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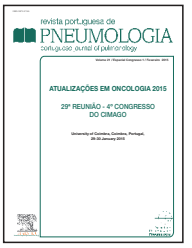

POSTER PRESENTATIONS

# Atualizações em oncologia 2015

# 29ª Reunião-4º Congresso do CIMAGO

## University of Coimbra, Coimbra, Portugal, 29-30 January 2015

The abstracts are the author's responsibility.

### P1. EXPRESSION OF ALDH AND P53 IN BREAST CANCER STEM CELLS

M. Laranjo[1], T. Costa[1], M.J. Carvalho[1,2], J. Casalta-Lopes[1,3], A.M. Abrantes[1], M.F. Botelho[1]

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**Introduction:** In clinical practice, breast cancer (BC) is stratified into 3 groups: tumors that express hormonal receptors, tumors overexpressing Her2, and finally those which do not express hormonal receptors and do not overexpress Her2, triple negative (TN) BC. Estrogen receptor-$\alpha$ (ER- $\alpha$) and progesterone receptor (PR) are hormonal receptors that are used in clinic as markers for diagnostic and treatment purposes. The recent theory of cancer stem cells (CSC) refers to a small tumor cell population that has the main characteristics of stem cells. It is believed that CSC are responsible for tumor progression, recurrence as well as resistance to therapy. The protein aldehyde dehydrogenase (ALDH) is a common a marker for both normal and malignant stem cells and p53 has been involved in the regulation of the process of dedifferentiation. With this work we intend to evaluate the expression of ALDH and p53 in HR positive (HR+) and TN mammospheres.
**Methods:** The breast cancer cells lines MCF-7, HR+, and HCC1806, TN, were submitted to the mammosphere (MM) forming protocol. The first MM generation (MS1) was cultured in adherent conditions. This procedure was repeated in order to obtain successive generations of MM (MS1, MS2 and MS3) and the MM-derived cells in adherent conditions (G1, G2 and G3). After obtaining the various generations of MM and MM-derived cells total protein extracts were prepared in order to evaluate the expression of ALDH and p53 by Western blot.
**Results:** The expression of ALDH is three fold higher in TN than HR+. In HR+ MM the expression of ALDH increases in the three generations with statistical significance for MS1 ($p < 0.01$). Relative to MM-derived adherent cells ALDH expression is similar to the parental cell line. In TN populations the expression of ALDH has the same profile than in HR+ with significant increase for MS1 ($p < 0.001$), MS2 ($p < 0.05$) and MS3 ($p < 0.01$). Regarding p53 TN, MM and adherent-derived populations had null expression. In case of HR+, p53 is downregulated in MM generations but maintains a similar expression in MM-derived cells.
**Conclusion:** The upregulation of ALDH in MM generations in both cell lines prove that it was possible to isolate a population of CSC. The higher expression of ALDH in TN parental cell line comparing to HR+ confirms the poor prognosis of TN tumors. The downregulation of p53 in CSC shows that they might be able to avoid apoptosis, corroborating the resistant phenotype of these cells.

### P2. EXPRESSION OF HORMONAL RECEPTORS IN BREAST CANCER STEM CELLS

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**Introduction:** In clinical practice, breast cancer (BC) is stratified into 3 groups: tumors that express hormonal receptors, tumors overexpressing Her2, and finally those which do not express hormonal receptors and do not overexpress Her2, triple negative (TN) BC. Estrogen receptor-$\alpha$ (ER- $\alpha$) and progesterone receptor (PR) are hormonal receptors that are used in clinic as markers for

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diagnostic and treatment purposes. The recent theory of cancer stem cells (CSC) refers to a small tumor cell population that has the main characteristics of stem cells. It is believed that CSC are responsible for tumor progression, recurrence as well as resistance to therapy. With this work we intend to evaluate the expression of hormonal receptors in TN and hormonal receptors positive (HR+) CSC.
**Methods:** The breast cancer cells lines MCF-7, HR+, and HCC1806, TN, were submitted to the mammosphere forming protocol. The first CSC generation (MS1) was cultured in adherent conditions. This procedure was repeated in order to obtain successive generations of CSC (MS1, MS2 and MS3) and the CSC-derived cells in adherent conditions (G1, G2 and G3). After obtaining the various generations of CSC and CSC-derived cells total protein extracts were prepared in order to evaluate the expression of ER- $\alpha$ and PR by Western blot.
**Results:** In the cell line HCC1806, CSC and adherent-derived populations had null expression for both receptors. Regarding MCF7, the expression of ER- $\alpha$ was lower in MS1 ($p < 0.001$), in MS2 ($p < 0.001$) and MS3 ($p < 0.05$) than the parental cell line. However the adherent CSC derived populations had similar expression of this receptor comparing to MCF7. The expression of PR was also lower in MS1 ($p < 0.001$), in MS2 ($p < 0.001$) and MS3 ($p < 0.001$), however the adherent CSC derived populations showed no differences.
**Conclusion:** The lower expression of both hormonal receptors in CSC can represent an undifferentiated phenotype of these cells. However when submitted to adherent culture conditions cells regain the original phenotype. The undifferentiated phenotype of CSC suggest that targeting agents for HR may not be effective, at least in a long term perspective considering recurrence and distant metastization.

## P3. LOCAL ANESTHETIC LIDOCAINE AS A PROMISE APPROACH IN ORAL SQUAMOUS CELL CARCINOMA TREATMENT: AN IN VITRO STUDY

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**Introduction:** Oral squamous cell carcinoma (OSCC) represents the most frequent malignant neoplasia that affects the oral cavity, accounting for more than 90% of cases. The etiology of OSCC is multifactorial and involves intrinsic and extrinsic factors, namely tobacco and alcohol, which are the two primary environmental risk factors associated. Besides the new and advanced therapeutic strategies, patients with OSCC show poor survival rates. Local anesthetics, such as lidocaine, are usually used to control pain in patients with head and neck tumors, but recent reports have been shown that also can inhibit cancer cell proliferation, invasion and migration. In this work, we evaluated the potential therapeutic efficacy of the local anesthetic lidocaine, in OSCC cell lines, alone and in combination with conventional treatment (Cisplatin, 5-Fluorouracil) and studied the underlying mechanisms.
**Methods:** Two OSCC cell lines were maintained in culture, the HSC-3 (metastatic) and BICR-10 (*in situ*) cells, in absence and in presence of different concentrations of lidocaine in monotherapy (daily or single dose administration) or in association with conventional chemotherapeutic drugs (Cisplatin or 5-Fluorouracil). Cell viability was assessed by the rezasurin assay and cell death by Optical Microscopy (May-Grünwald staining) and flow cytometry using the Annexin V/Propidium Iodide double staining. The influence of these compounds in cell cycle (propidium iodide incorporation), mitochondrial membrane potential (JC-1 probe), caspases (apostat kit), reactive oxygen species production (hydrogen peroxide, $H_2O_2$; superoxide anion, $O_2^-$, evaluated by 2.7-diclorofluorescein and dihidroetidium, respectively) and in the level of the antioxidant defense Reduced Glutathione, GSH (using mercury orange) were performed by flow cytometry.
**Results:** Our results showed that lidocaine induced an antiproliferative and cytotoxic effects in a dose, time and cell type dependent manner. HSC-3 cells seemed to be more sensitive to lidocaine effect than BICR-10 cells, as the $IC_{50}$ at 48 hours was 4-4.5 mM and 5-6 mM, respectively. Moreover, in HSC-3 cell line, when we administrated lidocaine in combination with conventional chemotherapy, we observed a synergist cytotoxic effect. On the other hand, in BICR-10 cells, lidocaine in daily low doses administration was more effective when compared with equivalent dose in single dose administration. Furthermore, lidocaine induced cell death mainly by apoptosis and late apoptosis/necrosis, which might be related with the increase in caspases and superoxide anion levels and the decrease of mitochondrial membrane potential as well as with the pre-G1 peak in cell cycle. Lidocaine also increased GSH levels in HSC-3 and BICR-10 cells.
**Conclusion:** Our *in vitro* results showed that lidocaine alone or in combination with conventional treatment in OSCC cells may constitute a new complementary therapeutic approach, namely in metastatic cancer.
This work was supported by Center of Investigation in Environment Genetics and Oncobiology (CIMAGO).

