ABSTRACTS

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10. Cancer Pharmacology

10.001 Paclitaxel reeducates tumor-associated M2-like Macrophages to M1 phenotype in a TLR-4/NF-kB dependent-manner and reduces tumor growth. Wanderley CWS[1], Colón DF[2], Luiz JPM[2], Oliveira FFB[1], Viacava PR[2], Leite CAVG[2], Pereira JA[2], Silva CMS[1], Silva CR[2], Silva RL[2], Mota JMSC[2], Alvez-Filho J[2], Cunha TM[2], Cunha FQ[2], Lima-Junior RCP[1] [1]UFC – Fisiologia e Farmacologia, [2]FMRP-USP
Introduction: Paclitaxel (PCX), a drug commonly used to treat breast cancer, has been shown to induce a LPS-like effect observed in human and animals cells. However, whether PCX could modulate antitumor immune response is still a matter of debate. Herein, we investigated whether PCX could influence macrophage polarization and whether that mechanism would modulate tumor progression. **Methods**: Murine bone-marrow derived macrophages (BMDM) from wild-type (WT) or toll-like deficient mice ($TLR4^{-/-}$) were cultured in RPMI and treated with LPS (100ng/mL, M1-like condition), IL-4 (10ng/mL, M2-like condition) or PCX (10, 30, 100µM) alone or concomitantly to IL-4, after 48h M1 and M2 markers (TNF, IL-12, IL-6, IGF and CD206) were measure by ELISA or flow cytometry. Raw 264.7 cells reporter to NF-kB was treated with LPS (100ng/mL) or PCX (30µM), after 6h the NF-kB luciferase activity was measure. Furthermore, WT, or $Tlr4^{fl/fl}$/LysM-cre mice were inoculated with murine tumor cell line (B16), and were treated with saline or PCX (10 mg/kg, 6/6h, i.p.) after treatment the frequency of $F4/80^{+}/CD206^{+}$ cells and the gene expression analysis of M1 (*Il12*, *inos* and *Il6*) and M2 (*cd206, relmα, mmp9* and *arg1*) markers was evaluated in isolated tumor associated macrophages (TAMs). Patients with ovarian cancer had a biopsy obtained before and after 3 cycles of PCX, the tumor gene expression profile by array was obtained in GEO datasets (GSE15622) and analyzed by R system. $P<0.05$ was accepted. **Results:** *In vitro,* PCX increased the NF-kB activation and the production of M1 markers in a dose dependent manner, similarly to LPS (NF-kB - Medium: 1±0.05 vs. PCX: 8.9±0.3 or LPS: 9.5±0.2; TNF - medium 9.5±0.3 vs. PCX: 721±30 or LPS: 554±20 for example). Furthermore, PCX blocked the IL-4-driven M2-type and reverted M2 polarized cells to M1-like profile (IGF – IL-4: 672±17 vs. IL-4 + PCX 49±17 and TNF – IL-4: 1.1±0.7 vs. IL-4 + PCX: 190±5 for example). However, the immunomodulatory effect of PCX failure in $TLR\text{-}4^{-/-}$ cells. *In vivo*, the gene expression analysis in TAMs revealed that PCX treatment altered the signature of M2-like cells to M1-like profile (cd206 – saline: 1.3±0.2 vs. PCX: 0.5±0.1; IL-6 – saline: 1±0.1 vs. PCX: 1.7±0.2 for example). Next, to confirm if the immune mechanism of action of PCX involves TAMs polarization via TLR-4, we generated mice in which TLR-4 was specifically deleted in myeloid cells (eg. macrophages; $Tlr4^{fl/fl}$/LysM-cre). The antitumor effect of PCX was reduced in $Tlr4^{fl/fl}$/LysM-cre mice compared to control group (WT - saline: 363±126 vs. PCX: 84±26 and $Tlr4^{fl/fl}$/LysM-cre - saline 730±104 vs. PCX: 644±276). Additionally, tumor biopsies from patients with ovarian cancer treated with PCX showed an increase in the mRNA levels of pro-inflammatory markers (IL6ST, LITAF, NFkB1). **Conclusion:** PCX antitumor efficacy is also related to an immunomodulatory activity through reeducation of TAMs into an active M1-type. Furthermore, these effects were dependent on a direct stimulation of TLR-4/NF-kB signaling. **Founding**: CNPq and FAPESP. CEPA:56/2016

10.002 Identification of circulating microRNA signature as a potential non-invasive biomarker for prediction of colorectal cancer. Silva CMS[1], Nobre LMS[1], Lucetti LT[1], Mello JBH[2], Kuasne H[2], Barros-Filho MC[2], Muniz HA[1], Falcão AL[1], Fernandes C[1], Aguiar MG[1], Ferreira DPPF[3], Cunha MPSS[4], Santana RO[4], Souza MHLP[1], Quetz JS[4], Wong DVT[1], Rogatto SR[2], Ribeiro RA[1], Lima-Junior RCP[1] [1]UFC, [2]AC Camargo Cancer Center, [3]Cesar Cals Hospital, [4]Haroldo Juaçaba Hospital – Cancer Institute of Ceará

Introduction: Colorectal cancer (CRC) is the third leading cause of cancer deaths, with an estimated 50,000 deaths each year. CRC is often diagnosed at a late stage when tumor metastasis may have already occurred, which is associated with high mortality rates. CRC Alternative strategies are therefore urgently required. The gold standard for CRC screening and diagnosis are endoscopic tests, which are expensive and invasive procedures. Currently, the search for non-invasive biomarkers has been an alternative strategy for CRC diagnosis at early potentially curable stages. In that context, circulating microRNAs (miRNAs) are of interest. miRNAs are small posttranscriptional modifiers of gene expression that are frequently altered in disease conditions and are released into the circulation by pathologically affected tissues. In the present study, we aimed to clinically identify the molecular signature of miRNAs during the CRC carcinogenesis. **Methods**: TaqMan low-density array human miRNA Cards were used to analyze 377 miRNAs in plasma samples of a hundred volunteers (n= 20 patients/group) that represented CRC carcinogenic process, including healthy individuals as control group, and also patients with either hyperplastic polyp, adenoma, CRC (early CRC) or metastatic CRC (late CRC). Student's t-test was used to compare expression levels of miRNAs. $P<0.05$ was accepted. **Results:** The identification of miRNAs was performed through an unsupervised clustering analysis (hierarchical cluster analysis). Most of the samples from the early and late CRC groups were classified in the first cluster with 292 miRNAs, which were organized by consensus of hierarchical subtype. Through a supervised grouping analysis, a pattern of sample clustering from the CRC groups was organized in a cluster. Then, the nine most differentially expressed miRNAs were selected and combined to obtain the best classification comparing the plasma of patients with malignant and non-malignant lesions. The comparison between CRC patients and non-cancer led to the identification of 5 miRNAs differentially expressed (hsa-let-7e-5p, hsa-miR-106a-5p, hsa-miR-28-3p, hsa-miR-203a e hsa-miR-542-5p). For discrimination, sensitivity of 93% and specificity of 90% were determined. **Conclusions**: A potential circulating miRNA signature associated with CRC carcinogenesis was identified that could be used as biomarker for non-invasive disease screening. **Financial Support**: REBRATS/CNPq and CAPES. Ethics Committee: 1891627.

