proteins and eventually resulting in cell death. RNA can be cleaved by a family of enzymes called ribonucleases, one of which is the widely studied and well-characterized bovine ribonuclease A (RNase A). As a member of the pancreatic RNase family, bovine RNase A is not active inside cells due to the presence of a cytosolic protein called ribonuclease inhibitor (RI). Previous reports have shown that bovine RNase A variants which have diminished binding to RI but retain ribonucleolytic function can kill cancer cells *in vitro* and *in vivo*.
Our primary objective was to develop variants of human RNase 1 with a positive safety and efficacy profile and to advance a candidate into the clinic. Variants of mammalian RNases with diminished binding to RI are called EVade™ RNases. Safety data as well as efficacy in xenograft models will be presented with an emphasis on the lead candidate that has been selected for IND-enabling studies.
The EVade™ RNases are currently expressed in inclusion bodies in *E. coli* and purified by traditional column chromatography. A FRET-based assay, utilizing a mixed oligonucleotide with quenching fluorophores at either end as the substrate, is used to determine enzymatic activity. A similar activity assay can be used to measure the binding of RNases to RI. Our RNase variants are tested for the inhibition of human tumor growth in tumors implanted in the flanks of nude mice (xenograft models).
QBI-188 is a variant of human RNase I that retains ~99% sequence identity with the native protein. QBI-188 has demonstrated tumor regression in a non-small cell lung cancer (A549) xenograft model. Additional variants that have significantly inhibited tumor growth (>60%) with broader efficacy will also be presented. Additional xenograft models to be presented are: prostate (DU145), pancreas (Bx-PC-3), and ovarian (SKOV-3) cancer. Xenograft study data for FDA approved therapeutics will also be provided as positive controls. In addition to efficacy data, pharmacokinetic data and clinical chemistry for EVade™ RNases will be provided.
EVade™ RNases created from the human pancreatic RNase 1 have potent anti-tumor activity *in vivo* compared to standard therapies and an RNase from another species. These data suggest that the engineered ribonucleases are strong candidates for development as therapeutic agents.