## P4. INFLUENCE OF INSULIN IN TOXICITY MEDIATED BY EVEROLIMUS IN ORAL CANCER CELLS

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**Introduction:** Oral cancer (OC) is a subtype of head and neck cancer (HNC) that arises from the oral cavity. OC holds the eighth position in cancer incidence ranking worldwide, with oral squamous cell carcinoma (OSCC) being responsible for more than 90% of all of them. The current curative treatment modalities are usually surgery and radiation, with chemotherapy added to decrease the risk of metastasis. Despite recent progress in therapeutic approaches, the five-year survival rate for OC has not improved significantly over the past several decades. On the other hand, some studies have shown a higher prevalence of cancer in diabetic patients, namely oral cancer. Oral carcinogenesis is a multistep process in which gene alterations in oncogenes and tumor suppressor genes lead to the disruption of the normal regulatory pathways that control basic cellular functions including cell division, differentiation, and apoptosis. PI3K/Akt/mTOR is a very important pathway, involved in the regulation of many of this processes and its deregulation is considered pivotal for oral cancer, and so being a potential candidate for therapeutic targeting. Everolimus inhibits cell signaling through the PI3K/Akt/mTOR pathway and

has been shown to reduce cell proliferation and angiogenesis. It is also known that insulin can indirectly activate PI3K/Akt/mTOR pathway, enhancing the effects of growth factors, such as PDGFR that may lead to an increasing resistance to anti-cancer drugs, even though the mechanisms are, yet, not well understood. With this work, we intend to study the role of Insulin, as an activator, and its interference in Everolimus toxicity, an inhibitor of the PI3K/Akt/mTOR pathway in oral cancer cell lines and study the underlying mechanisms.
**Methods:** For this purpose of the study, two oral cancer cell lines (HSC-3 and BICR-10) were used, incubated with Insulin (100 nM) and mTOR inhibitor (Everolimus) in different therapeutic protocols. The cytotoxicity was evaluated by the Alamar Blue assay. Flow cytometry was used to analyze cell cycle, cell death mechanisms (using Annexin V/Propidium Iodide assay), and Cyclin D1 and PDGFR expression.
**Results:** Our results showed that mTOR inhibitor, Everolimus, had a cytotoxic effect in monotherapy in a dose, time and cell-dependent manner, inducing cell death preferentially by apoptosis. By cell cycle analysis, everolimus showed an antiproliferative effects, with a G2 phase increase and decrease in S phase. On the other hand, Insulin had no cytotoxic effect, showing an increase in proliferation on HSC-3 cells, with an increase in both S and G2 phase. We could also observed that Insulin plays a protective role in oral cancer cells, since cells pre-treated with insulin showed a decrease of toxicity mediated by Everolimus. There was an overall decrease in Cyclin D1 and PDGFR expression, when comparing to control, for both mono and combined therapies.
**Conclusion:** Our work showed that Insulin can have an important role in chemotherapy resistance in oral cancer which can affect therapeutic protocols in diabetic patients with oral cancer.
This work is financed by Center of Investigation on Environment Genetics and Oncobiology (CIMAGO) and a scientific research grant is supported by Núcleo Regional do Centro da Liga Portuguesa Contra o Cancro (NRC-LPCC) in partnership with CIMAGO.

## P5. PILOT STUDY ON MGMT GENE METHYLATION STATUS BY MS-MLPA IN PATIENTS WITH GLIOBLASTOMA

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**Introduction:** Glioblastoma (GBM) is one of the most common and aggressive primary brain tumors with an annual incidence of about three per 100 000. The median survival time is only 14 months after diagnosis and the standard treatment for patients with newly diagnosed glioblastoma consists of surgery followed by temozolomide chemoradiotherapy, an alkylating agent. The DNA repair protein O6-Methylguanine-DNA methyltransferase (*MGMT*), located on chromosome 10q26, encodes a DNA repair protein that removes alkyl groups, and is an important prognostic factor in glioblastoma since its presence has been associated with decreased survival and resistance to alkylating chemotherapy.
**Methods:** Tissue samples fixed in paraffin collected from resective surgery or biopsy procedures from 80 patients with high-grade gliomas were subject to DNA extraction. The DNA samples were analyzed by methylation-specific multiplex ligation dependent probe amplification (MS-MLPA) to determine the promoter methylation status of the *MGMT* gene. The panel used for evaluation contains 6 probes specific for the *MGMT* promoter region.
**Results:** Of the 80 patients, 4 are not possible to analyze due to insufficient DNA, and 57 have been analyzed by MS-MLPA for *MGMT* gene methylation status. Of those, 26 presented a non-methylated promoter region, defined by the average methylation value of the 6 probes ≤ 25%; and 31 presented a methylated promoter region, defined by the average methylation value of the 6 probes > 25%. Of these 31 samples, 3 levels of methylation can be considered: low, moderate and extensive methylation.
**Conclusion:** The next step will be to correlate the methylation levels to the patients' response to the temozolomide alkylating agent. It is expected that patients subject to temozolomide treatment present a favorable prognostic, namely a higher overall survival.

## P6. ASTHMA AND RHINOSINUSITIS: GENETIC AND CLINICAL LINKS AND DIVERGENCES

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**Introduction:** Asthma and rhinosinusitis are related complex phenotypes, depending on multiple genetic and environmental risk factors.
**Objectives:** To evaluate whether polymorphisms of *IL4R* (rs1805015), *IL13* (rs20541), *IL17A* (rs2275913) and *GSTP1* (rs1695) genes are associated with rhinosinusitis and/or asthma in adults.
**Methods:** 192 unrelated healthy individuals and 232 patients, 83 with rhinosinusitis and 149 with asthma, were studied. Polymorphisms were detected by real time polymerase chain reaction (PCR) using TaqMan assays.
**Results:** Comparing patients and controls, we found AA genotype of rs1695 (GSTP1) to be associated with susceptibility to asthma (Odds Ratio (OR) – 1.96; 95%CI - 1.18 to 3.25; p = 0.010). The association sustained for allergic asthma (OR – 2.17; 95%CI - 1.23 to 3.80; p = 0.007). GG genotype of rs20541 (IL13) showed to be a protective factor to asthma (OR – 0.55, 95%CI - 0.33 to 0.94, p = 0.028). Among patients, the polymorphisms of IL17A (rs2275913) and IL13 (rs20541) genes were differently distributed between asthma and rhinosinusitis groups. In fact, both AA genotype of rs2275913 (IL17A) and GG genotype of rs20541 (IL13) were less associated with asthma than with rhinosinusitis (OR – 0.20; 95%CI of 0.07 to 0.56; p = 0.002 and OR – 0.48; 95%CI of 0.25 to 0.93; p = 0.031; respectively). There were no significant differences in the distribution of allelic and genotypic frequencies between patients and controls for the IL4R polymorphism' analysed.
**Conclusion:** These results support the existence of a significant association between *GSTP1* rs1695 and *IL13* rs20541 SNPs, with susceptibility to asthma. Different genetic profiles of *IL17* and *IL13* genes seem to influence the localization of affected airways.

