nM affinity. Pharmacokinetic studies and antitumor evaluation indicate that efficacy is dependent on sustained plasma concentrations rather than high C_{max} levels followed by rapid elimination.
Methods: A study was conducted to assess the potential toxicity and toxicokinetic profile of ECO-4601 when administered to cynomolgus monkeys for 14 consecutive days by continuous intravenous infusion (CIV). This route and schedule of administration was chosen to achieve sustained plasma concentrations and is similar to that planned for the clinical Phase I trials. ECO-4601 was administered to cynomolgus monkeys by CIV at doses of 5, 15 and 30 mg/kg/day, during a 14-day period, followed by a 14-day recovery period.
Results: The highest dose level (30 mg/kg/day) was very well tolerated. This dose resulted in sustained drug plasma concentrations of 10−20 μM, which is well above expected therapeutic and target drug concentrations to be achieved in human (2–5 μM). Furthermore, when treatment was stopped, drug plasma concentrations declined quickly and there was no persistence in tissues. There were no effects on body weight, blood pressure and electrocardiographic activity, and no treatment-related ocular or neurologic abnormalities. Treatment-related changes observed were limited to: (1) occasional inappetance; (2) a modest degree of regenerative (reversible) anemia with no other hematologic abnormalities noted; (3) elevations in serum cholesterol and triglycerides, and a decrease in serum albumin (all reversible); and (4) diffuse vacuolization of hepatocytes and accumulation of foamy histiocytes in the spleen, which appeared to reflect clearance of the vehicle.
Conclusions: An adequate margin of safety for ECO-4601 was established under clinically relevant dosing conditions in monkeys, which supports advancement into clinical trials.

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Marked inhibition of tumor growth, MMP secretion and invasion by a nutrient mixture on head and neck squamous carcinoma cell line FaDu: in vitro and in vivo studies

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Background: Head and neck squamous cell carcinomas (HNSCC), the sixth most common malignancy in the United State, are known for their aggressive growth and propensity to invade and metastasize. We investigated the effect of a novel nutrient mixture (NM) containing ascorbic acid, lysine, proline, and green tea extract on human HNSCC cell line FaDu *in vitro*, evaluating viability, MMP secretion, invasion and morphology. *In vivo* studies were carried out in athymic nude mice bearing HNSCC FaDu xenografts.
Methods: After one week of isolation, 5–6 weeks old athymic male nude mice were inoculated with 3×10^6 FADU cells subcutaneously and randomly divided into two groups; group A was fed a regular diet and group B a regular diet supplemented with 0.5% NM. Four weeks later, the mice were sacrificed and their tumors were excised, weighed, and processed for histology. We also tested the effect of NM *in vitro* on FaDu cells, measuring cell proliferation by MTT assay, invasion through Matrigel, morphology by H&E staining, and secretion of MMPs by gelatinase zymography. Cells were also treated with PMA for MMP-9 induction.
Results: NM strongly inhibited the growth of tumors by 50%. *In vitro*, NM exhibited dose response toxicity with maximum toxicity of 50% over the control at 100 μg/ml. Zymography showed only a faint band representing MMP-2 and PMA-induced MMP-9. NM inhibited secretion of both MMP-2 and MMP-9 in a dose dependent fashion, with virtual total inhibition at 1000 μg/ml. Invasion through Matrigel was inhibited at 50, 100 and 500 μg/ml by 75%, 85% and 100% respectively. H&E staining did not indicate changes even at the highest concentration.
Conclusions: In conclusion, NM has a great potential for therapeutic use in the treatment of HNSCC by suppressing tumor growth and significantly inhibiting MMP secretion and invasion of HNSCC cells *in vitro*.

