Material and Methods: We have studied routine cervical smears stained by Leishman's stain. For each smear a definitive diagnosis was made by light microscopy. Then all specimens with known diagnoses were investigated by AFM (NTEGRA Prima, NT-MDT Co., Russia). We measured heights of cytoplasm and nucleus, nuclear-cytoplasmic ratio, the parameters of koilocytes perinuclear cavity in case of HPV infection.
Results: See the table.

	Nucl. height nm	Cytoplasm height nm	nuclear–cytoplasmic ratio (vertical)
Superficial cells of squamous epithelium	1290±488	511±169	2.56±0.53
Dysplasia	1153±433	381±157	3.33±1.45
Non-keratinizing squamous cell carcinoma	758±164	225±65	3.48±0.82
Keratinizing squamous cell carcinoma	1607±549	986±291	1.61±0.22

Koilocytes perinuclear cavity: depth – 212±69 nm; width – 3.78±1.86.

Conclusions: There are tendencies in 3D-parameters of cells according to malignization's progression: decrease in heights of nuclei and cytoplasm and increase in nuclear-cytoplasmic ratio. The only exclusion is keratinizing squamous cell carcinoma.
Some of differences in 3D-parameters of squamous epithelium of different nozologies are statisticaly significant ($P < 0.05$) and could have differential diagnostics meaning: nuclear-cytoplasmic ratio for superficial cells of squamous epithelium and squamous cell carcinomas (Keratinizing and Non-keratinizing).

283 The trifunctional antibody catumaxomab: mode of action

D. Seimetz[1], R. Linke[2], J. Atz[3], M. Essing[4], A. Klein[4]. [1]*Fresenius Biotech GmbH, CSO, München, Germany*, [2]*Fresenius Biotech GmbH, Clinical Development, München, Germany*, [3]*Fresenius Biotech GmbH, Preclinical Research and Development, München, Germany*, [4]*Fresenius Biotech GmbH, Medical Affairs, München, Germany*

Background: Catumaxomab (Removab®) is a trifunctional bispecific monoclonal antibody which is presently the only approved therapeutic antibody targeting EpCAM, a surface antigen that is widely expressed in the most frequent forms of human cancer. With its trifunctional mode of action, catumaxomab represents a new generation of antibodies activating the body's own immune system for tumour cell elimination.
Material and Methods: For preclinical testing a targeted program was designed taking into account that catumaxomab binds to human antigens. Non-clinical properties were assessed in vitro using human cells and using in-vivo models including a surrogate antibody. Several clinical findings support the proposed mode of action.
Results: Catumaxomab differs from other antibodies by the ability to bind three different cell types: One specific antigen-binding site binds T cells via CD3, the other site tumour cells via the EpCAM antigen. The Fc-region provides a third functional binding site that is able to bind and activate Fcγ receptor-positive accessory cells. Preclinical studies identified different mechanisms of action including T cell-mediated lysis, phagocytosis and ADCC mediated by accessory cells. These complex immune reactions lead to an effective activation of immune cells against the tumour.
The mode of action is underlined by several clinical studies. Patients had cytokine release related symptoms like fever, nausea or vomiting as a main side effect which also demonstrate an activation of the immune system. Moreover, a positive trend between cytokine-release-related symptoms and clinical outcome was observed.
Due to the fact that catumaxomab is a murine antibody the majority of patients develop anti-drug antibodies after the end of treatment. Anti-drug antibody-positive patients seemed to have an improved clinical outcome possibly due to their better immunological response which on the other hand is a precondition for the functioning of the mode of action of catumaxomab.
Conclusions: Catumaxomab simultaneously recruits and activates different types of immune cells resulting in an efficient destruction of tumour cells. Positive treatment effects led to an approval in the European Union for catumaxomab in April 2009 for the intraperitoneal treatment of malignant ascites in patients with EpCAM-positive cancer.