## P7. GENETIC VARIABILITY OF DNMTS AND FOLATE/METHIONINE METABOLISM GENES – ROLE IN METHYLATION STATUS AND MYELOID NEOPLASIAS RISK

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**Introduction:** Global hypomethylation and targeted hypermethylation are considered defining characteristics of human tumours, including myeloid neoplasias (MN). Folate, methionine and vitamin B12 can influence the supply of methyl groups and, therefore the biochemical pathways of methylation processes. Moreover, single nucleotide polymorphisms in genes that encode DNA methyltransferases (DNMTs) as well as enzymes from folate/methionine metabolism can influence the DNA methylation status, as well as the individual susceptibility to develop MN, namely Acute Myeloblastic Leukemia (AML), Myelodysplastic Syndromes (MDS) and Myeloproliferative Neoplasias (MPN), including Chronic Myelogenous Leukemia (CML). In this context, we analyzed the influence of the polymorphisms in *DNMT1*, *DNMT3a*, *DNMT3b*, *RFC1*, *CBS* and *MTRR* genes in DNA methylation status and as a risk factor for myeloid neoplasia development.
**Methods:** This study enrolled 333 patients diagnosed with myeloid neoplasia (80 AML, 106 MDS, 147 MPN, being 77 CML) and 261 healthy controls. The genetic polymorphisms of *DNMT1* (rs759920), *DNMT3a* (rs2289195), *DNMT3b* (rs2424908), *RFC1* (rs1051266), *MTRR* (rs162036) and *CBS* (844ins68) were assessed by RFLP-PCR and Tetra-primer-ARMS-PCR. The localized methylation status was analyzed through *p15*, *p16*, *p53*, *MGMT* and *KEAP1* methylation profile by MSP. Global methylation status was assessed by 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC) levels and LINE-1 methylation. The statistical analysis was carried out by variance analysis, $\chi^2$ test and Fisher exact test ($p < 0.05$).
**Results:** Our results show that *CBS*, *DNMT1* and *DNMT3b* genes influence the localized and global methylation status in a genotype-dependent manner. Moreover, *DNMT1* AA and *DNMT3b* CT genotypes were protector factors for MDS development [OR 0.49 (CI95% 0,27-0.90), p = 0.03; OR 0.50 (CI95% 0.28-0.83), p < 0.001]. The *DNMT3a* AG genotype was also a protector factor for AML development [OR 0.16 (CI95% 0.05-0.52), p < 0.001]. Besides that, individuals with *DNMT1* GG genotype have an increased risk for SMD development about 1.78-fold (CI95% 1.03-2.01; p = 0.04), while those with *DNMT3a* GG and *DNMT3b* CC genotypes have an increased risk for AML development about 6.82-fold (CI95% 1.22-37.95; p = 0.03) and 2.01-fold (CI95% 1.07-11.48; p = 0.03), respectively. The *RFC1* GG genotype also increases the risk of CML development about 2.65-fold (CI95% 1.53-4.57; p < 0.001). Moreover, *DNMT3a* genotype can influence prognosis since MDS patients with *DNMT3a* GG genotype had lower survival (p = 0.032).
**Conclusion:** These results suggest that genetic polymorphisms in DNMTs and folate/methionine metabolism genes can influence the DNA methylation status, the susceptibility to develop MN and the prognosis of MDS patients.
This work was supported by CIMAGO (Project 22/09) and R. Alves is supported by the FCT fellowship FRH/BD/51994/2012.

## P8. GENOME-WIDE COPY NUMBER ANALYSIS IN ORAL SQUAMOUS CELL CARCINOMA

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**Introduction:** Oral squamous cell carcinoma (OSCC) is a multistep disease of progressive genomic imbalances, which often occur before a phenotypic manifestation and are not detectable by the diagnostic methodologies currently available. Thus, the identification of the genomic profile of these tumors is a great promise for early diagnosis, prediction of disease progression and response to therapy. Taking this in mind, the main goal of this study was to characterize the genomic profile of OSCC through array-Comparative Genomic Hybridization (aCGH).
**Methods:** Biopsies of oral tumors were acquired from 75 patients and aCGH was performed using an Agilent oligonucleotide microarray 4x180K. Healthy donors were used as controls.
**Results:** With this whole genome approach we detected imbalances in almost all chromosomes, being the sizes of these imbalances often variable between patients. However, the identification of the chromosomal regions and genes more frequently altered was possible through the application of data analysis and data visualization techniques to untangle the complexity of genomic profiles of these samples. Thus, the most common copy number alterations were observed in chromosomes 3, 5, 8, 11 and 17, where are mapped important genes for oral carcinogenesis process.
**Conclusion:** With this high-throughput approach we identified the most prevalent chromosomal regions reported in literature as altered in oral cancer and also in other chromosomal regions that seems mapped important genes related to disease initiation and progression. The correlation between molecular and clinic-pathological data has the power to identify putative biomarkers with possible diagnostic and prognostic value. Specific statistical framework designed to analyze chromosomal aberrations in cancer is pivotal to distinguish meaningful events from random background aberrations in order to identify biologically significant aberrations.

## P9. CHROMOSOME 1Q21.1 RECURRENT MICRODELETIONS AND MICRODUPLICATIONS IN PATIENTS WITH DEVELOPMENTAL DELAY REVEALED BY ARRAY-CGH

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**Introduction:** Chromosomal region 1q21.1 is structurally complex with many segmental duplications (SD) that mediate recurrent breakpoints (BPs) and make it prone to non-allelic homologous recombination (NAHR). Chromosomal region 1q21.1 can be divided into two distinct regions: a proximal region from 144.1 to 144.5Mb (NCBI36/hg18), containing 16 genes and flanked by BP2 and BP3 and a distal region located from 145 to 146.35Mb (NCBI36/hg18), containing 13 genes, flanked by BP3 and BP4. Array-Comparative Genomic Hybridization (array-CGH) has led to the identification of several new syndromes including microdeletion 1q21.1 (OMIM ID: 612474) and the reciprocal microduplication 1q21.1 (OMIM ID: 612475). Clinical features of patients with 1q21.1 distal deletion include developmental delay, microcephaly, facial anomalies and congenital heart defect. Patients with 1q21.1 duplication usually

