intermediate growing and sensitive as well as chemoresistant solid tumor types. The cell lines resemble the original patient tumor and xenograft histology after s.c. injection into nude mice. Each drug was tested in 2 concentrations (0.3, 3 μg/ml). Of 10 registered anticancer agents within the natural product pool, 9 were found active applying our screening criteria. 1.5 % of the total number of novel compounds screened, possessed potent and differential *in vitro* cytotoxicity profiles. Eleven of these hits were forwarded for *in vivo* testing. To save precious compound, we developed a model system, which would give a hint of *in vivo* antitumor activity and enable estimation of the maximal tolerated dose. Two or four different xenografts with similar doubling times were implanted s.c. between the fore and hind flanks of nude mice. They were validated for stable growth, response to clinical agents and maintaining the morphology. The xenograft doublet was composed of mammary (MAXF 401) and lung cancer models (LXFL 529) which had also been used for *in vitro* screening. Against these tumors, leads were tested i.p. on days 1,5, and 9 in a single mouse and subsequently in tumor quartets composed of fast growing cancers (RXF 944, MX-1, OVXF 899, XF 575), or slower growing tumors (LXFL 529, MAXF 401, MEXF 989, CXF 280). Six of 11 compounds showed a reproducible *in vivo* activity. They are candidates for preclinical development. By using this approach, clinical candidate agents will be discovered on an economical and fast track.

513

The silybin-phospholipid complex IdB 1016 inhibits human ovarian cancer growth in athymic nude mice

D. Gallo[1], S. Giacomelli[1], C. Ferlini[1], G. Raspaglio[1], D. Travaglia[1], A. Riva[2], P. Morazzoni[2], E. Bombardelli[2], S. Mancuso[1], G. Scambia[1].
[1]*Catholic University of the Sacred Heart, Department of Obstetrics and Gynaecology, Rome, Italy;* [2]*INDENA S.p.A., Milan, Italy*

We have recently reported that the flavonoid silybin, and its more bioavailable derivative IdB 1016, are able to potentiate, both in *in vitro* and *in vivo* experimental models, the antiproliferative activity of optimal or sub-optimal doses of cisplatin against human ovarian cancer cells (A2780); we also showed for the first time, an antiangiogenic effect of IdB 1016 in an *in vivo* experimental model. In the present study we provide evidences for the growth inhibitory activity of IdB 1016 when used as single agent against human ovarian cancer. IdB 1016 (450 mg/kg/day) was administered by oral route for 20 days to athymic nude mice bearing A2780 xenografts, starting from the day of tumour cell inoculation. This treatment significantly inhibited tumour growth (TWI=78% and LCK=1.1) and, importantly, treated animals did not show any signs of toxicity such as weight loss or reduced food consumption. Bioavailability of silybin after administration of a pharmacologically active dose of IdB 1016 was also assessed: free plasma silybin levels were found to be in the range of 0.2 to 13.4 mcg/ml. Tumour samples were also taken from treated animals and analysed to determine silybin tissue levels and to evaluate the modulation exerted by IdB 1016 on angiogenesis gene expression profile. Furthermore, the effect of IdB 1016 on secretion of VEGF by human ovarian cancer cells has been studied by analysing human VEGF levels in serum from athymic mice bearing A2780 xenografts. Due to this interesting pre-clinical anti-tumour profile, a clinical trial is currently undergoing in order to evaluate the efficacy of the drug in patients with serological recurrence of ovarian cancer.

514

The intravenous administration of rViscumin induces cytokine release and antibody formation. Results of EORTC New Drug Development Group trial 16002

P. Schöffski[1], P. Fumoleau[2], M. Campone[2], M. Herold[5], H. Zwierzina[5], G. Beutel[1], B. Vandael[3], A.-S. Govaerts[3], K. Wilhelm-Ogunbiyi[4], B. Mockel[4], C. Twelves[3]. [1]*Hannover Medical School, Hannover, Germany;* [2]*Centre Rene Gauducheau, Nantes-St. Herblain, France;* [3]*EORTC, NDDP, Brussels, Belgium;* [4]*VISCUM AG, Zwingenberg, Germany;* [5]*Leopold-Franzens-Universität, department of Internal Medicine, Innsbruck, Austria*

