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.

520 POSTER

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

M.W. Roomi, V. Ivanov, A. Niedzwiecki, M. Rath. *Dr. Rath Research Institute, Oncology, Santa Clara, CA, USA*

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*.

521 POSTER

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

M. Switalska[1], J. Wietrzyk[1], D. Nevozhay[1], G. Grynkiewicz[2]. [1]*Institute of Immunology and Experimental Therapy, Department of Experimental Oncology, Wroclaw, Poland;* [2]*Pharmaceutical Research Institute, Warsaw, Poland*

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.
This work was supported by the Foundation for Development of Pharmaceutical Sciences (grant 8/FB/2004, Poland).

522 POSTER

Selective action of reveromycin A, a novel anti-resorptive agent, on osteoclasts

M. Kawatani, N. Kanoh, H. Osada. *RIKEN, Antibiotics Laboratory, Saitama, Japan*

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.

523 POSTER

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

S. Elluru[1,2], J. Duong Van Huyen[3], S. Delignat[1], F. Prost[1], J. Bayry[1], S. Kaveri[1]. [1]*INSERM UMRS 681, Institut des Cordeliers, Paris, France;* [2]*Genie Enzymatique et Cellulaire, Université de Technologie de Compiègne, Compiègne Cedex, France;* [3]*Laboratoire d'Anatomie Pathologique, Hôpital Européen Georges Pompidou, Paris, France*

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

QuSpez extracts (12.5 and 50 μg/ml), the cell growth and bi-dimensional organization were analyzed after 24h.
In vivo angiogenic assay: In vivo angiogeneic assay was performed in female Balb/C mice (6–8 weeks old) by analyzing the growth of blood vessels from subcutaneous tissue into a Matrigel plug. Matrigel was mixed with or without VA extracts and was injected into the abdominal subcutaneous tissue. The mice were also injected with VA preparations intraperitonially (IP) (20 μg/day). Mice were sacrificed after 7 days, and the Matrigel plugs were excised and processed for histological analysis.
Apoptosis assay: EA-hy926 cells were incubated for 24 hrs with varying concentrations of VA extracts (12.5 and 50 μg/ml). The induction of apoptosis by VA extracts was analysed by Annexin V labeling that recognizes exposed phosphotidyl serine on apoptotic cells and PI that binds to DNA.
Results: Treatment of the cells with VA Qu Spez was associated with a reduction in capillary network, in a dose dependant manner. VA Qu Spez at 50 μg/ml induced a nearly complete disruption of the capillary tube formation. The area of angiogenesis network was also reduced by 33%. In our in vivo studies, there was a dramatic reduction in the vascular density in the matrigel treated with VA Qu Spez at the time of the implantation (intra-matrigel treatment) and followed by systemic (IP) treatment as compared to control untreated mice. VA QuSpez also induced apoptosis (upto 60%) of EA-hy926 cells as analysed by Annexin V and PI staining.
Conclusions: Our results show that VA QuSpez reduces angiogenesis in vitro and in vivo and the induction of apoptosis is the one of the underlying mechanisms. The anti-angiogenic properties of VA extracts may explain at least in part to their efficacy as adjuvant therapy in cancer patients.

524 POSTER

Stimulatory effect of eucalyptus essential oil on macrophage/ granulocyte phagocytic activity: in vitro and in vivo evidences

A. Serafino[1], P. Pierimarchi[1], F. Andreola[1], M. Zonfrillo[1], L. Mercuri[1], M. Federici[1], G. Rasi[1], P. Sinibaldi-Vallebona[2]. *[1]Institute of Neurobiology and Molecular Medicine, Rome, Italy; [2]University of Rome "Tor Vergata", Department Experimental Medicine and Biochemical Science, Rome, Italy*

Background: many species of the genus *Eucalyptus* from the Myrtaceae family are used in folk medicine for a variety of pathologies. Monoterpenoid oil components of aromatic constituents are traditionally used as analgesic, anti-inflammatory, and antipyretic remedies and are commercially available for the treatment of the common cold and other symptoms of respiratory infections. Phytochemical analysis have shown, that the profile of the monoterpenoids changes among the *Eucalyptus* species with potential variations in medicinal properties. In *Eucalyptus globulus* the major monoterpenoid component is eucalyptol, constituting the 60–90%. Macrophages constitute one of the primary cellular mechanisms of the immune response playing a pivotal role in the detection and elimination of foreign body such as pathogenic microorganisms. To our knowledge, in literature actually there is no available data, concerning the influence of *Eucalyptus* essential oil in the cell components of the immune system, the only exception is for the effect of some cytokine production. In this study we investigated whether essential oil from *Eucalyptus globulus* (EO) is able to affect the phagocytic activity of human monocyte-derived macrophages (MDMs) *in vitro* and of rat peripheral blood monocytes/granulocytes *in vivo*.
Materials and Methods: analysis of morphological changes, characteristic of activated MDMs, was performed by scanning electron microscopy. The evaluation of phagocytic activity was carried out: a) in EO treated and untreated MDMs *in vitro* with confocal microscopy after fluorescent beads administration; b) in monocytes/granulocytes from peripheral blood of BDIX rats, after *in vivo* EO administration, with cytofluorimetric analysis using the phagotest kit from ORPEGEN Pharma. Immuno-suppression in BDIX rats was induced by administration of the chemotherapeutic agent 5-fluorouracil (5-FU).