display mental retardation, autism, macrocephaly, and dysmorphic features. Clinical variability, incomplete penetrance, and lack of distinct facial dysmorphisms have been reported in individuals affected by these imbalances, making genetic counseling very challenging.
**Methods:** Oligonucleotide array-CGH analysis using an Agilent 180K platform was performed in 1,000 patients with intellectual disability (ID), autism spectrum disorders (ASD) and congenital anomalies. Fluorescence *in situ* hybridization (FISH) analysis using a SureFISH (Agilent) probe was performed in patients with abnormal microarray results for validation and in parents to determine the origin of 1q21.1 rearrangements.
**Results:** We identified 8 probands with rearrangements involving the distal 1q21.1 region (6 deletions and 2 duplications) limited by BP3 and BP4 and 1 proband with a duplication involving the proximal region flanked by BP2 and BP3. 3 subjects had the common distal 1.35 Mb microdeletion and one had the reciprocal microduplication. 3 patients had a larger microdeletion and one patient with autistic features revealed a 2.5 Mb duplication, but still limited by BP3 and BP4. Patient 8 was the exception revealing a 444 Kb duplication involving the proximal region associated with Thrombocytopenia-Absent Radius syndrome region. Inheritance was unknown in 5 patients, 2 microdeletions had arisen *de novo* and 2 microdeletions was inherited from an unaffected mother. All patients with microdeletion revealed mental retardation, microcephaly or/and dysmorphic features. The phenotype of patients with microduplication included mental retardation and/or macrocephaly.
**Conclusion:** Array-CGH is a useful tool for 1q21.1 rearrangements screening. Duplications 1q21.1 were less frequent in our cohort, consistent with recent studies showing that rates of deletion mediated by NAHR are higher than that for duplications. Patients with 1q21.1 rearrangements have a considerable phenotypic diversity that could be associated with incomplete penetrance and variable expressivity. Family studies and further clinical data will be essential to improve genetic counselling in 1q21.1 affected individuals.

## P10. GENETIC AND EPIGENETIC CHANGES IN TONGUE SQUAMOUS CELL CARCINOMA: DETECTION BY METHYLATION-SPECIFIC MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION

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**Introduction:** Tongue squamous cell carcinoma (TSCC) is the most common malignancy in the oral cavity, characterized by high recurrence rates, reduced overall survival and increasing incidence worldwide. Identification of genetic markers, as well as epigenetic alterations, involved in tongue malignant transformation and progression is a crucial step towards a better understanding of the disease biology, thus improving diagnosis and prognosis.
**Methods:** DNA was extracted from fresh tissue samples of 31 primary tongue tumors, collected from patients with TSCC, upon resection surgery. Copy number alteration and methylation status were assessed by Methylation-specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA). Gingival samples from 11 healthy donors were used as controls.
**Results:** From the 41 genes analyzed in this study, the ones exhibiting a higher frequency of copy number losses were present at chromosomal arms 9p, 11p and 11q, whereas those exhibiting gains were more frequent at 2q, 11q, 16p, 17q and 19p. DNA methylation was found in 15 of the studied genes. *WT1*, *PAX5* and *MSH6* genes were aberrantly methylated in 80.65%, 48.39% and 35.48% of the tumor samples, respectively.
**Conclusion:** Our study revealed several genetic and epigenetic alterations that may play a role in TSCC development.

## P11. MOLECULAR CHARACTERIZATION OF PRENATALLY DETECTED SMALL SUPERNUMERARY MARKER CHROMOSOMES: IMPROVING THE GENOTYPE – PHENOTYPE CORRELATIONS

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**Introduction:** Small supernumerary marker chromosomes (sSMC) are defined as structurally abnormal chromosomes that cannot be characterized by conventional banding cytogenetics and are generally equal in size, or smaller, than the chromosome 20 of the same metaphase spread. The identification of a sSMC at prenatal diagnosis is a great challenge for genetic counseling because the lack or little clinical and prognostic information. Array-CGH (aCGH) has been shown to be a valuable tool in genetic counseling for prenatally detected sSMC allowing the identification of sSMC, its euchromatic content and the associated genes involved, contributing for a better genotype-phenotype correlation.
**Methods:** Three cases of mosaic *de novo* sSMC, detected by conventional cytogenetics, were analysed, during the last year, with Agilent whole genome array; two of them using the 180K oligonucleotide array-CGH and the other with the 60K oligonucleotide array-CGH.
**Results:** aCGH analysis on DNA from cultured amniocytes characterized two mosaic alterations of chromosome 18 (one case with triplication of 14 Mb at 18(p11.32p11.21) and the other case with an amplification involving whole 18p and part of the 18q of about 26 Mb) and a 17 Mb amplification at 6(p12.1q13).
**Conclusion:** The majority of sSMC are derived from the acrocentric chromosomes. The sSMC derived from the autosomes, as the ones in the present study, generally have poor genotype-phenotype correlations, with phenotypes ranging from normal to severely abnormal, being their euchromatic content the most accurate predictor of phenotype. Array-CGH analysis is an effective tool to characterize sSMC. Using this approach the origin and euchromatic content of sSMC can be identified. The measurement of the sSMC size, genetic material and associated genes allows the improvement of prenatal genetic counseling with a more accurate delineation of the genotype-phenotype correlation.

## P12. CHALLENGES OF MOLECULAR KARYOTYPING IN PRENATAL DIAGNOSIS

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**Introduction:** Conventional cytogenetics has been the main approach for the detection of chromosomal abnormalities in prenatal diagnosis, however is being supplemented and in some cases, especially in cases with ultrasound anomalies, replaced by chromosomal microarray analysis.
**Methods:** Array-CGH was performed in 67 prenatal samples. The indications for the analysis were diverse, ranging from ultrasound anomalies/major abnormalities (37), medical interruptions due to major anomalies (13), carrier progenitors of genomic imbalances (4) and even to clarify conventional cytogenetic findings (13). Array-CGH was performed using Agilent oligonucleotide 180K in DNA obtained in the majority of the samples from amniotic fluid and chorionic villus, but also from fetal blood and skin biopsy of the death fetus. Each sample was hybridized against a sex-matched commercial control and the analysis was performed to detect imbalances above 400 Kb in size, except in the cases where a specific familiar imbalance was being evaluated.
**Results:** Of the 13 samples analyzed due to cytogenetic findings, we were able to: characterize 2 marker and 4 derivative chromosomes, exclude genomic imbalances in 2 translocations and 2 inversions, detect genomic imbalances in 2 apparently balanced translocations and detect mosaic trisomy for chromosomes 13 and 21 in a 13;21 Robertsonian translocation. Of the 4 samples tested due to carrier progenitors of genomic imbalances, 1 fetus was normal for the imbalance observed in the progenitor, while the other 3 were carriers of the same imbalance. In the remaining 50 samples, 10 imbalances were observed, 3 maternal, 4 paternal, 1 *de novo*, 1 imbalance resulting from a maternal balanced translocation and 1 sample where inheritance was not available.
**Conclusion:** Array-CGH is valuable not only to establish a diagnosis in samples with ultrasound anomalies but also to characterize cytogenetic findings, showing to be a good toll for genetic counseling. The use of CGH array in high-risk pregnancies in conjunction with the karyotype analysis seems to be best strategy in prenatal diagnosis.

