**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

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**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

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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

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**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

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**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