10.003 Abatacept, a CTLA4-Ig complex which inhibits T-cell activation, enhances irinotecan-induced intestinal mucositis. Nobre LMS[1], Silva CMS[1], Silva FWL[1], Silva RL[1], Pereira VBM[1], Paiva IKD[1], Fernandes C[1], Alves APNN[2], Lima-Júnior RCP[1] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Farmácia, Odontologia e Enfermagem

Introduction: Intestinal mucositis (IM) is a common side effect of irinotecan, a drug used in first-line treatment regimens for colorectal cancer. Over the last decades, many aspects of the pathogenesis of IM have been discovered. However, little is known regarding the role of lymphocytes in IM. Then, we aimed to investigate the effect of abatacept, a CTLA4-Ig complex which targets T-cell activation, during IM. **Methods** Swiss male mice (20-25g) were divided into groups (n = 6-8) and injected with either saline (5 mL/kg, i.p.), irinotecan (75 or 45 mg/kg, i.p. for 4 days), abatacept (10mg/kg, i.p. 1h before first dose of irinotecan) or abatacept (10mg/kg, ip). The animals were analyzed daily for loss of body mass, diarrhea and survival. The animals were euthanized on day 5 (protocol 1, irinotecan dose = 75mg/kg) or day 7 (protocol 2, low dose of irinotecan = 45 mg/kg). Blood samples were collected for the total leukocyte count. Following animal euthanasia, ileum samples were collected for myeloperoxidase assay (MPO), histopathology and cytokine levels (pg/mg tissue). ANOVA / Bonferroni, Kruskal-Wallis / Dunn's or Log-rank tests were used for statistical analysis. $p < 0.05$ was accepted (CEUA: 11/17). **Results** In protocol 1, Irinotecan induced a pronounced leukopenia (564.3 ± 77.99), intestinal damage [1 (1 – 2)] and inflammatory response (2,769 ± 297.4) versus saline group (2,158 ± 148.6; 0 (0 - 0); 821.5 ± 167.5). In addition, pre-treatment with abatacept reduced ($p < 0.05$) the animal survival (62.5% ± 17.11) and induced a pronounced loss of body mass (84.33 ± 4.15) ($p < 0.05$) compared with their inotecan group. Besides, abatacept enhanced diarrhea [1 (0 – 4)], intestinal damage (0.69 ± 0.04), as detected by reduced villi/crypt ratio and inflammatory reaction (4,6 ± 743.9) versus irinotecan injection alone (0 [0 – 2];0.98 ± 0.05; 2,769 ± 297.4;$p < 0.05$). In the second protocol, irinotecan subdose induced mild to moderate intestinal damage versus the saline group (1.15 ± 0.07; 1.94 ± 0.08, $p<0.05$), but the pre-treatment with abatacept enhanced the damage, as detected by diarrhea scores (2 [0 – 3]), intestinal damage (1.15 ± 0,07), MPO (1,240± 180.2) and KC (107.3 ± 23.9) and IL-1 levels(123.8 ± 33.6) versus irinotecan group[0 (0 – 2); 1.59 ± 0.07; 717.1 ± 59.60; 43.9 ± 25.4; 27.5 ± 6.4]. **Conclusion** Abatacept aggravated irinotecan-induced intestinal mucositis in mice. **Financial Support**: CNPq, Capes and Funcap. CEUA: 11/17

10.004 Autophagy promotes cell survival in Temozolomide/SAHA treated glioma cells. Gonçalves RM, Zanotto-Filho A UFSC – Farmacologia

Introduction: Glioblastoma multiforme (GBM) is the most frequent and aggressive type of primary brain tumor. In this study, we tested the efficacy of combining the classical anti-GBM chemotherapeutic temozolomide (TMZ) with suberoylanilide hydroxamic acid (SAHA) - an inhibitor of histone deacetylase (HDAC) 1, 2, 3, and 6 – upon the viability and cell death mechanisms in C6 and U251MG glioma cells in vitro. **Methods:** MTT assays were used to evaluate potential synergy between SAHA and TMZ. Annexin-V-FITC, caspase-3 activity, cell cycle analysis and acridine orange staining as well as LC3 and p62/SQSTM1 immunoblots were performed to evaluate apoptosis and autophagy in SAHA/TMZ-treated cells. **Results:** The data showed that synergy between TMZ and SAHA was not achieved due to activation of protective autophagy *in vitro*. The SAHA/TMZ treatment promoted arrest of 50 % in the G2/M phase of the cell cycle as soon as 24 h whereas apoptosis was only detected after long-lasting drug exposure (≥96 h), associated with increased from sub-G1 of 10 % in 24 h to 43 % in 96 h. Time-course of autophagy preceded apoptosis as detected by increased formation of acidic acridine orange-stained vacuole, increased immunocontent of lipidated LC3 and decreased p62/SQSTM1 protein levels. By blocking the termination step of autophagy with chloroquine we were capable of promoting a reduction in the viability of 15 % in glioma cells which was accompanied by increased apoptosis of 20 % in SAHA/TMZ treatment. **Conclusion:** This study demonstrates that autophagy impairs the efficacy of combined TMZ/SAHA in vitro, and inhibiting this process can improve the therapeutic potential of these compounds. References: JOHNSON, D.R. et al. J. Neurooncol, v. 107, p. 359, 2012; CHIAO, M.-T. et al. Autophagy, v. 9, p. 1509, 2013. Acknowledgements: Brazilian funding agencies CAPES, FAPERGS and CNPq.

10.005 Bioguided fractionation of acetone extract from *Annona muricata* L. seeds for prospecting of molecules with cytotoxic potential *in vitro*. Rocha GGG[1], Santos RS[1], Silva MFS[2], Moura AF[1], Brito LM[1], Santos CC[3], Pessoa OC[4], Moraes OM[1]
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Introduction: Graviola (*Annona muricata*) is a tropical fruit tree of the family Annonaceae that is widely used as a traditional drug and studies conducted with its bioactive secondary metabolites have shown remarkable anti-cancer properties associated with Annonaceous acetogenins (SACRAMENTO et al., 2003; MISHRA et al., 2013). In this context, the present study aimed the bioguided fractionation of the acetonic extract of *A. muricata* seeds to prospect for molecules with cytotoxic potential in human tumor cell lines. **Methods:** The fractionation of acetone extract of A. muricata seeds (AMSA) was executed by column chromatography and was guided by the cytotoxic activity of the most active fractions and sub-fractions using the MTT assay, after 72 hours of incubation. Cell growth was quantified by the ability of living cells to reduce MTT to a blue formazan product. The fractions were tested against three cancer cell lines, HCT-116 (colon), HL-60 (leukemia) and SF-295 (brain), the sub-fractions against two cancer cell lines, HCT-116 and HL-60, and the compound isolated against nine cell lines, HCT-116, HEPG2 (liver), HL-60, NCI-H460 (lung), PC3 (prostate), PC9 (lung), SF-295, SNB-19 (brain), SW620 (colon) and one non tumoral cell line, L-929 (murine fibroblast). High performance liquid chromatography (HPLC) was used to purify the sample and Nuclear Magnetic Resonance, mass and infrared spectrometry were used to determine the compound. **Results:** The crude extract was fractionated into four fractions, hexane (AMSA-H), dichloromethane (AMSA-D), ethyl acetate (AMSA-A) and methanolic (AMFA-M). The fractions AMSA-D, AMSA-A and AMSA-M showed antiproliferative effects against all tested tumoral cell lines with IC_{50} values ranged from 0.01 to 0.6 µg/mL in HL-60; 0.5 to 120 ng/mL in HCT-116 and 1 to 400 ng/mL in SF-295. AMSA-A showed best activity and was fractionated in 32 sub-fractions which were grouped according to chemical profile. The sub-fraction AMSA-A (8-9) showed IC_{50} values ranged from 0.02 to 0.08 µg/mL in HCT-116 and HL-60, respectively and was fractionated in 59 sub-fractions. The sub-fraction AMSA-A (8-9)(49-57) showed IC_{50} ranged from 1,2 to 2.2 µg/mL in HCT-116 and HL-60, respectively and was purified by HPLC. The isolated compound was identified as annonacinone, a mono-THF acetogenin. The annonacinone were tested against nine cancer cell lines with IC_{50} values ranged from 3.7 to 0.19 µM in SW620 and SF-295, respectively. Annonacinone showed no cytotoxic effect in L929 at the maximum concentration tested (5 µM). **Conclusion:** The results showed that the acetonic extract of *A. muricata* seeds is a promising source for the elucidation of compounds with cytotoxic activity in tumor cells. The annonacinone showed relevant cytotoxic activity *in vitro* against different human tumor lines, with greater effect in SF-295 cells, however, other tests should be performed to better evaluate the therapeutic potential of this molecule. **Supported by:** CNPq, PRONEX, CAPES, and FUNCAP. **References:** SACRAMENTO, C.K. et. al., Rev. Bras. Frutic. v. 25, p. 329, 2003; MISHRA, S. et. al., Glob. J. Pharm. Res. v.2, p. 1613, 2013.