## P13. CHROMOSOME 13Q31.3 DELETION INVOLVING MIR17HG GENE: MIRNA INVOLVEMENT IN HUMAN DEVELOPMENT

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**Introduction:** Feingold syndrome (FS) is an autosomal dominant disorder characterized by variable combinations of microcephaly, relative short stature, limb malformations, digital anomalies and intellectual disability. In approximately 70% of affected families, FS is caused by germline loss-of-function mutations of the *MYCN* gene located at 2p24.1. More recently, microdeletions at chromosome 13q31.3 involving *MIR17HG* gene were identified in patients with this clinical syndrome. The *MIR17HG* locus encodes a polycistronic miRNA cluster from which six distinct miRNAs are produced.
**Methods:** Oligonucleotide array-CGH analysis using an Agilent 180K platform was performed in a 30 year old female patient with severe intellectual disability, microcephaly and short stature, in her newborn son with microcephaly and digital anomalies and in her 7 year old daughter with global developmental delay, but with normal stature and no digital anomalies.
**Results:** Array-CGH revealed a normal result for the daughter and a 2.3 Mb deletion at chromosome 13q31.3 containing 14 genes, 8 in OMIM and including *MIR17HG* gene in both mother and son. As both progenitors of the mother have deceased, inheritance was not possible to ascertain.
**Conclusion:** The observed deletion reinforces the data that *MIR17HG* gene deletions are responsible for developmental abnormalities, such as microcephaly, digital anomalies and short stature. The intellectual disability observed might be caused by deletion of adjacent genes, such as *GPC5*, which is involved in cell growth and morphogenesis within the central nervous system, kidneys and limbs. This result provides further evidence for the involvement of a miRNA gene in human development and of the diagnostic power of array-CGH for the study of patients with multiple anomalies.

## P14. IMPORTANCE OF REVISITING PREVIOUSLY CLASSIFIED COPY NUMBER VARIANTS: TOWARDS BUILDING STRONGER CNVS DATABASES

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**Introduction:** Microarray-based comparative genomic hybridization (array-CGH) allows the possibility to screen the whole genome at once and with high resolution, allowing the detection of a large number of Copy Number Variants (CNVs). Taking into account the existence of known syndromes, the existence of relevant genes for the phenotype and the existence of CNVs in healthy population, we have proposed the classification of CNVs into different classes: Class I are deletions or duplications in a region associated with a microdeletion or a microduplication syndrome; Class II are deletions or duplications not reported in normal subjects and involving known coding genes; Class III are deletions or duplications reported in low frequency in normal subjects or not involving genes; and Class IV are deletions or duplications reported in healthy subjects that are considered common variants.
**Methods:** We have revisited 250 array-CGH cases from 2011 and the first trimester of 2012 in order to evaluate if the observed CNVs would be reclassified according to the update of databases.
**Results:** In 174 cases no classification was changed. In the other cases: 1 classification changed from Class II to I, 10 from II to IIIA, 5 from II to IV, 17 from IIIA to IV, 3 from IIIB to IIIA, 45 from IIIB to IV. In 70% of the cases reviewed there were no changes in the CNVs' classification. About 95% of the classification changes made reduced the probability of the imbalance being responsible for the phenotype. There was one case in which an imbalance classified as a class II was reclassified as class I, since the imbalance is nowadays considered a genomic syndrome.
**Conclusion:** Although progress and knowledge have improved the content of databases, the findings of this revision have not changed the clinical strategy neither the management of patients. We can conclude that our CNV classification system is robust.

## P15. PROTEOME ANALYSIS IN LARYNGEAL SQUAMOUS CELL CARCINOMA

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**Introduction:** Laryngeal cancer represents about 25% of malignant tumors affecting this area and 2% of malignancies. It occurs preferentially in women, being one of the most frequents tumors of the head and neck. It can occur in the: supraglottis, glottis and subglottis. Approximately two thirds of the tumors arising in the true vocal cord, located at the glottis and supraglottic can done third is located above the vocal cords. The most common histological type and achieves more than 90% of patients, is the squamous cell carcinoma (SCC). The SCC is classified according to the degree of cell differentiation. Gene expression studies have been conducted in various tumors to allow some of the proteins involved in tumor development as well as to determine severity markers. The objective of the present study was verify the proteomic context in tumor and normal cells of SCC larynx patients.
**Methods:** After to collect the cancer sample, the cancer confirmation was made by hematoxylin-eosin. There after the cells were selected and captured in Laser Mircodissection and the proteomic analysis was performed using the mass spectrometer coupled in liquid chromatograph. Statistical test (t test) was applied and used a software to process the analysis; standardization was made in another software and compared to the database of the Human UniProt, which has 88,429 reads and 35,079,223 residues.
**Results:** Eleven patients were evaluated (normal and tumor tissue) and were matched for age and sex. In the group of males with an average age of 56 without chemotherapy and radiotherapy, were isolate 1,057 proteins in normal tissue and 1,088 in tumor tissue.
**Conclusion:** After clustering analysis, with $p < 0.05$, were isolated 81 proteins, 38 with higher expression in tumor tissue and 43 with lower expression in the tumor tissue. In the future, these proteins should be analyzed by their function, and could be candidates for biological markers in laryngeal squamous cell carcinoma.

## P16. SLC23A2-05 AND KRAS-LCS6 POLYMORPHISMS IN PATIENTS WITH HEAD AND NECK SQUAMOUS CELL CARCINOMA

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**Introduction:** Cancer is a genetic disease that is influenced by environmental factors. To determine the risk factors in head and neck squamous cell carcinoma, two polymorphisms - solute carrier family 23 member 2 [SLC23A2-05 (rs4987219)] and v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog [KRAS-LCS6 (rs61764370)],- and environmental factors, including smoking and alcohol consumption, were analyzed in a population.
**Methods:** The study enrolled 165 males diagnosed with head and neck squamous cell carcinoma. The control group consisted of 230 healthy male subjects without cancer or family history of cancer. The SLC23A2-05 and KRAS-LCS6 polymorphisms were analyzed by polymerase chain reaction followed by enzymatic digestion. All patients and healthy subjects were assessed with regard to their smoking habit and alcohol consumption, which are risk factors for cancer.
**Results and conclusion:** For Kras-LCS6 polymorphism, the allele frequency for the T and G alleles in patients were 0.91 and 0.09 while in the control group was 0.90 and 0.10, respectively. For the SLC23A2-05 polymorphism, the frequency of the C and G alleles were 0.47 and 0.53 respectively in both groups. In the analysis of the logistic regression test, no association was observed between the studied polymorphisms and squamous cell carcinoma of the head and neck, as well as the staging of the disease.