284 Withdrawn

285 Gene array analysis of anticancer agents in breast cancer cell lines

M. Alokail[1]. [1]*King Saud University, Biochemistry, Riyad, Saudi Arabia*

Taxotere belongs to a group of anti-cancer drugs known as taxanes. It is a recently developed taxoid representing a novel class of compounds with a unique mechanism of action on the cytoskeleton. Ukrain is a derivative of alkaloids from the greater celandine plant. It is a semi-synthetic compound made up of three molecules of the deravitve alkaloids and one molecule of Tri-ethylene thio-phosphoric acid trimide (thio TEPA). We investigated here the molecular regulation of taxotere and ukrain treatments in MCF-7, MDA-MB231 breast cancer cell lines compared with normal breast cell line, MCF-12A. Cell viability have shown in MCF-7 high sensitivity for both agents, whereas, MDA-MB231 was resistant to both drugs. Douwn regulation of cell cycle genes have shown in MDA-MB231 compared with MCF-7 in both treatments. In case of MCF-7 treated with taxotere, cyclins (B1 and D1), CDKs (CDK4 and CDK5) were suppressed. Regarding the anti-apoptotic proteins; Bag1 level was low in MCF-7, but it was high in MDA-MB231 and in MCF-12A. It was also noticed that taxotere treatment had no affect in Bcl2 level in MCF-7, while it was significantly repressed in MDA-MB231. However, the expression level of Bag1 and Bcl2 was significantly repressed in ukrain treated MDA-MB231. Gene array analysis of cell proliferation and cell cycle genes have show down regulation of some genes like cyclins A1, B, D1 and D3, and some transcription factors like E2F5, TFDp-1, TPp73 and inhibitor of DNA binding 1 in both MCF7 and MDA-MB231, but unaffected in MCF-12A. In conclusion, taxotere and ukrain as breast cancer chemotherapy have shown to be useful predictive for the gene markers associated with breast cancer and the induction of apoptosis.

286 In vitro susceptibility of triple negative breast cancer cells to docetaxel, epirubicin and carboplatin

M. Laranjo[1], M.J. Carvalho[2], A.M. Abrantes[1], A. Brito[1], I. Torgal[3], C.F. Oliveira[4], M.F. Botelho[1]. [1]*Biophysics and biomathematics Institute IBILI CIMAGO, Faculty of Medicine of Coimbra University, Coimbra, Portugal*, [2]*Gynaecology Service University Hospitals of Coimbra Biophysics and biomathematics Institute IBILI CIMAGO, Faculty of Medicine of Coimbra University, Coimbra, Portugal*, [3]*Gynaecology Service University Hospitals of Coimbra, Faculty of Medicine of Coimbra University, Coimbra, Portugal*, [4]*CIMAGO, Faculty of Medicine of Coimbra University, Coimbra, Portugal*

Background: Triple negative (TN) tumours represent 10 to 17% of all breast cancers. These tumours are more prevalent in young women (under 50), are diagnosed in the interval between screenings (interval tumours) and have a more aggressive clinical behavior. This group of tumours are challenging considering systemic therapy, as they have a poor prognosis despite responding to conventional chemotherapy. The interest in the study of TN was first based on the lack of targeting therapies and overlap with the profile of basal-like tumours. Recent studies considered a particular susceptibility of TN cells to platinum derivatives.
Material and Methods: Human breast cancer cell lines HCC1806 (non-expressing estrogens, progesterone and HER2) were purchased to ATCC and cultured according to recommended procedures. Cells were incubated in absence and presence of the docetaxel, epirubicin and carboplatin in several concentrations ranging from 50nM to 150μM. The sensitivity of the cell line to the drugs studied was analyzed using the MTT colorimetric assay, performed 24, 48 and 72 hours after incubation. Cytotoxicity was expressed as the percentage of inhibition of cell proliferation correlated with untreated cultures. Dose-response curves were established and the half maximal inhibitory concentration (IC50) was calculated in Origin7 software.
Results: The cytotoxic capacity of epirrubicin revealed a IC50 of 2.3 μM and 1.15 μM respectively at 24 and 72 hours. Considering the results for carboplatin, it was found higher IC50 than epirrubicin. The IC 50 values for the former at 24 hour and 72 hours were 224.4 μM and 8.6 μM respectively. The IC50 for docetaxel was lower than the other drugs evaluated, 0.03 μM at 24 hours.
Conclusions: TN cells seem to harbor more susceptibility to epirrubicin than to carboplatin, according with a higher IC 50 testing the last drug. The lowest IC50 was reported with taxotere, what emphasizes its importance in association adjuvant therapy.