10.006 Probiotics prevents irinotecan-induced experimental steatohepatitis. Melo AT[1], Aragão KS[1], Wong DVT[1], Fernandes C[1], Freitas JA[1], Gurgel DC[1], Pereira MA[2], Almeida PRC[3], Lima-Júnior RCP[1] [1]UFC – Farmacologia e Fisiologia, [2]VP, [3]UFC – Patologia e Medicina Legal

Introduction: Nonalcoholic steatohepatitis (NASH) is a severe side effect of the anticancer agent irinotecan. Preliminary results suggest that the intestinal microbiota plays an important role in the development of this condition. In addition, probiotics have been reported to prevent NASH in nutritional and genetic models. In the present study we aimed to evaluate the role of probiotics in the prevention of irinotecan-induced NASH. **Methods:** C57BL/6 mice (25-30g) were divided into experimental groups (n = 8-10) and injected thrice a week, every other day, with saline (5 ml/kg, ip) or irinotecan (50 mg/kg, ip) alone or in combination with daily injection of probiotics suspension (Simfort®, which contains *Lactobacillus acidophilus, Lactobacillus casei, Lactococcus lactis, Bifidobacterium bifidum e Bifidobacteirium lactis;* $1x10^7$ CFU/mL, p.o.). At week 5, blood samples were collected for total leukocyte count and, following euthanasia, liver and intestinal samples were obtained for assessment of histopathology, inflammatory parameters (number of neutrophils/mg of tissue), *real time* PCR to evaluation of Toll-Like Receptor 4 (TLR4) and TNF-□ receptor (TNFR) expression and f*low cytometry to quantification* of *lymphocyte* subsets. One-Way ANOVA or Kruskal Wallis test was used. *P*<0.05 was accepted. (CEUA: 21/12) **Results:** When compared to saline group, irinotecan caused a pronounced leukopenia (IRI: 3206 ± 398.6 *vs* SAL: 6400 ± 902.4) and intestinal damage [IRI: 10 (7-12) *vs* SAL: 4 (3-5)], which were significantly prevented by probiotics [6113 ± 683.5; 5 (3-8), respectively]. In addition, liver histopathological scores [IRI: 4.5 (2-6) *vs* SAL: 3 (2-3)] and the number of inflammatory foci (IRI: 7.6 ± 2.0 vs SAL: 0.6 ± 0.4) were increased in irinotecan group when compared to saline. These parameters were prevented in the animals treated with probiotics [3 (2-3); 2.7 ± 0.6, respectively]. In addition, irinotecan (39.67±2.40%) increased the number of $CD45^+CD25^+CCR6^+$ lymphocytes vs. saline (31.32 ± 1.20%). Furthermore, probiotics increased the % of lymphocytes subpopulations of $CD3^+CD4^+$ (40.09±1.18%), $CD45^+CD25^+CCR6^-$ (4.37±0.85%) and $CD45^+CD25^-CCR6^+$ (5.18± 0.63%) when compared to the irinotecan group (28.81±3.33%; 2.00±0.40% and 2.96±0.28%, respectively). On the other hand, probiotics did not change TLR4 (2.72 ± 0.17) and TNF-α receptor expression (4.47 ± 0.71), which were increased in the irinotecan group (TLR4: 3.51 ± 0.75,; TNFR: 11.68 ± 3.49) when compared to the saline group (TLR4: 0.97 ± 0.03; TNFR: 1.93 ± 0.69). **Conclusion:** Probiotics prevented irinotecan-related NASH partially by modulating systemic immune cells.**Financial support**: CAPES, CNPq and FUNCAP. **Keywords:** Steatohepatitis. Irinotecan. Intestinal microbiota. Probiotics.

10.007 Melatonergic system enzymes ASMT and CYP1B1 as biomarkers and pharmacological targets for solid tumors Kinker GS[1], Ostrowski LH[1], Muxel SM[1], Oba-Shinjo SM[2], Marie SKN[2], Markus RP[1], Fernandes PA[1] [1]IB-USP – Physiology, [2]FM-USP – Neurology

Introduction: Melatonin synthesized and released by the pineal gland at night is responsible for translating the environmental dark phase to the organism. The production of melatonin has also been detected in many extra-pineal tissues, including retina, gastrointestinal tract and brain. Generally, extra-pineal melatonin is poorly released into the circulation, acting locally in an autocrine and paracrine manner. Currently, it is well established that melatonin exerts oncostatic effects across a wide variety of tumors, impairing tumor growth, invasion and angiogenesis. In this sense, we have recently demonstrated that the ability of gliomas to synthesize and accumulate melatonin negatively correlates with their overall malignancy (Kinker et al., 2016). Using luzindole, a non-selective antagonist of melatonin membrane receptors, we have shown that glioma-synthesized melatonin exerts an autocrine anti-proliferative effect. Additionally, based on The Cancer Genome Atlas (TCGA) glioma RNAseq data, we designed a predictive model of the content of melatonin in the tumor microenvironment, the ASMT:CYP1B1 index, combining the gene expression levels of melatonin synthesis and metabolism enzymes. The ASMT:CYP1B1 index negatively correlates with tumor grade and represents an independent prognostic factor. Even when considering only high-grade gliomas, a low ASMT:CYP1B1 score, suggestive of low levels of melatonin in the tumor microenvironment, is strongly associated with poor survival. Therefore, here we investigated the clinicopathological relevance of the ASMT:CYP1B1 index in fifteen other types of solid tumors. **Methods:** We obtained level 3 TCGA RNAseq data and clinical information of 7,002 patients from sixteen types of solid tumors (including gliomas). Based on single sample gene set enrichment analysis (ssGSEA), we created a melatonin signature combining the expression of genes involved in tumor development/progression that are known to have their expression levels modulated by melatonin. Classical GSEA was used to evaluate the correlation between the index value and the expression of gene sets obtained from the Reactome pathway database. Survival analysis was performed using univariate and multivariate Cox regressions. **Results:** In all tumors analyzed, the ASMT:CYP1B1 index negatively correlated with the melatonin signature score and with the expression of Reactome gene sets involved in tumor growth, migration and invasion. Importantly, as observed for gliomas, the ASMT:CYP1B1 index was an independent positive prognostic factor for bladder urothelial carcinoma, colorectal adenocarcinoma, lung squamous cells carcinoma, pancreatic adenocarcinoma, paraganglioma/pheochromocytoma, stomach adenocarcinoma. **Conclusion:** Overall, our data reinforce the role of the ASMT:CYP1B1 index as a robust predictive model of the content of melatonin in the tumor microenvironment and highlight the potential of ASMT and CYP1B1 as clinically relevant biomarkers and pharmacological targets. Moreover, we provide support for further investigations of the biological role of melatonin synthesized by malignant cells. **Financial Support**: FAPESP (2010/52687-1, 2013/13691-1, 2014/27287-0), CNPq (480097/2013-5, 162670/2014-1).