## P17. DNA BINDING AND PHOTOCLEAVAGE PROPERTIES OF PLATINUM-PORPHYRINS

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**Introduction:** Considering the important roles of DNA during tumor development, it is a valuable target on the development of new anticancer compounds. While the clinical application of platinum (II) based is widespread, their efficacy has been hampered by mechanisms related with cellular resistance. Therefore, it is necessary to develop new targeting platinum (II) based drugs. One way to achieve this goal has been the incorporation of porphyrin macrocycles (Pors) into platinum (II) based complexes. After binding with DNA, photo-activated Pors are able to generate reactive oxygen species (ROS), namely singlet oxygen ($^1O_2$) in the presence of molecular oxygen, photocleaving DNA. The aim of this study was to characterize the binding ability and photocleavage activity of two novel platinum-Pors ($H_2TPPF_{16}(SPyPt)_4$ and its correspondent zinc complex $ZnTPPF_{16}(SPyPt)_4$) with DNA.
**Methods:** DNA from salmon sperm and DNA from calf thymus were used to determine (by fluorescence and UV-Vis spectroscopy) the binding properties of platinum-Pors with DNA. Photostability of platinum-Pors was followed by UV-Vis spectroscopy after light irradiation. Singlet oxygen generation was determined by a chemical method using 1.3-diphenylisobenzofuran as $^1O_2$ quencher. Electrophoresis on agarose gel was used to determine the photoclevage activity of platinum-Pors against pMT123 plasmid DNA.
**Results:** A general trend of hypochromism and fluorescence emission quenching for both Pors after addition of DNA solutions was observed. In addition to hypochromicity, ZnTPPF16(SPyPt)4 exhibited a red shift in the absorbance of the Soret-band after addition of DNA. In PBS solutions both compounds showed high photostability. Additionally, both Pors exhibited high ability to generate 1O2. No cleavage of pMT123 plasmid DNA was observed, if platinum-Pors were mixed with DNA without illumination. Otherwise, after photo-excitation, the compounds were able to convert supercoiled DNA plasmid (form I) into circular relaxed form (form II).
**Conclusion:** Herein, $H_2TPPF_{16}(SPyPt)_4$ revealed the typical electrostatic surface binding with DNA. In addition to cationic-anionic binding with DNA, $ZnTPPF_{16}(SPyPt)_4$ demonstrated a particular intercalation binding mode, high ability to generate $^1O_2$ and to cleavage DNA after photo-excitation. A strong pattern of interaction between DNA and $ZnTPPF_{16}(SPyPt)_4$ could be a potential methodology to kill cancer cells using photodynamic therapy.
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## P18. 18F-FDG PET/CT AND NORMALIZED UPTAKE FRACTION: A SUV ALTERNATIVE?

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**Introduction:** Functional imaging with 18F-FDG PET/CT has become a valuable tool in Oncology. Standardized uptake value represents quantification in PET/CT and there are recommendations for its inclusion in clinical reports. Nevertheless, SUV is affected by multiple variables, related to the equipment or to the operator, among others, with negative impact in the comparative analysis between exams from different labs or in radiotherapy planning. Our goal is to demonstrate a new methodology of quantification, much more precise and less dependent.
**Methods:** We studied, with 18F-FDG PET/CT previously to treatment, 30 women with cervical cancer in stage IIB, IIIA and IIIB. Images were included in MEVISLAB® platform, an image processing and visualization software, and we obtained images of independent visualization, with possibility of optimizing the visualization by interacting with color scale and transparencies, after the application of, what we called, a normalized uptake fraction (FCN), as the expression: FCN = CxW(g)/Nt, where C represents counts/pixel, W mass in grams and Nt total counts. We selected the most representative cases.
**Results:** With the method of independent visualization, after the application of FCN, we obtained normalized images of regions, with FCN above a certain limit, allowing a new approach of metabolic imaging of tumour, lymphatic dissemination and distant lesions. We reported an evidence-case.
**Conclusion:** There is evidence that, through visual navigation in CT-mode but mainly in PET/CT-mode after application of FCN, we were allowed to analyze quantification images of PET/CT, from different patients and different equipment's, without the controversial ROI definition and measurement of SUV. For radiotherapy planning this method can improve the determination of the biological target volume.

## P19. OSTEOSARCOMA STEM CELLS ARE FUNCTIONAL HETEROGENEOUS POPULATIONS AND HAVE DISTINCT GENETIC PROFILES

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**Introduction:** Osteosarcoma is a bone tumour of mesenchymal origin displaying significant heterogeneity and a complex genetic phenotype. Although evidence suggests the presence of cancer stem cells (CSCs) in osteosarcoma a consensus on their genetic and phenotypic profile is still missing, and we therefore aimed to characterize CSCs population in osteosarcoma cell lines.
**Methods:** We used a combination of distinct functional approaches (sphere-forming, Aldefluor and side-population assays) for identification of CSC populations on a panel of osteosarcoma cell lines derived from two distinct histological subtypes. Gene expression profile of stemness-related transcription factors, multilineage differentiation and quiescent nature were evaluated.
**Results:** Different CSC populations co-exist on osteosarcoma cell lines exhibiting distinct functional properties. Osteosarcoma spheres derived from fibroblastic-like cell lines are slow-proliferative cell populations overtime and negative for the Ki-67 proliferation marker, differentiated into multiple mesenchymal lineages (adipocytes, osteocytes and chondrocytes) and overexpressed *SOX2* and *KLF4* transcription factors (genes involved in maintenance of pluripotency in embryonic stem cells). Moreover, we found expression of these genes in primary osteosarcomas suggesting that they provide markers of stemness signatures in these tissues. Osteoblastic-like cell lines did not form spheres in serum-free medium but had a subset of Aldefluor-positive cells. Aldefluor-positive cells overexpressed *SOX2* but not *KLF4*. Aldefluor-positive populations and CSC-enriched spheres significantly overlapped but this trend was not observed for side-population combination with sphere assay. Side-population phenotype was also correlated with *ABCG2* drug-efflux transporter expression.
**Conclusion:** Distinct functional methods identify CSC populations with dissimilar molecular characteristics. Intrinsic heterogeneity seems to exist on osteosarcoma CSCs and can have important implications on the design of targeted therapies aiming to eradicate this important cell subset among tumours.
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## P20. ENDOMETRIAL CANCER 18F-FDG UPTAKE: IMPORTANCE OF THE STEM CELLS

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**Introduction:** Endometrial cancer, the most common gynecologic malignancy in western countries, is usually symptomatic and diagnosed in initial stages. However, the risk of recurrence is largely influenced by prognostic factors, particularly molecular characterization. Cancer stem cells (CSC), a small proportion of tumor cells, have self-renewal capacity and generate a differentiated progenitor cells that origin the majority of tumor cells. The characterization of this population in endometrial cancer can contribute to stratify clinical prognosis and early diagnosis. With this work we aim to evaluate the $^{18}$F-FDG uptake in CSC and CSC-derived population in endometrial tumor cells.
**Methods:** Endometrial cancer cell line ECC1 was submitted to sphere forming protocol. The first spheres generation (ES1) was cultured in adherent conditions. This procedure was repeated in order to obtain successive generations of spheres (ES1, ES2 and ES3) and the spheres-derived cells in adherent conditions (G1, G2 and G3). The populations were submitted to $^{18}$F-FDG uptake at 5, 30, 60, 90 and 120 minutes.
**Results:** The mean uptake for ECC1 ant 5 minutes was 0.55 ± 0.12%, not different from the results at 90 and 120 minutes (0.55 ± 0.24% and 0.54 ± 0.23%, respectively). Considering the first spheres generation (ES1), there is an increase in the uptake from 5 minutes (0.70 ± 0.16%) to 1.30 ± 0.38% at 120 minutes. This tendency seems similar in the second and third generation. For the sphere derived adherent population, there is a mean uptake at 5 minutes for G1 of 0.45% ± 0.11 and at 120 minutes of 0.55% ± 0.14. Considering the second and third generation of adherent population (G2 and G3), the results were 0.64 ± 0.15% and 0.59 ± 0.13% at 5 minutes and 0.55 ± 0.27% and 0.68 ± 0.18% at 120 minutes.
**Conclusion:** There seems to be an increase in $^{18}$F-FDG uptake in spheres populations comparing with endometrial tumor cells

of origin. The adherent populations acquire a similar behavior comparing with the adherent endometrial cell line. These finding point an important role of $^{18}$F-FDG in staging and recurrence detection regarding endometrial CSC profile.