287 In vitro study of the antitumour effect of Artemisia annua tea

Z. Juranic[1], I. Matic[1], P. Lutgen[2], M. Juranic[3]. [1]*Institute of Oncology & Radiology of Serbia, Experimental Oncology, Belgrade, Serbia*, [2]*IFBV, Hostert, Luxembourg*, [3]*Galenika-Phytopharmacy, Belgrade, Serbia*

Background: One of the main goals in modern cancer research is to find chemotherapeutic agents that selectively suppress survival of malignant cells, with minimal cytotoxic effect against healthy cells. Bioactive phytochemicals of Chinese medicinal plant species *Artemisia annua* have been demonstrated to perform antimalarial, bactericidal and anticancer effect. The aim of this *in vitro* cytotoxic study was to elucidate whether the *Artemisia annua* tea possess anticancer potential.
Material and Methods: *Artemisia annua* tea for the each experiment was prepared by adding 100 ml of boiling distilled water to 5 g of dry herb leaves. The mixture was covered, stayed for 10 min and the leaves were removed by filtration. After cooling at room temperature, the tea was filtered through Millipore filter, 0.22 μm, before use. Cytotoxicity of *Artemisia annua* tea was evaluated against malignant cell lines: human cervix adenocarcinoma HeLa, human malignant melanoma Fem-x and BG, human myelogenous leukemia K562, human breast adenocarcinoma MDA-MB-361, human colon carcinoma LS174, normal human immunocompetent peripheral blood mononuclear cells

(PBMC), unstimulated and stimulated to proliferate by mitogen phytohemagglutinin, using MTT cell survival test, 72 h after the continuous tea action.
Results: Tea prepared from *Artemisia annua* dry leaves exhibited selective dose-dependent cytotoxic effect against malignant cell lines and both unstimulated and stimulated PBMC. The strongest cytotoxic action was observed against K562 cells ($IC_{50} = 1.33 \pm 0.38$ mg/ml). Moreover, tea exerted pronounced cytotoxic effect on melanoma BG and Fem-x cells ($IC_{50} = 3.20 \pm 0.65$ and $IC_{50} = 3.76 \pm 1.20$ mg/ml respectively), and to HeLa cells ($IC_{50} = 3.06 \pm 0.62$ mg/ml). Cytotoxic activity was found to be weaker against MDA-MB-361 and LS174 cells ($IC_{50} = 8.86 \pm 0.42$ and $IC_{50} = 10.45 \pm 0.26$ mg/ml respectively). Cytotoxicity of tea on human healthy immunocompetent PBMC, was slightly weaker on unstimulated PBMC in comparison to stimulated PBMC ($IC_{50} = 10.38 \pm 0.49$ and $IC_{50} = 9.27 \pm 0.54$ mg/ml respectively).
Conclusions: The present research clearly demonstrates stronger and selective (Ksel > 6.95) antitumour effect of *Artemisia annua* tea to leukemia K562 cells in comparison to healthy PBMC. To melanoma BG and Fem-x cells and to HeLa cell tea was also selective in its antitumour action but to a less extent (Ksel > 2.24), pointing that some tea constituents might have choosy anticancer action.