10.008 Cytotoxic effect of telocinobufagin on human colorectal ileocecal adenocarcinoma cells. Godoy TM, Castelo-Branco MTL, Quintas LEM UFRJ – Ciências Biomédicas

Introduction: Na/K-ATPase (NKA) is a plasma membrane protein currently recognized as a target for antitumor therapy, since this membrane protein plays an important role in cell adhesion and its increased expression and activity accompanies tumor progression. Cardiotonic steroids (CTS) are a class of compounds that has NKA their single receptor in cells. In addition, through the interaction with NKA, these substances are able to trigger the transduction of intracellular signals that underlie different effects such as proliferation, differentiation and apoptosis, depending on the cell type. In vitro and in vivo studies report antiproliferative and antitumor activity of several CTS in tumor cells. Currently, there are no studies on the effect of telocinobufagin (TCB), a bufadienolide present in the parotoid secretion of Rhinella toads and one component of Chan'Su, a traditional Chinese medicine, on tumor cells. Here, we are investigating the effect of TCB on the human colon cancer HCT8 cell line. **Methods**: HCT8 cells were cultured in DMEM + 10% FBS and antibiotics, and cells were serum-starved for 24 h before treatment. In 24-well plates, cells were treated with 30, 100 and 300 nM TCB for 24, 48 and 72 h, when cells were trypsinized, centrifuged and Trypan blue viable cells were counted in Neubauer chamber. For experiments with [3H]thymidine, HCT8 cells were grown in 96-well plates and treated with the same concentrations of TCB. After 24 h, 50 nCu of [3H]thymidine was added to each well for 6 h, followed by trypsinization and radioactivity counting in liquid scintillation counter. **Results**: Compared to control, decrease of cell number was significantly observed after 24 h for 100 and 300 nM TCB (35 and 42%, respectively; $p<0.05$, $n=6$) and with 30, 100 and 300 nM TCB after 48 (36, 42 and 57%, respectively; $p<0.05$, $n=5$) and 72 h (55, 63 and 79%, respectively; $p<0.05$, $n=3$). The experiments of [3H]thymidine incorporation showed a significant reduction at 100 nM (40%) and 300 nM (76%, $p<0.05$, $n=4$). **Conclusion**: TCB displays an antiproliferative effect in a concentration- and time-dependent fashion. More studies are underway to characterize the type of cell death and signaling pathways involved. **Financial support**: FAPERJ, CAPES, e CNPq.

10.009 Cytotoxic and genotoxic effects of a *Stevia urticifolia* extract on animal and plant cells. Sousa IJO[1], Ferreira JRO[2], Silva JN[2], Machado KN[3], Nascimento AM[3], Costa MP[1,2], Cavalcante AACM[4], Ferreira PMP[5,2] [1]UFPI – Farmácia, [2]UFPI – Cancerologia Experimental, [3]UFOP – Química, [4]UFPI – Genética Toxicológica, [5]UFPI – Departamento de Biofísica e Fisiologia

Introduction: To overcome the resistance of many tumors, the search for new antineoplastic agent alternatives becomes a constant necessity. **Aims:** To evaluate the *in vitro* and *ex vivo* antioxidant, cytotoxic and antiproliferative activity of ethyl acetate extract from *S. urticifolia* aerial parts. **Methods:** Phytochemical and antioxidant (DPPH) evaluations were performed by colorimetric assays. For preliminary toxicity studies, *Artemia salina* larvae were treated with the extract for 24 h. Death *nauplii* were quantified to obtain CL_{50} value. For hemolytic capacity, blood was collected from anesthetized female Swiss mice (Protocol 008/2015-UFPI) to obtain a cell suspension of erythrocytes 10 % in 0.85% NaCl solution. Liberated hemoglobin was measured at 540 nm. Meristematic cells from *A. cepa* roots were examined after 72 h exposure to determine Mitotic Index (MI) and chromosomal alterations. Next, ascitic Sarcoma 180 cells were treated for 24h and submitted to the trypan blue exclusion test to investigate antiproliferative action. **Results:** Qualitative phytochemical evaluation revealed the presence of flavonoids, terpenoids and saponins. The extract showed concentration-dependent antioxidant capacity (EC_{50} of 253.0 ± 3.6 µg/mL), toxicity upon *A. salina* nauplii [LC_{50} of 68.9 (63.7-74.5) µg/mL), r^2= 0.971], slight hemolytic action [EC_{50} of 131.5 (108.6-159.1) µg/mL, r^2= 0.990] and antiproliferative activity on Sarcoma 180 cells [CI_{50} of 16.5 (13.1-20.9) µg/mL]. The extract also inhibited root growth of *A. cepa* (33.2 ± 8.1, 30.2 ± 8.1 and 64.3 ± 5.4 %) and mitotic index (33.0 ± 7.7, 28.3 ± 5.5 and 21.1 ± 2.9 %) at concentrations of 25, 50 and 100 µg/mL (1.53 ± 0.1, 1.54 ± 0.1 and 1.07 ± 0.1 cm, respectively). Chromosomal alterations were found at 100 µg/mL (bridges: 10.8 ± 4.6, lost: 12.0 ± 1.2; fragmentations: 27.8 ± 2.6; anaphase delays: 16.0 ± 3.6) ($p < 0.05$). **Conclusion:** *Stevia urticifolia* extract showed antiproliferative activity on vegetal and animal cells and clastogenic effects on growing *A. cepa* roots, indicating its pharmacological potential for the development of antineoplastic drugs. **Financial support:** FAPEPI and CNPq (CEEA-UFPI - Protocol 008/15) **Keywords:** Allium cepa. Antiproliferative action. *Artemia salina*. Chromosomal alterations. Sarcoma 180.

10.010 Effects of prodigiosin and derivatives in melanoma lineages. Pontes CA[1], Branco PC[1], Jimenez PC[2], Costa-Lotufo LV[1] [1]ICB-USP – Farmacologia, [2]Unifesp

Introduction: The growing incidence of skin cancer melanoma, as well as resistance to conventional anticancer therapy, becomes one of the most challenge question in oncology, therefore search for new therapies is constant. BRAF mutations are present 40-60% of the melanomas while NRAS mutations are found in 15-20%. These mutated genes are essential components of the MAPK pathway, participating of normal processes of growth and survival of cells. The mutation in this pathway leads to its constitutive activation causing exacerbated proliferation. The actual therapies for treatment of melanoma present as pharmacological target the mutated $BRAF^{V600E}$. Although promising, the more recent results show that much of the patients demonstrate pharmacological resistance. The natural marine products are significant source of anticancer compounds. Prodiginines are bacteria products, isolated from several species of both gram-negative and gram-positive bacteria. These substances are recognized as potent antiproliferative agents. Our research group isolated four prodiginines: prodigiosin (PG, m/z 324.2059 [M + H]+), cyclononylprodigiosin (CNP, m/z 364.2294 [M + H]+), nonylprodigiosin (NP, m/z 366.2453 [M + H]+) and methylcyclooctylprodigiosin (MCP, m/z 366.2383 [M + H]+) from marine bacteria. Thus, the aim of the present work is to evaluate the cytotoxicity of prodigiosin and derivatives against a panel of melanoma cells including $BRAF^{V600E}$ mutated cell lines. **Methods**: The cytotoxicity of the substances was evaluated by MTT assay using two cell lines with mutated $BRAF^{V600E}$, Sk-mel-19 and Sk-mel-28, and one NRAS mutated cell line, Sk-mel-147. The viability of treated cells was assessed by trypan blue exclusion assay. To analyze whether the treatment interferes in cell cycle the DNA was stained with propidium iodide and its fluorescence measured in a flow cytometer. To analyze the morphology, the cells were dyed by fast panoptic staining. **Results**: PG, NP and CNP presented a potent activity against the melanoma cell lines, while MCP was not active. The obtained IC_{50} values ranged from 0.02 to 0.06 µM in Sk-mel-19, from 0.02 to 0.32 µM in SK-Mel-28, and from 0.52 to 2.7 µM in Sk-mel-147. Sk-mel-19 with mutated BRAF was generally more sensitive than Sk-mel-147 with mutation in NRAS. It was observed a significant decrease in the number of viable cells, but with no significance increase in non-viable cells for PG, NP and CNP, suggesting a cytostatic rather than a cytotoxic activity. The cell cycle experiments suggest an arrest in G1 phase. **Conclusion**: The substances presented an antiproliferative activity mainly against BRAF mutated cell lines, however more studies must be performed to comprehend their mechanisms. **Financial support**: CNPq (PróArquipélago, INCTBioNat and PIBIC) and FAPESP (2015/17177-6)

10.011 Investigation of cytotoxic mechanisms of synthetic derivatives of seriniquinone in melanoma cell lines. Hirata AS[1], Branco PC[1], Santos EA[2], Jimenez PC[3], Fenical W[4], Costa-Lotufo LV[1] [1]USP – Farmacologia, [2]USP – Biologia Celular e Molecular, [3]Unifesp – Ciências do Mar, [4]Scripps Institution of Oceanography