## P21. MUSCLE-INVASIVE BLADDER CANCER TUMORS CONTAINS CHEMORESISTANT SPHERE-FORMING CELLS EXPRESSING STEMNESS-RELATED MARKERS

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**Objectives:** Bladder Cancer (BC) is the fifth most common cancer in western world, currently treated by transurethal resection followed by chemotherapy/radiotherapy and intravesical administration of BCG. This tumor has high propensity for local recurrence and distant metastasis that appears to be related with the presence of Cancer Stem Cells (CSCs), displaying stemness features and unresponsiveness to standard therapies. We aim to isolate and characterize cells with stem-like properties from two BC cell lines as well as clinical samples of BC patients at different stages of malignancy.
**Methods:** CSCs from two invasive BC cell lines (HT-1376 and UM-UC3) were isolated using two different approaches (the matrigel sphere-forming assay and the enzymatic ALDH assay), and characterized by qRT-PCR, immunofluorescence and flow cytometry for the expression of cell surface and pluripotency related markers. Tumor samples isolated from BC patients were also analyzed for the expression of the same markers. Cell lines chemosensitivity to cisplatin (CIS) and methotrexate (MTX) was analyzed using the MTT-colorimetric assay, the Annexin V-apoptotic assay and Caspase 3/7 activity. The tumorigenic potential of CSCs was tested by subcutaneous injection in immunocompromised mice. We also tested the enrichment in CSCs after chemotherapeutical approaches in both cell lines using the ALDH assay.
**Results:** An enriched population of CSCs was identified in both BC cell lines expressing CD44, CD47 and pluripotency transcription factors SOX2, OCT4 and NANOG. These sphere-forming cells are enriched in ALDH+ cells and displayed high resistance to CIS and MTX. Our results also showed enrichment in CSCs following short-term chemotherapy. The animals inoculated with CSCs formed a tumor 100 times higher than the one inoculated with equal number of corresponding parental cells. Gene expression analysis in clinical samples showed an increased expression of stemness-related genes in muscle-invasive tumors relatively to non-invasive tumors.
**Conclusion:** Both BC cell lines contain a subset of cells exhibiting distinct stem-like features with enhanced tumorigenic potential and highly resistance to chemotherapy. These CSCs due to their chemoresistant and tumorigenic abilities may be the main responsible for tumour relapse and progression to more invasive forms. CSCs may be considered as a valuable marker in BC prognostic and treatment.
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## P22. A MULTIFACTORIAL INTEGRATIVE MODEL FOR ORTHODONTIC-INDUCED EXTERNAL APICAL ROOT RESORPTION

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**Introduction:** Orthodontic-induced external apical root resorption (EARR) is a complex phenotype, being determined by poorly defined genetic and clinical factors. This work aimed to construct a multifactorial integrative model to analyze the risk of developing this common orthodontic complication.
**Methods:** This retrospective study included 195 orthodontic patients. Using a multiple linear regression model, where the dependent variable was the maximum% of root resorption for each patient, we evaluated the contribution of nine clinical variables and 4 polymorphisms of genes involved in bone and tooth root remodeling (rs1718119 from *P2RX7*, rs1143634 from *IL-1B*, rs3102735 from *TNFRSF11B*, encoding OPG, and rs1805034 from *TNFRSF11A*, encoding RANK).
**Results:** In this sample, clinical and genetic variables explained 30% of the maximum % of root resorption variability. The variables with more significant unique contribution to the model were: gender ($p < 0.05$), treatment duration ($p < 0.001$), premolar extractions ($p < 0.01$), Hyrax appliance ($p < 0.001$) and GG genotype of rs1718119 from P2RX7 gene ($p < 0.01$). Age, overjet, tongue thrust, skeletal class II and the other polymorphisms had minor contributions.
**Conclusion:** This study identified a *P2RX7* polymorphism and Hyrax appliance as new susceptibility factors to EARR. To be of clinical utility, a more extensive panel of etiologic factors should be included in the model.

## P23. IS TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-INDUCING LIGAND AND DEATH RECEPTORS RELEVANT IN MONOCLONAL GAMMOPATHIES?

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**Introduction:** Tumor Necrosis Factor-related Apoptosis-inducing Ligand (TRAIL) was identified in 1995 and, since then, growing interest has emerged in oncology due to its reported ability to selectively trigger cancer cell death. Pro-apoptotic TRAIL signaling is mediated through DR4 (also TRAIL-R1) and DR5 (also TRAIL-R2) receptors. Despite intensive investigations very little is known regarding expression of TRAIL and pro-apoptotic TRAIL receptors in plasma cells (PCs) of patients (pts) with monoclonal gammopathies (MG). The aim of this investigation is to contribute to clarify the involvement of TRAIL and death TRAIL receptors in the development of MG, in the progression of MGUS to MM and in the prognosis of MM.
**Methods:** Between April 2010 and July 2013, we evaluated bone marrow PCs from 125 pts with MG, 59 symptomatic multiple myeloma (MM), 66 monoclonal gammopathy of uncertain significance (MGUS) and 11 healthy controls (Ctr). PCs were analysed by flow cytometry and the 2 populations were identified by gating CD138+/CD19- and CD138+/CD19+. We evaluated the expression of TRAIL, TRAIL-R1 and -R2 with monoclonal antibodies in both populations by flow cytometry. The results are expressed as percentage of PCs expressing these proteins and expression levels in mean intensity of fluorescence (MIF).
**Results:** In our population, median age was 70 (39-86) years, 52% were male. We found that the mean percentage of CD138+/CD19+ PCs expressing TRAIL and TRAIL-R1 in MM (70.8% and 73%, respectively) is significantly higher than in MGUS (54.8% and 46.1%, respectively) pts ($p = 0.0001$) and in MGUS compared to Ctr (46.2% and 13.2%; $p = 0.043$ and $p = 0.0001$, respectively); TRAIL-R2 was also higher in MGUS *vs* Ctr (56.7% *vs* 17%, $p = 0.0001$). TRAIL expression level in PCs with the same phenotype was higher in MM (46.6 ± 3.4 MIF) than in MGUS (35.31.4 MIF) pts ($p = 0.026$), as well

as the level of expression of TRAIL-R1 and -R2 in MM (32.71.1 MIF and 44.82.2 MIF, respectively) and in MGUS (37.11.7 MIF and 60.91.9 MIF, respectively) pts compared to Ctr (27.50.65 MIF and 24.80.7 MIF, respectively; p = 0.0001). We also evaluated the expression of TRAIL and TRAIL pro-apoptotic receptors in CD138+/CD19- PCs in MGUS and in MM pts. In MM, we found increased expression levels of TRAIL and TRAIL-R2 in CD138+/CD19+ (46.6 ± 3.4 MIF and 44.8 ± 2.2 MIF, respectively) compared to CD138+/CD19- (29.21.8 MIF and 34.22 MIF, respectively) PCs (p = 0.0001). Similar data were observed in MGUS pts only for TRAIL-R2 (60.91.9 MIF in CD138+/CD19+ *vs* 54.82.4 MIF in CD138+/CD19- PCs; p = 0.001). In our investigation, we also searched for prognostic markers in MM pts and we found a significant better survival for pts with a percentage of CD138+/CD19- PCs expressing TRAIL superior to 13.5% (p = 0.008).