Background: rViscumin (proposed INN: aviscumine) is a recombinant E.coli-derived type II ribosome-inactivating protein with potent antitumor activity *in vitro* and *in vivo*. The drug is currently being investigated in the first intravenous dose-escalating phase I clinical trial performed by the EORTC New Drug Development Group (NDDG) in Hannover and Nantes (EORTC 16002). rViscumin is given twice weekly as a 1 hour i.v. central line infusion in patients with advanced solid tumors not previously exposed to natural mistletoe preparations. Translational research includes the determination of cytokine levels in plasma and monitoring of a possible anti-rViscumin antibody induction.
Methods: Sequential plasma levels of interleukin (IL)-1β, IL-6, IL-10, IL-12, interferon (IFN)gamma and TNFalpha were determined by standard ELISAs (Becton Dickinson) at baseline and on the days of the first and eleventh administration of the drug. Anti-rViscumin antibodies were detected in plasma at baseline, repeated every 3 weeks.
Results: Increased concentrations of IL-1β and IL-6 in plasma of treated patients were observed over the entire dose range administered to date (10-4800 ng/kg). Increased release of IFNgamma as a marker of activated T-cells occurred after higher doses of 3200 and 4000 ng/kg. T-cells might be activated by the cytokine release of the innate immune system. Increase in these cytokine levels was highest 4 and 8 h after the first infusion and declined with further treatment duration. No increases of IL-10, IL-12 or TNFalpha were noted. During the course of treatment, IgG and IgM anti-rViscumin antibodies were mainly detected in plasma of patients treated with rViscumin doses * 800 ng/kg. No clear dose dependency was observed. The titers measured are low (serum dilution of 1:50 and 1:100) and of as yet unclear clinical relevance.
Summary: The i.v. administration of rViscumin stimulates the immune system with a significant release of cytokines such as IL-1β, IL-6 and IFNgamma and is associated with an induction of anti-rViscumin antibodies of IgM and IgG class of unclear clinical significance.

515

Pharmacokinetics of the intravenous administration of rViscumin in patients with solid tumours - First results from EORTC Phase I Study 16002

P. Schöffski[1], M. Campone[2], S. Riggert[1], S. Mali[3], A.-S. Govaerts[3], K. Wilhelm-Ogunbiyi[4], M. Langer[4], P. Fumoleau[2], C. Twelves[3]. [1]*Medical School Hannover, Haematology/Oncology, Hannover, Germany;* [2]*Centre Rene Gauducheau, Nantes-St. Herblain, France;* [3]*EORTC Data Center, New Drug Development Group (NDDG) and New Drug Deve, Brussels, Belgium;* [4]*VISCUM AG, Zwingenberg, Germany*

Background: rViscumin (proposed INN: aviscumine) is a recombinant E.coli-derived type II ribosome-inactivating protein composed of a binding (B-) and an active (A-) chain. The compound has potent antitumor activity *in vitro*, in syngeneic and xenograft models. The drug is currently being investigated in the first intravenous dose-escalating phase I clinical trial performed by the NDDG (Early Clinical Studies Group) in Hannover, Germany, and Nantes, France.
Methods: Pts with progressive solid tumors refractory to conventional treatment are eligible for the trial. rViscumin treatment is given twice weekly by a central i.v. infusion over 1 hour. The number of pts required per dose level is defined by a Continuous Reassessment Method. Assessment of human pharmacokinetics is a key research endpoint of the trial. PK samples were obtained at 0, 1, 1.25, 1.5, 2, 4, 8 and 24 h after start of the 1st and 11th infusion. Sample analysis was performed by immuno-PCR using a polyclonal antibody allowing to detect both the intact holoprotein and each chain of the recombinant molecule.
Results: To date, 26 pts have been entered and doses have been escalated interindividually from 10 to 4800 ng/kg per administration. The maximum tolerated dose has not been reached so far. Pharmacokinetic data of 19 pts are being processed. According to this preliminary analysis, the intact protein has a t1/2 alpha of 30 min. The terminal t1/2 is currently estimated to be in the range of 24 h, but may be shorter because of the polyclonal nature of the antibody which may also detect degradation products. Comparing PK data after the 1st and 11th infusion there is no evidence of drug accumulation with the twice weekly administration. The correlation of AUC and Cmax values with doses indicates a linear relationship for rViscumin dose levels *1600 ng/kg. In pts given 4800 ng/kg per dose, plasma levels of about 20 ng/ml were achieved and maintained briefly. These plasma concentrations were effective in animal models. In human tumor xenograft clonogenic assay, the mean *in vitro* IC70 is 3 ng/ml. Based on our pharmacokinetic findings and the short t1/2, EORTC NDDG is currently considering two additional phase I trials with more frequent or more prolonged administration of rViscumin.