Introduction: Through the bioprospection of microbial secondary metabolites, Seriniquinone (SQ) was isolated from a marine bacterium of the genus *Serinicoccus*. Further, it was shown to regulated the expression of the DCD gene and induced cell death by autophagy selectively in melanoma cell lines (TRZOSS, 2014), the most serious skin tumor due of its aggressiveness and high resistance to chemotherapy. Several therapeutic targets have been explored from the molecular basis of tumorigenesis and the DCD gene, which encodes the dermcidin protein, is an example that has been studied more recently. It is known that this protein has an important role in skin defense, where antimicrobial peptides are derived from dermcidin. On the other hand, its role in neoplasias remains poorly understood, with punctual observations related to proliferation, migration, invasion, survival and resistance. Considering that, 4 structural analogs (LT402, LT432, LT406 and LT4120) of SQ were synthesized in order to increase cytotoxicity and selectivity for melanoma cells and to improve SQ pharmacokinetic properties. In view of this, the present work aimed to evaluate the cytotoxicity of these molecules in tumor and non-tumor cell lines, as well as to study the mechanism of action of them. **Methods**: MTT assay (MOSMMAN, 1983) was used to indirectly measure the cytotoxicity of the compounds on tumor and non-tumor cell lines and to determine their IC_{50}. SK-MEL-28 melanoma cell line was used as model to investigate on viability (by Trypan blue), cell cycle and two protocols of clonogenic assays, with SQ and LT406 treatments. **Results**: SQ and its synthetic derivatives showed, in general, high cytotoxicity in the tested cell lines, with lower IC_{50} for melanoma cells, as well as higher selectivity. On the viability and clonogenic assays with SK-MEL-28, SQ and LT406 decreased the number of viable cells and increased the number of non-viable ones. However, SQ was the best inhibitor of colony formation. These treatments showed to modulate cell adhesion; still, only cells treated with LT406 were able to adhere again. Through cell cycle analysis, it was observed that SQ and LT406 generated cell accumulation at S/G2 phases. **Conclusion**: Seriniquinone and its synthetic derivatives showed, in general, high cytotoxicity in the tested cell lines, with selectivity towards melanoma cells. Therefore, it can be concluded that the intention of the synthetic molecules has been met. Then, more studies are needed to elucidate the mechanism of action of these molecules. References: TRZOSS, et al. Proc Natl Acad Sci USA. v. 111. p. 14687. 2014. MOSMMAN, T. J. Immunol Methods: v. 65. p. 55.1983. Acknowledgments: FAPESP (Process numbers: 2015/17177-6 and 2016/10854-5) and CNPq (INCTBioNat).

10.012 Versican: **A differential marker and a potential pharmacological target for gestational trophoblastic diseases**. Souza LPG[1], Oliveira HGS[1], Pires KSN[1], Santos JC[1], Gonçalves CM[1], EMP Silva[1], Botelho RM[1], Silva ALM[1], Pendelosky KPT[2], Daher S[2], Sun SY[2], Borbely KSC[1], AU Borbely[1] [1]UFAL – Health and Biological Sciences, [2]Unifesp – Obstetrics

Introduction: Gestational trophoblastic diseases (GTD) are characterized both by benign tumors, such as partial and complete moles, as well as malignancies, such as invasive moles and choriocarcinomas. They are considered rare conditions in developed countries, but their incidence and morbidity are rising in undeveloped countries. Current diagnoses are based on routinary dousage of beta-hCG and transvaginal ultrasound, followed by curettage and chemotherapy with methotrexate. Although rather efficient, they can cause serious or life-threatening side effects and novel therapeutic targets are in dire need. As such, versican, a danger-associated molecular pattern was investigated in order to establish its differential expression among GTD and the possible outcomes of its gene silencing. **Methods:** Partial, complete and invasive moles, and choriocarcinomas had versican isoforms V0, V1, V2 and V3 expression evaluated by immunohistochemistry and RT-PCR. Choriocarcinoma-derived BeWo cells were employed for versican mRNA silencing, which was confirmed by RT-PCR, immunofluorescence and flow cytometry. Cell death, hCG and cytokines production were analyzed by flow cytometry and forskolin-induced syncytialization by hematoxylin-eosin staining. **Results:** Versican V0 and V1 isoforms were mainly expressed in syncytiotrophoblast and more expressed in benign tumors in comparison to healthy placenta and malign tumors, disappearing completely in invasive moles. BeWo cells also expressed V0 and V1 isoforms, only in cells undergoing syncytialization. Versican expression reduced 70% after gene silencing. Versican gene silencing greatly decreased spontaneous and forskolin-induced syncytialization, decreased hCG secretion and increased cell death. **Conclusion:** Versican expression might be used as a differential marker for different types of GTD. Furthermore, versican expression pattern in consonant with the functional results after gene silencing suggest versican could be a novel therapeutic target in benign tumors, since it is important for syncytialization and survival. **Financial Support** and Acknowledgements: Cell Biology Laboratory from ICBS/UFAL Ethical Approval: CAEE 43605515.9.0000.5013

10.013 Investigation of the effect of Synadenium grantii on the inhibition of breast cancer cell proliferation Oliveira JS, Martins MM, Montor WR FCMSCSP – Ciências Fisiológicas

Introduction: *Synadenium grantii* popularly known as "leiterinha" is an euphorbiaceous shrub, rich in biologically active compounds such as flavonoids, alkaloids, terpenes and others, popularly used in regions of Brazil in the treatment of gastric diseases, allergies and cancer. Our objective is to evaluate the antitumor effect of the latex of this plant in breast cancer cell lines, the second most common type of cancer among women. **Methods:** This study aims to analyze the antiproliferative properties of the latex in MCF-7, representative of a less aggressive tumor type, and MDA-MB231, representative of triple negative tumors, the most aggressive breast cancers in the clinics, with no specific therapy so far, initially using the MTT assay and growth curves. **Results:** To determine if the homemade formulation used by the population (18 drops/L in water) can promote proliferation inhibition, different concentrations of the latex, freshly extracted from plants cultivated in our laboratory were prepared in culture medium. Solution C corresponds to what the population uses and solutions A and B are 5x and 2x more concentrated than C, while solutions D and E correspond to ½ and $^{1}/_{3}$ of solution C, respectively. In the pilot experiment, morphological alterations and cell viability were evaluated along the harvesting of cells for the growth curve with solutions A, B and C. For C, MCF-7 presented itself enlarged after 24h of treatment, while the influence on MDA-MB-231 was the presentation of cytoplasmic projections after 72h. Concentrations A and B were very toxic to both cell lines, killing all cells within the first days of treatment, while C left about ¼ of viable cells in the case of MCF-7 and showed almost no effect on MDA- MB-231, which is classically more resistant to any treatment attempt. For the MTT assay, solutions C, D and E were used for a 24 h treatment. C and D seemed to promote some growth inhibition on MDA-MB-231, although not statistically significant and being further checked. Preparation E had no effect on this cell line. Nonetheless, for MCF-7, activity was decreased to 49.4% under the effect of C ($p=0.0022$), 38.5% under the effect of D ($p=0.0176$) and 42.2% under the effect of E ($p=0.0088$), showing that MCF-7 is more sensitive to the latex preparation. **Conclusion:** So far it was possible to conclude that the latex promotes cell death as acutely visualized in higher concentrations and also changes the morphology and proliferation curve of the tested cell lines, possibly by the inhibition of proliferation beyond death by the observed pattern. Potentially, it also promotes the inhibition of metabolic activity, evidenced by the MTT assay for MCF-7. Other experiments on cell proliferation, death and migration are underway **Financial support** – CNPQ/PIBIC; FAP - Santa Casa **CEP Number**: 951.081