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Influence of new analogs and complexes of genistein on the expression of αvβ3 integrins on the A498 cell line

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Integrins comprise a large family of heterodimeric cell-surface receptors that present in many species. They are expressed on the wide variety of cells and mediate cell–cell and cell–extracellular matrix interaction. Dysregulation of the β3 integrins is involved in the pathogenesis of many diseases including cancer and transplant rejection.
The aim of our study was to investigate the influence of new analogs of genistein IFG-027 (7-O-alkenyl) and IFG-043 (7-O-arylmethyl) and its two polysaccharide complexes: schisophillan–genistein (SCH) and xyloglucan–genistein (XYL) on the expression of αvβ3 integrins.
Human kidney carcinoma A498 cell line was used (ATTC). Cells were incubated for 72 hours with tested compounds in concentration of 10 μg/ml. The cells were then labeled by αvβ3-specific antibodies conjugated with FITC and expression of integrins was analyzed by flow cytometry (Becton Dickinson, San Jose, CA, USA).
We have found that genistein, its new analogs and complexes have antiproliferative effect against many human cancer cell lines. We have also showed in our studies that these compounds had also influenced the expression of αvβ3 integrins. Genistein and XYL complex decreased the expression of integrins by 20%, whereas IFG-027 analog and SCH complex decreased it by 38%. IFG-043 analog revealed only low influence on the expression of the integrins (decrease by 10%).
We suggest that potential antitumor (antimetastatic) properties of genistein and its derivatives IFG-027, SCH, and XYL are worth of further research.
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Selective action of reveromycin A, a novel anti-resorptive agent, on osteoclasts

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Background: Bone destruction by osteoclasts plays an important role in the establishment and progression of osteolytic bone metastasis, which causes pain, pathologic fractures, and hypercalcemia. Therefore, osteoclasts are the ideal therapeutic target of osteolytic bone metastasis. Recently, we found that reveromycin A (RM-A), a polyketide-type natural product with three carboxylic groups in its structure, inhibited bone resorption through inducing apoptosis specifically in osteoclasts in vitro and in vivo. Moreover, we showed that RM-A inhibited the formation of bone metastasis in an experimental multi-organ metastasis mouse model of human lung cancer cells. Here, we investigated the mechanism of selective action of RM-A on osteoclasts using tritium-labeled RM-A ([^{3}H]RM-A).
Methods: [^{3}H]RM-A was prepared from RM-A by oxidation of C-5 OH followed by 1,2-reduction of the resultant enone using NaB[^{3}H]$_4$ and $CeCl_3$.
Results: RM-A inhibited the survival of osteoclasts with an IC50 value of 0.2 μM, and the ED50 of RM-A on bone marrow cells, osteoblasts, and a number of other cell lines, was at least 100-fold higher than that for osteoclasts. [^{3}H]RM-A was selectively incorporated into osteoclasts among various cells, and the uptake of [^{3}H]RM-A was prevented by disruption of the acidic microenvironment, a prominent characteristic of osteoclasts. [^{3}H]RM-A was dramatically incorporated into murine monocytic cell line RAW264 in acidic culture medium (pH 5.5), but not in neutral culture medium (pH 7.5). In addition, the apoptotic effect of RM-A was also increased under acidic conditions in RAW264 cells. RM-A inhibited protein synthesis in osteoclasts by selectively blocking enzymatic activity of isoleucyl-tRNA synthetase.
Conclusions: These results suggest that the specific sensitivity of osteoclasts to RM-A is due to their acidic microenvironment, which increases cell permeability of RM-A by suppressing the dissociation of protons from the carboxylic acid moieties, and the inhibitory effect of RM-A on bone resorption is caused by apoptosis through the inhibition of isoleucyl-tRNA synthetase in osteoclasts. This unique mechanism suggests that RM-A may represent a new type of therapeutic agent against osteolytic bone metastasis.

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The anti-angiogenic properties of Mistletoe extracts is associated with endothelial cytotoxicity

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Background: Viscum album (VA) preparations are used as adjuvant therapy in cancer patients. Angiogenesis plays an important role in the growth and sustenance of the tumors and their metastasis. Inhibition of angiogenesis is explored as a new therapy for cancer. We hypothesize that the anti-angionenic properties of VA extracts are due to their cytotoxic properties.
Materials and Methods: *In vitro angiogenesis assay*: Unpolymerised matrigel (10 mg/ml) was placed in the wells (400 μl/well) of a 24-well plate and allowed to polymerize for 1 h at 37ºC. EA-hy926 cells (Endothelial cell line) were then seeded (50×10^3 Cells/well). After the incubation with VA