288 Effect of curcumin on vascular endothelial growth factor levels in rat fibrosarcoma

M. Serter[1], K. Ergin[2], E. Demir[3]. [1]*Adnan Menderes University, Biochemistry, Aydin, Turkey*, [2]*Adnan Menderes University, Histology and Embryology, Aydin, Turkey,* [3]*Adnan Menderes University and Eskisehir Government Hospital, Biochemistry, Aydin, Turkey*

Background: Fibrosarcoma is a malignant tumour derived from fibrous connective tissue and characterized by immature proliferating fibroblasts. This cancer invades long or flat bones such as femur or tibia. It also involves the periosteum and the overlying muscle. Fibrosarcoma usually seen in males in ages between 30 and 40. It could not treated with surgery, radiotherapy or chemotherapy always. Vascular endothelial growth factor (VEGF) stimulates vasculogenesis (new formation of a vessel) and angiogenesis (the growth of blood vessels from pre-existing vasculature). Solid cancers that can express VEGF are able to grow and metastasize because of their dependency to blood supply. The aim of this study was to determine the VEGF levels in rat fibrosarcoma.
Materials and Methods: In this study eighteen Wistar male rats were used and were divided into three groups: I. Control group (C, n = 6), II. Fibrosarcoma group (F, n = 6) and III. Curcumine treated Fibrosarcoma group (F+C, n = 6). Fibrosarcoma was induced by 3-metilcholantren and curcumin was given in a dose of 200 mg/per rat, via oral gavaj for ten days. After the experiment, the homojenized tissues were analysed with Western blot and ELISA with anti-VEGF antibodies.
Results: VEGF levels were increased in the F group ($p \leqslant 0.05$) and decreased after curcumine (F+C group) ($p \leqslant 0.05$) by both methods.
Conclusion: We thought that curcumine have an antiangiogenic effect of fibrosarcoma and it could possible act as an alternative assistant therapy.

Sunday 27 June 2010 09:45–17:30

Poster Session
Tumour Immunology

289 Indoleamine 2,3-Dioxygenase (IDO) silencing for improved antitumour vaccination

M. Macagno[1], E. Bolli[1], C. Marchini[2], A. Amici[2], C. Riganti[3], A. Bosia[3], G. Forni[1], F. Cavallo[1]. [1]*Molecular Biotechnology Center, Scienze Cliniche e Biologiche, Torino, Italy*, [2]*University of Camerino, Department of Molecular Cellular and Animal Biology, Camerino, Italy,* [3]*University of Turin, Department of Genetics Biology and Biochemistry, Torino, Italy*

Background: As tumour progresses the efficacy of vaccination is tuned down by suppressive activities. The administration of adjuvants or the silencing of specific immune regulatory molecules will optimize the function antigen presenting cells (APC) and will permit the immune response elicited to be active at the tumour site. Indoleamine 2, 3-dioxygenase (IDO) the enzyme that degrades the essential amino acid tryptophan in mammals is overexpressed in both tumour cells and APCs in tumour-draining lymph nodes, where it promotes the establishment of peripheral immune tolerance to tumour antigens. IDO seems to be an ideal target to be silenced for the optimal induction of an antitumour immune response. We plan to use plasmids coding short shRNA specific for IDO to be administered together with the plasmid coding portion of Erbb-2, or plasmids containing both the shRNA module and the oncoantigen module, in vaccination-protection tests in BALB-neuT mice transgenic for the rat Erbb-2.
Material and Methods: Retroviral vectors (pLKO.1, Open Biosystem®) including five shRNA sequences targeting IDO mRNA have been used as template to amplify the interference cassettes (pU6-shRNA-IDO) that we cloned into the Eco72I site of both pVAX1 (Invitrogen®). The gene silencing efficacy of the various interference cassettes was evaluated in a kynurenine assay using N11 microglial cells (Grant et al. 2000). The most efficacious cassettes were subcloned into a pVAX vector containing the sequence of the extracellular and transmembrane domains of rat Erbb-2 (pVAX-ratECTM) and used for vaccination of BALB-neuT mice carrying different stages of mammary carcinogenesis.
Results: All the five interference cassettes were able to reduce kynurenine release from N11 cells, confirming their ability to silence IDO expression. Two cassettes were chosen to be subcloned into pVAX-ratECTM, and used to vaccinate BALB-neuT mice bearing atypical hyperplasia and in situ carcinomas (weeks 10 and 12 of age) or microscopic invasive carcinomas (weeks 16 and 18). The in vivo observation of mammary cancer progression is still ongoing.
Conclusions: We expect that this simultaneous alteration of tumour microenvironment and induction of an immune response against Erbb-2 elicits an anti-tumour response of therapeutic significance, in that it halts the progression of lesions that cannot be inhibited by Erbb-2 vaccination alone.