10.014 Prodigiosin effects on melanoma cell lines: **survivin as a possible target**. Branco PC, Pontes CA, Bauermeister A, Alves-Fernandes DK, Hirata AS, Jimenez PC, Lopes NP, Engler SSM, Lotufo LVC ICB-USP

Introduction: Cancer is one of the diseases with the highest mortality in the world. One hallmark of cancer is its ability to evade apoptosis, justifying studies aimed to validate targets that modulate the disease in this pathway, such as inhibitory apoptosis proteins (IAPs). Survivin is IAP member that is highly expressed in tumors and has an essential action for tumor maintenance, the search for drugs that modulate its action is of vital importance. Among cancer types, melanoma is a skin cancer with high mortality rate and that develops chemoresistance to conventional therapy. The search for new alternatives is necessary. The marine environment, an exceptional reservoir of bioactive natural products. Prodigiosin (PG) is a pigment produced by microorganisms and an effective pro-apoptotic agent against several cancer cell lines, including multiple drug resistant cells with little toxicity against normal cell lines. In the productive chain of drug development, elucidation of the molecular target is still one of the main obstacles, adding to the process high costs and time. Functional chromatography is a very innovative approach that aims to isolate bioactive substances already based on their interaction with a molecular target of relevance in the treatment of cancer. We aim to study the effects of PG on melanoma cell lines, naïve and resistant to therapy emphasizing the role of survivin as a possible molecular target. **Methods:** Cell lines MCF-7, HCT116, RPE, NGM, SKMel 147 besides SKMel 19 and 28, both naive and resistant to vemurafenib were tested against prodigiosin, MTT assay was performed. Using the IC_{50} we also evaluated cell cycle, viability and cell migration assays. For determining the prodigiosin target, functional chromatography (Lau et al., 2015) and thermoforesis assay. **Results:** Prodigiosin showed a selective citotoxity against melanoma cell lines, besides that it is clear that cell lines presenting BRAF mutation are more sensitive when compared to NRAS mutation, with IC50 (µM) values ranging from 0.02 in SKMel 19 cells to 3.13 in RPE cells. Interestingly, prodigiosin was active against SKMel 19R (0.05 µM) but not against SKMel 28 resistant to BRAFi (>5µM). Prodigiosin also promoted a significative reduction of cell area and number of colonies in clonogenic assay in SKMel 19 cell lines but not in SKMel 28. We also showed an increase in cell fragmentation (sub G1) population in SKMel 19, both naïve and resistant. Regarding prodigiosin target, we both checked that survivin is a main candidate, functional chromatography demonstrated a hit when evaluating this interaction, what was also suggested by thermoforesis demonstrating a Kd of 2.2 □M in our preliminary data. **Conclusion:** We conclude that prodigiosin is active against BRAF melanoma, including a resistant to BRAFi cell line. Studies of heterolog survivin suggested that survivin, an IAP protein, is the potential target for prodigiosin. Further studies are necessary to describe its molecular mechanism of action and to better comprehend the difference obtained for the two cell lines resistant to chemotherapy. **Reference:** Lau EC; Mason DJ; Eichhorst N; Engelder P et al. Functional chromatographic technique for natural product isolation. ***Org. Biomol. Chem.***, 2015,**13**, 2255-2259 **Finantial Support:** Capes; CNPq and Fapesp (2015/17177-6)

10.015 Spontaneously hypertensive rats were more resistant to the 4NQO-induced oral carcinogenesis than normotensive rats. Tjioe KC[1,2], Lopes FYK[1,2], Valente VB[2], Soubhia AMP[3], Oliveira SHP[1], Bernabé DG[2] [1]FOA-Unesp – Basic Sciences, [2]FOA-Unesp – Pathology and Clinical Propaedeutics, [3]FOA-Unesp – Pathology and Clinical Propaedeutics

Introduction: Strong evidence suggests that sympathetic nervous system activation accelerates cancer progression however its role in the carcinogenesis process remains controversial. Spontaneously hypertensive rat (SHR) is a widely used model of primary hypertension and it presents increased tissue and circulating norepinephrine levels than Wistar rats. The aim of this study was to investigate the incidence and clinic-pathological profile of chemically-induced oral cancer in SHR. **Methods:** Thirty SHR and 30 Wistar rats (control) were treated with the carcinogen 4NQO for 5 months. After euthanasia, tongue specimens were microscopically diagnosed and analyzed. P16, VEGF, TNF-alpha, IL-6, MMP-2, and MMP-9 expressions were verified by RT-PCR. Mast cells and eosinophils were quantified in hematoxylin and eosin cuts and natural killer (NK) cells, in anti-CD57 immunohistochemically stained cuts. The anxiety levels of both groups were verified by the elevated zero maze (EZM) and opened-field tests before the carcinogenic induction, 75 and 150 days after tumor induction. All results were statistically confronted and p values below 0.05 were considered significant. **Results:** Tongue cancer incidence was lower in SHR (28%) than Wistar rats (60%). MMP-9 expression was reduced in the SHR tongue cancer than Wistar ($p<0.05$) while p16, VEGF, TNF-alpha, IL-6, and MMP-2 levels did not differ statistically. Despite of SHR presenting an average of 6 times less eosinophils per field than Wistar rats, this difference was not significant. Neither the amount of mast cells nor of NK cells differed between the groups. SHR spent more time and frequency at the central area of the open-field test than the Wistar rat ($p=0.0001$) in the 3 analyzed periods. Interestingly, at the 150-day following the carcinogen induction no Wistar rat went to the central area of the apparatus, indicating a strong anxious behavior. EZM test corroborated the previous results at the pre-carcinogen period but not in the 150-days treatment. Additionally, both anxiety tests showed a progressive increase of anxiety levels of both groups during 4NQO treatment. **Conclusion:** Our results suggest that the SHR presented higher resistance to 4NQO-induced oral carcinogenesis and a lower anxious behavior than the Wistar rats.. Moreover, the SHR tumors presented lower expression of genes related to the neoplastic progression.. **Financial Support**: Fundação de Amparo à Pesquisa do Estado de São Paulo (process # 14/25393-8) and Pró-Reitoria de Pesquisa da UNESP (ProPe) (#12/2015/Prope). This study was approved by the animal research ethical committee from Araçatuba School of Dentistry, São Paulo State University, protocol #2014- 01446.

10.016 Antitumor, **hepatic and hematological effects of the mesoionic compound SIDNONE-1 in mice with Ehrlich tumor**. Agnes JP[1,2], Corso C R[2], Adami ER[2], Echevarria A[3], Acco A[2] [1]UFSC – Farmacologia, [2]UFPR – Farmacologia, [3]UFRRJ – Química

Introduction: Due to the lack of selectivity of the chemotherapeutic agents used in cancer treatment, several side effects are observed, reducing the quality of life of the patients. Research with new compounds demonstrating antitumor effects and few adverse reactions that do not compromise the life quality is essential. The present work evaluated the antitumor, hepatic and hematological effects of the mesoionic compound Sydnone-1 (SYD-1) in Swiss mice inoculated with Ehrlich tumor. **Methods:** The tumor cells ($2x10^6$ cells) were subcutaneously implanted in the right pelvic member. The treatment started in the next day and continued for 20 days, with oral SYD-1 (75 mg/kg) or vehicle (basal control), or intraperitoneal methotrexate (MTX, 1,5 mg/kg, positive control). To evaluate the antitumor effect the tumor volume was checked daily, and the tumors were removed and weighed in the end of the treatment. Whole blood was used for haematological analysis and plasma for biochemical analysis. Liver and tumor tissues were applied for measurements of oxidative stress parameters. Organs such as kidneys, lungs, liver and spleen were weighed and evaluated macroscopically and staining to assess possible morphological alterations. This study was approved by the Ethics Commitee on Animal Use (CEUA) of the Federal University of Paraná, certificate nº 934. **Results**: The treatment with SYD-1 reduced the tumor volume in 97%, while the MTX group reduced 72%, in comparison to the vehicle. SYD-1 also reduced the tumor weight in 48% comparing with the vehicle group, and reduced the amount of protein in the tumor. In analyzes of oxidative stress was observed an increase in the activity of hepatic superoxide dismutase and catalase, which act against reactive oxygen species, in animals treated with SYD-1.In the tumor tissue the rate of lipoperoxidation was higher and the GSH levels were reduced in the same group, making the tumor microenvironment more pro-oxidative. In the evaluation of kidneys, lungs, spleen and liver, no differences were found between groups. In hematology, the red series did not show changes, while leukocytes and lymphocytes were increased in the SYD-1 treated group. **Conclusion:** SYD-1 showed expressive antitumor effect against the solid Ehrlich tumor. SYD-1 showed an antioxidant effect on the liver and a pro-oxidant tendency in tumor; increased the count of leukocytes and blood lymphocytes; with no toxic effects in blood cells, as well as in the evaluated organs. SYD-1 showed relevant antineoplastic potential against Ehrlich solid tumor. Thus, additional studies with other tumor models and different therapeutic protocols should be encouraged. **Financial support:** Brazilian National Research Council (CNPq).