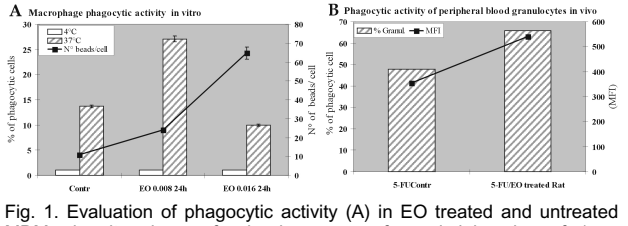

Fig. 1. Evaluation of phagocytic activity (A) in EO treated and untreated MDMs *in vitro*, by confocal microscopy, after administration of 1 μm fluorescent beads; and (B) in BDIX rat peripheral blood granulocytes, after *in vivo* EO treatment, in absence or in presence of 5-FU administration, by cytofluorimetric analysis.

Results: Our results demonstrate that EO is able to activate MDMs and peripheral blood monocytes/granulocytes both *in vitro* (Fig. 1A) and *in vivo*, stimulating their phagocytic activity. EO is also able to induce a dramatic recovery of granulocyte phagocytic activity after bone marrow suppression induced by 5-FU (Fig. 1B).
Conclusion: Our results suggest that the components of essential oil extracts from eucalyptus represent a possible new class of immunoregulatory agents useful in chemotherapy.

Prodrugs

525 POSTER

Targeting Doxorubicin to LHRH-receptor positive tumors by the cytotoxic hybrid ZEN-008 (AN-152)

E. Guenther[1], M. Teifel[1], J. Engel[2], K. Paulini[1], A. Schally[3]. *[1]Zentaris GmbH, Drug Discovery, Frankfurt, Germany; [2]Universitaet Wuerzburg, Frauenklinik, Wuerzburg, Germany; [3]Veterans Affairs Medical Centers, Dept. of Medicine, New Orleans and Miami, USA*

ZEN-008 (AN-152) is a cytotoxic analog of the luteinizing hormone releasing hormone (LHRH) in which doxorubicin (DOX) is linked to [D-Lys^6]LHRH. ZEN-008 binds to LHRH-receptors, which are found on a variety of tumors including breast, prostate, ovarian and endometrial cancers. After binding, ZEN-008 is internalized and transported to the nucleus where it induces apoptosis upon release of DOX. The activity of this compound has been demonstrated in experimental models of a variety of human cancers. Here we report on the antitumor activity of ZEN-008 in experimental human endometrial cancers.
LHRH receptors were determined in the HEC-1A human endometrial cancer cell line. The efficacy of ZEN-008 was evaluated and compared to its cytoxic radical DOX in athymic nude mice bearing HEC-1A tumors. The safety and tolerability of ZEN-008 was evaluated in series of studies including safety pharmacology studies and acute and subchronic toxicity studies.
43 days after the injection of ZEN-008 HEC-1a tumor growth was significantly inhibited by 54.2%, while treatment with an equimolar dose of DOX only resulted in a nonsigificant tumor inhibition by 23.4%. WBC 8 days after application was signifcantly suppressed by DOX, but not by ZEN-008.
The good safety profile was confirmed in safety pharmacology studies evaluating the effects of ZEN-008 on respiratory and cardiovascular parameters in the dog as well as in the Irwin and Rotarod test. In the cardiovascular safety study in beagle dogs, no evidence of QT prolongation was seen at any dose administered. Superior tolerability of ZEN-008 as compared to DOX was confirmed in acute and subchronic toxicity studies in mice, rats and dogs, respectively. In contrast to DOX, where lymphohistiocytic myocarditis with intramuscular fibrosis was observed, ZEN-008 did not induce any cardiotoxicity.
Targeted chemotherapy with ZEN-008 is significantly more effective than DOX itself. ZEN-008 is less toxic than DOX as reflected by a consistently higher LD50 and reduced cardiotoxicity. Due to the attractive mechanism of action and the overall promising safety and toxicity profile, ZEN-008 was selected to be evaluated in clinical phase I trials. ZEN-008 is available as a red powder (50 mg) lyophilisate for i.v. application as a solution.

526 POSTER

A Prostate-Specific Antigen (PSA) activated channel-forming toxin as therapy for prostatic disease

S.A. Williams[1], N. Merchant[2], J.T. Buckley[2], S.R. Denmeade[1]. *[1]Johns Hopkins University, Oncology, Baltimore, USA; [2]ProTox Therapeutics, Inc., Vancouver, Canada*

Background: While the exact physiologic function of the prostate is unknown, it is a gland associated with significant morbidity in the aging male. The prostate is the most common site of non-skin cancer diagnosed in American men, with one in six developing the disease during their lifetimes. In addition, approximately 80% of men will present with a symptomatic benign overgrowth of the prostate known as benign prostatic hyperplasia (BPH) by age 80. Prostate-specific antigen (PSA) is a serine protease that is secreted at high levels (micro to mg/ml) by the normal and diseased prostate. To develop effective prostate tissue-selective therapy for localized prostatic disease we modified proaerolysin (PA), the inactive precursor of a bacterial cytolytic pore-forming protein, to produce a PSA-activated protoxin (PRX302).
Materials and Methods: PRX302 was generated by replacing the wild type furin protease activation site within PA with a 6 amino acid PSA-selective activation site. PRX302 was tested for in vitro toxicity against PSA positive and negative prostate cancer cell lines. Intratumoral efficacy of PRX302 was evaluated in PSA-producing xenograft models. Since the PSA gene