290 The role of tetraspanins in antigen presentation to CD4+ T cells via exosomes

S. Petersen[1], G. Taylor[1], A.B. Rickinson[1], F. Berditchevski[1]. [1]*University of Birmingham, School of Cancer Sciences, Birmingham, United Kingdom*

Exosomes are membrane vesicles released by various cell types. When derived from antigen-presenting cells, exosomes are MHC class II-positive and can induce CD4+ T cell responses. Tetraspanins are a family of transmembrane proteins which might play a role in MHV II delivery to the cell surface and/or exosomes.
We have prepared exosomes derived from Epstein–Barr virus (EBV)-infected human B lymphoblastoid cell lines (LCLs) and shown by Western blotting and immunoelectron microscopy that they contain MHC class II and tetraspanins including CD63, CD81 and CD82.
Such LCL-derived exosomes can mediate immunologically specific recognition by MHC class II matched EBV antigen-specific CD4+ T cell clones (1) when directly added to the T cells in the absence of antigen-presenting cells, and (2) when added to B cells lacking the EBV antigen but expressing the MHC class II matching alleles. Using shRNA, we have decreased CD63 expression in LCLs and we are studying the effect of such downregulation on LCL and LCL-derived exosome function.

291 Do regulatory T cells require cognate MHC/peptide recognition for endothelial transmigration?

A. Popple[1], J. Ramage[1], I. Spendlove[1], L.G. Durrant[1]. [1]*The University of Nottingham, Academic Oncology, Nottingham, United Kingdom*

Background: The endothelium acts as a selective barrier for leukocyte migration into tissue, including tumour tissue, requiring recognition of non specific adhesion molecules, chemokine gradients and possible cognate MHC peptide. While it is accepted that higher numbers of Regulatory T cells (Tregs) can be found within tumour microenvironments there still remains uncertainty as to which conditions promote Treg recruitment into tumours. The aim of this study was to investigate the conditions which favour Treg transmigration.
Materials and Methods: Treg migration in response to tumour-associated chemokines and self MHC recognition was modelled using a murine model to mimic T cell transmigration across syngeneic (cognate MHC) and allogeneic (non-cognate MHC) murine lung endothelium. In addition the level of CXCL12 expression and T cell infiltration within tumours was examined by immunohistochemical analysis of ovarian tumour TMA samples.
Results: Our data shows that the level of CXCL12 expression by tumour cells can affect patient survival by potentially altering the balance of T cell subset infiltration into the tumour. Including a novel mechanism for Treg transmigration where cognate antigen-specific recognition of self-peptides is required for transmigration with preferential transmigration of Tregs across syngeneic endothelium, under conditions of inflammation and CXCL12.
Conclusion: Regulatory T cells recognising self antigen may preferentially accumulate within tumours where recognition of self peptides presented by self MHC allows migration of antigen-specific Tregs in response to CXCL12.

292 T-cell based identification of tissue antigens by automated two-dimensional protein fractionation

C. Herold-Mende[1], R.W. Warta[2], M. Schnölzer[3], R. Ahmadi[1], G. Dyckhoff[2], T. Woelfel[4], A. Unterberg[1], P. Beckhove[5]. [1]*University of Heidelberg, of Neurosurgery, Heidelberg, Germany*, [2]*University of Heidelberg, of Head and Neck Surgery, Heidelberg, Germany,* [3]*German Cancer Research Center, Functional Proteome Analysis, Heidelberg, Germany,* [4]*University of Mainz, III. Medical Department, Mainz, Germany,* [5]*German Cancer Research Center, Translational Immunology Unit, Heidelberg, Germany*

Background: Here we describe a new method to comprehensively identify candidate tissue antigens that spontaneously cause T-cell responses in disease situations.