10.017 Polymeric nanocarrier systems containing lapachol derivatives: **development and evaluation of anticancer activity** Vieira JB[1], Oliveira FC[2], Nunes da Silva EJ[3], Dias GG, Pessoa C[1] [1]UFC – Fisiologia e Farmacologia, [2]UFC, [3]UFMG

Introduction: Cancer is one of the major Public Health problems, being one of the leading causes of morbidity and mortality worldwide. An intensive search for new anticancer drugs that are more potent, less toxic, and with fewer side effects to the patient is therefore crucial. Numerous plant-derived compounds were reported to possess robust anticancer activity. Lapachol is a compound obtained from the bark of the Lapacho tree (*Tabebuia avellanedae*). A large number of studies have demonstrated that the lapachol and its derivatives have significant cytotoxic activity against several human cancer cell lines. Nor-β-lapachone (NβL) and ENSJ39 are semisynthetic naphthoquinone derivate from β-Lapachone. Several investigations have shown the potential anticancer these chemical group of drugs, and structural changes have been proposed to make them more efficient and less toxic. Different strategies for cancer treatment based on nanoencapsulation have been the focus of many investigations to optimize the therapeutic efficacy, reducing toxic side effects and resistance of the anticancer drugs. OBJECTIVE: To improve its cytotoxicity and overcome its liposolubility and non-specific toxicity, these compounds were nanoencapsulated. New approaches to cancer treatment based on drug delivery systems have been the focus of many investigations to overcome the problems of liposolubility and/or low bioavailability, optimizing the therapeutic efficacy and reducing toxic side effects. In this work was investigated the physicochemical characteristics, and cytotoxicity activity of two diffrentes polymeric nanoparticles prepared (NβL-loaded PLGA nanoparticles and ENSJ39-loaded PLGA nanoparticles). **Methods**: PLGA nanoparticles with anticancer compound (NBL and ENSJ39) were prepared by nanoprecipitation. The average diameter of the nanoparticles, zeta potential and Fourier transform infrared (FT-IR) spectroscopy. The *in vivo* cytotoxicity assays of the drug-loaded nanoparticles were determined by MTT cell proliferation assay in several human cancer cell lines (SF-295, HCT-116, HL-60 and PC3M). **Results**: The nanoparticles obtained had a uniform appearance, with homogenous suspension, without lumps polymer filaments or crystals of the drug visible by microscopy. The nanoparticles with NβL and ENSJ39 showed a size range of 192.70 ± 5.58 and 192.23 ± 5.25 nm, respectively. NβL-loaded nanoparticles exhibited a zeta potential of -48.50 ± 1.10 mV and the nanoparticles with ENSJ39, -51.53 ± 0.48 mV. After incubation for 72 h, both encapsulated drugs showed anticancer activity. The IC_{50} (CI 95%) values of the encapsulated NβL ranged from 0.2 to 1.4 µ g/mL, while the IC_{50} values of the ENSJ39-loaded nanoparticles ranged from 1 to 16.6 µg/mL. **Conclusion**: The nanocarries obtained had satisfactory characteristics for use in drug delivery. NβL-loaded nanoparticles were more sensitive in all cancer cells tested. Based on these results, we can conclude that these polymeric nanoparticles could be a promising drug delivery system for anticancer applications. **Acknowledgment:** CNPq, CAPES and FUNCAP.

10.018 Cytotoxic effect of a synthetic benzodiazine entitled PACP08A in non-small cell lung carcinoma cell line NCI-H460. Santos RS[1], Maranhão SS, Lima DJB, Rocha GGG, Moraes OM[1], Souza MVN[2], Pessoa C[2] [1]UFC – Fisiologia e Farmacologia, [2]Fiocruz

Introduction: Cancer is responsible for a significant and growing number of deaths, besides is the second cause of death worldwide. Synthetic products have increasingly taken up space in research and in pharmaceutical industry due to reduced production time and independence of natural raw materials. Although some heterocyclic compounds such as benzodiazines are rarely found in nature, they are easily synthesized. In addition, they have a broad spectrum of biological activity. **Methods:** The IC_{50} (half maximal inhibitory concentration) values were calculated from the colorimetric MTT assay, where its salt is reduced to formazan by mitochondrial enzymes. This experiment was carried out against eight human tumoral cell lines and one murine normal cell line. To investigate a pattern of cell death, microscopic assays were performed by acridine/ethidium bromide (LA/BE) and hematoxin/eosin (HE) staining. In the first, three hundred cells were counted in a fluorescence microscope. The second staining is based on the charge difference between nucleus and cytoplasm. The treatments used in the work were of 48 hours for all the tests. Doxorubicin and cells with no treatment were used as positive and negative controls, respectively. **Results:** The benzodiazine in study showed a cytotoxic effect against the cell lines tested, however the NCI-H460 non-small cell lung carcinoma cell line had one of the smallest IC_{50}, 7.5μM (± 1.07). The murine fibroblast L-929 presented the highest IC_{50} of all, 24.40μM (± 2.40). As one of the most sensitive cell lines against toxicity of the molecule in study, we used the NCI-H460 cell and its value of IC_{50} in the other assays. In LA/BE staining, a 30% reduction in the number of viable cells of the treatment was observed, 189 cells (± 9.8), compared to the negative control, 270 cells (± 5.5). The number of apoptotic cells remained statistically similar, while the number of necrotic cells increased significantly from five cells (± 1.1) of the negative control to 100 (± 6.5) in the treatment. Besides that, the analysis by HE showed that cells under incubation with the benzodiazine in the concentration of 7.5 μM showed undelimited morphology, size increase and cytoplasmic extravasation, similar to the necrosis process. These data corroborate with previous findings. **Conclusion:** The synthetic benzodiazine PACP08A in study has cytotoxic potential against all the tumor cell lines tested and less activity against the murine cell line. Morphological assays suggest that for 48 hours incubation with 7.5 μM treatment cells developed a necrotic cell death profile. However, more studies are required to design the citotoxic profile and mechanism by which the molecule exerts its cytotoxic activity. **Financial Support**: CNPq LAHLOU, M. Pharmacol Pharm v. 4, p.17, 2013. AJANI, O. European J. Of Med. Chem., v. 85, p 688, 2014.

10.019 The tumor suppressor, **micro-RNA-137**, **inhibits malignant melanoma migration by targetting TBX3** Peres J[1], Maliepaard EM[1], Rambow F[2], Larue L[3], Prince S[1] [1]University of Cape Town – Health Sciences, [2]VIB-Campus Gasthuisberg – Molecular Cancer Biology, [3]Institut Curie – Normal and Pathological Development of Melanocytes

Introduction: Malignant melanoma is a highly aggressive and treatment resistant cancer that has a rising incidence surpassing all cancers. The transcription factor, TBX3, is a key driver of melanoma progression and any drug that impacts its expression / activity is likely to have a major impact on the treatment of this cancer. MicroRNAs repress gene expression by primarily binding the 3 'untranslated region (3'UTR) of genes and their downregulation has been associated with the upregulation of oncogenes and cancer progression. Replacement of miRNAs that target oncogenes has gained much attention as a therapeutic intervention. This approach is anticipated to have little side-effects and to be effective since miRNAs are naturally occurring and they have a large set of targets which are often part of the same oncogenic pathway. The aim of this study was to determine whether TBX3 is regulated by miRNA-137 in advanced melanoma. **Methods** The levels of miR-137 and TBX3 were compared in primary and metastatic melanoma tumors using data from the Cancer Genome Atlas (TCGA) and in a panel of melanoma cell lines using qRT-PCR. Luciferase reporter assays were performed to test the direct binding of miR-137 to TBX3. The effect of overexpressing or knocking down on miR-137 on TBX3 expression was examined by western blotting and scratch motility assays were performed to determine the impact of miR-137 targeting TBX3 on melanoma cell migration. **Results** We show that miR-137 is downregulated in a panel of melanoma cell lines and in a cohort of patients with primary melanoma and that miR-137 levels correlate inversely with TBX3 mRNA levels. Low levels of miR-137 and high levels of TBX3 are shown to be associated with poor patient survival. We show that miR-137 directly binds to a highly conserved site in the TBX3 3'UTR and that miR-137 mimic in malignant melanoma cells significantly reduces endogenous levels of TBX3 and cell migration. Evidence is provided that this occurs through the repression of the TBX3 target, E-cadherin. **Conclusions** Here we provide a novel data on the microRNA-137 / TBX3 / E-cadherin axis plays an important role in melanoma progression and that miR-137 replacement is a potential therapeutic approach to treating TBX3-driven melanomas. **Acknowledgments** This work was supported by grants from the MRC, NRF, CANSA, Ligue Nationale Contre le Cancer et comité de l'Oise, INCa, Pair Melanoma and Cancéropole IdF, and is under the program «Investissements d'Avenir» launched by the French Government and implemented by ANR Labex CelTisPhyBio (ANR-11-LBX-0038 and ANR-10-IDEX-0001-02 PSL)

10.020 *Chuquiraga spinosa* Less (Huamanpinta) ethanol extract of leaves Chemopreventive effect in cancer gastric NMU-induced rat. Arroyo J[1], Rojas J[1], Herrera O[2], Chavez R[3], Chumpitaz V[4], Enciso E[5], Tinco A[6] [1]Universidad Nacional Mayor de San Marcos – Farmacologia, [2]Universidad Nacional de Ica, [3]Facultad de Medicina Universidad Nacional Mayor de San Marcos – Farmacologia, [4]Facultad de Odontología Universidad Nacional Mayor de San Marcos – Farmacologia, [5]Dirección de Farmacia y Bioquímica Universidad Nacional San Cristobal de Huamanga de Ayacucho – Farmacologia e Toxicologia, [6]Dirección Facultad de Farmacia y Bioquímica de la Universidad Nacional San Cristobal de Huamanga de Ayacucho – Farmacologia e Toxicologia

Introduction: Gastric cancer is the third leading cause of death in the world. Medicinal plants are an alternative source for the search for new anticancer agents, the aim of the present investigation was to determine the chemopreventive effect of Chuquiraga spinosa Less ethanolic extract (EE-Cs) on gastric cancer induced by N-methyl-nitroso-urea (NMU) in rats. **Methods:** Holtzmann strain albino rats were used and 5 groups (n = 10) were considered: 1) normal; 2) induction control (NMU); 3) NMU + EE-Cs 50 mg / kg; 4) NMU + EE-Cs 250 mg / kg; 5) NMU + EE-Cs 500 mg / kg. The protective efficacy was determined by quantifying nitric oxide (ON), malondialdehyde (MDA), superoxide dismutase (SOD), micronuclei (antigenotoxic), liver profile, urea, creatinine, glucose and coronary profile. At the end of the experiment the stomach was removed to observe tumor characteristics, tumor cell type and percentages. The lethal dose 50 was established in mice (by administering increasing dose of the extract).

Results: Comparing groups with treatments and the toxic cancer inducer showed a reduction of PCR (70-87%, $p < 0.0001$); the increase in SOD (121-161%, $p < 0.0001$); hematological and biochemical values were within normal limits. The NMU-only stomach came to show in greater degree cells with anaplastic structural disorder, presence of pycnotic nuclei and dysplasia; being lower in treated animals, where they also presented congestion. **Conclusion:** In the experimental conditions a gastric chemoprotective effect of EE-Cs was demonstrated in rats. Key words: Gastric cancer, chemopreventive, *Chuquiraga spinosa* Authorization of the Ethics Committee Faculty of Medicine of *San Marcos National Major University*: 0260 of 04-04-2016 Funded by the Vicerectorate of investigation of San Marcos National Major University

10.021 Targeting Stathmin 1 and microtubule dynamics in acute lymphoblastic leukemia. Machado-Neto JA, Rodrigues Alves APN, Fernandes JC, Coelho-Silva JL, Scopim-Ribeiro R, Fenerich BA, Silva FB, Simões BP, Rego EM, Traina F FMRP-USP

Introduction: Stathmin 1 is a phosphoprotein that participates in microtubule dynamics, cell cycle progression and survival. Previously, our research group reported that Stathmin 1 is a proliferation marker for normal lymphocytes and that *Stathmin 1* is overexpressed in a small cohort of adult acute lymphoblastic leukemia (ALL) patients. Stathmin 1 silencing reduced cell proliferation in an ALL cell line, supporting a functional role for Stathmin 1 in leukemia phenotype. Recent studies have suggested that paclitaxel, a microtubule stabilizer drug, leads to Stathmin 1 serine 16 phosphorylation, which inhibits its microtubule destabilizing activity. The aims of the present study was to investigate *Stathmin 1* expression and its impact on clinical outcomes in an independent cohort of ALL patients, and to verify the effects of paclitaxel on Stathmin 1 phosphorylation and cell viability in ALL cell lines. **Methods**: Bone marrow samples were collected from 22 healthy donors (median age 28 years [range 11–68]). Bone marrow or peripheral blood samples were collected from 45 ALL patients (median age 35 years [range 18–79]) at the time of diagnosis or relapse. Gene expression was evaluated by qPCR. For functional assays, Jurkat (T-ALL) and Namalwa (B-ALL) cells were with graded concentrations of paclitaxel for 72 hours and the IC_{50} estimated by nonlinear regression analysis. Apoptosis was determined by annexin V/PI staining and flow cytometry. Protein expression and activation was evaluated by Western blot. For statistical analyses, Mann–Whitney test or Student t test were used for measured factors, Fisher's exact test was used for categorical factors, Spearman test was used for correlation tests, and Cox regression analysis was used to estimate overall survival (OS). A p value <0.05 was considered as statistically significant. **Results**: *Stathmin 1* transcripts were significantly higher in primary cells from ALL patients compared with healthy donors (median=3.50 [minimum=0.11–maximum=21.29] versus 1.01 [0.01 - 2.62], p<0.0001). High *Stathmin 1* expression was associated with lower age onset for ALL and *Stathmin 1* levels positively correlate with white blood cell (WBC) count (r=0.48; p=0.001). However, Stathmin 1 expression did not impact on clinical outcomes of ALL patients. Age significantly affected OS of our cohort. Cell viability and apoptosis assays reveal that Jurkat and Namalwa cells present high sensibility to paclitaxel treatment (IC_{50} of 5 nM and 6.5 nM, respectively). Western blot analysis indicates that paclitaxel treatment strongly induces Stathmin 1 phosphorylation at serine 16 site (an inhibitory site), α-tubulin acetylation (a microtubule stability marker) and caspase 3 cleavage (an apoptosis marker) in ALL cell lines. **Conclusion**: Our data confirm increased levels of Stathmin 1 in an independent cohort of ALL patients. Paclitaxel promotes Stathmin 1 phosphorylation at serine 16 site, microtubule stability and apoptosis in ALL cell lines. Taken together, these findings suggest that Stathmin 1 plays a role in ALL biology and may be a potential target for this disease. Supported by CNPq and FAPESP. Ethics Committee Approval: CEP 845.854.