**Conclusion:** Our study showed that TRAIL and TRAIL pro-apoptotic receptors, R1 and R2, are highly expressed in CD138+/CD19+ PCs of MG pts compared to Ctr with increased levels in MM *vs* MGUS, suggesting a significant role of these proteins in the development and progression of MG. We also demonstrated a prognostic impact of TRAIL expression in MM pts, with a better prognosis associated to a higher percentage of CD138+/CD19- PCs expressing TRAIL.

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## P24. THE INTERPLAY BETWEEN OXIDATIVE STRESS AND DNA METHYLATION – ROLE IN DEVELOPMENT, DIAGNOSIS AND PROGNOSIS OF MYELOID NEOPLASIAS

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**Introduction:** The pathogenesis of myeloid neoplasias (MN), such as Myelodysplastic Syndrome (MDS) and Myeloproliferative Neoplasias (MPN), is complex and involve multiple genetic and epigenetic events. Oxidative stress (OS), resulting from an imbalance between Reactive Oxygen Species (ROS) production and antioxidant defenses, contributes to cell proliferation and damage, apoptosis and dysfunctional hematopoiesis. Furthermore, aberrant methylation patterns are other mechanisms common in hematopoietic neoplasias. In this context, we evaluate the interplay between oxidative stress and epigenetic profile in MN patients, and analyzed their possible role as a risk factor and prognostic marker in these hematological neoplasias.

**Methods:** This study enrolled 96 MN patients (71 MDS and 25 MPN) and 26 controls (CTL). Oxidative stress was analyzed by: glutathione peroxidase (GL-PX) and glutathione reductase (GL-Red) activity; glutathione erythrocyte levels (total GS, GSH, GSSG); and vitamin A (vitA) and E (vitE), uric acid, total antioxidant status (TAS) and nitrate plus nitrite plasma levels. Oxidative damage was analyzed through malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) plasma levels. The localized methylation status was analyzed through *p15*, *p16*, *p53*, *MGMT* and *KEAP1* methylation profile by MS-PCR. Global methylation status was assessed by 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC) and LINE-1 methylation levels. Statistical analysis was carried out by variance analysis, ROC curves and $\chi^2$ test ($p < 0.05$).

**Results:** MN patients had decreased antioxidant defenses (TAS: 1.2 mM MN *vs* 1.0 mM CTL, p = 0.039; total GS: 8.7 μmol/gHb MN *vs* 10.2 μmol/gHb CTL, p = 0.05) and increased oxidative damage levels (MDA: 58 nmol/gHb MN *vs* 49 nmol/gHb CTL, p = 0.04; 8-OHdG: 35 ng/mL MN *vs* 29 ng/mL CTL, p < 0.001), relatively to controls. Moreover, 76% of MN patients presented at least one methylated gene and show increased levels of 5 mC and 5 hmC (5 mC: 9.0% *vs* 2.6%, p = 0.027; 5 hmC: 3.5% *vs* 2.2%, p < 0.001), as well as a decrease in LINE-1 methylation (MN 69.0% *vs* CTL 77.8%, p < 0.001), comparatively to controls. Moreover, MN patients with high 8-OHdG had higher levels of 5 mC and 5 hmC. We also observe that 8-OHdG, LINE-1, 5-hmC and 5-mC may be used as diagnostic biomarkers of MN, being 5-mC the most accurate biomarker (AUC: 0.919 and 0.971, respectively for MDS and MPN) and that patients with high 8-OHdG (p = 0.003) and 5-hmC levels (p = 0.02), as well as those with low LINE-1 methylation (p = 0.019) had lower survival.

**Conclusion:** In summary, oxidative stress is correlated with aberrant methylation status and these mechanisms may contribute to the development of hematological neoplasias, such as MDS and NMP. Moreover, 8-OHdG, LINE-1, 5-hmC and 5-mC could be new diagnostic and prognostic biomarkers in these hematological neoplasias.

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## P25. CHARACTERIZATION OF LEUKEMIC STEM CELLS IN PATIENTS WITH MDS

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**Introduction:** MDS is considered a disease of clonal hematopoietic stem cell (HSC), whereby the existence of changes in these type of cells, besides being based on the aetiology of the disease can also affect the progression to AML. Recently, a small subset of these cells was identified with unique ability to auto renovate and differentiate into different lineages of cancer cells that comprise the tumour, the cancer stem cells (CSC). These cells have been identified in many types of cancers, in particular cancer of the brain, breast, prostate, colon and pancreas, as well as in multiple myeloma and leukaemia (leukemic stem cells, LSC). Moreover, the development of MDS is accompanied by immune changes and production of antibodies and cytokines, such as IL-6 and TNF-α. However, the evaluation of these cytokines in the LSC and HSC has not been examined in patients with MDS.

**Objectives:** To assess the relevance of IL-6 and TNF-α in HSC and LSC in MDS patients, to identify new prognostic and predictive markers for new therapeutic targets.

**Methods:** Analysis of clinical and analytical data of 102 patients with *de novo* MDS. The distinction between HSC and LSC was performed by flow cytometry using a panel of monoclonal antibodies conjugated with the following combinations: CD34/CD117/CD123/GlicoP and CD34/CD117/CD123/IL-6/TNF -α.

**Results:** The sample consisted of 102 patients with median age of 74 years (22-89) and with a ratio Male/Female 0.8. The MDS subtypes according to the WHO are: refractory cytopenia with

multilineage dysplasia (RCMD) (n = 52), refractory cytopenia with unilineage dysplasia (RCUD) (n = 12), refractory anemia with excess blasts (RAEB) -1 (n = 8), RAEB-2 (n = 8), refractory anemia with ringed sideroblasts (RARS) (n = 6), 5q- syndrome (n = 4) chronic myelomonocytic leukemia and (CMML) (n = 12) with IPSS: low (n = 37), intermediate-1 (n = 39), intermediate-2 (n = 10) and high (n = 1). Eleven patients progressed to AML: 7 patients with RAEB-2, 2 patients with RCMD, 1 patient with RAEB-1 and another with CMML. In our study we noticed an increase in CD34 + cells in MDS patients in more advanced stages (RAEB-2) as well as in cells that co-express CD117, which can be related to the evolution of these patients to AML. On the other hand, the increase in CD34 +/CD117 + cells observed in patients with RCMD may identify those who have a higher risk of leukemic transformation. Analysis of stem cell also allowed the identification of two groups of cells, one phenotypically 'normal' (HSC) and other with neoplastic characteristics (LSC) that differentially express inflammatory cytokines IL-6 and TNF-$\alpha$. IL-6 production was observed in the HSC in RAEB-2 and in the LSC in RT subtype. TNF-$\alpha$ was also observed in the HSC in CMML and in the LSC in RAEB-2 subtype. The presence of both cytokines in patients with RAEB-2 ($p < 0.05$) is marked, although IL-6 predominates in HSC and TNF-$\alpha$ in LSC. On the other hand, the stem cell with IL-6 expression (CD34 +/CD117 +/+ IL-6) may influence the progression to AML ($p < 0.05$), since this group has the largest number of patients that progressed to AML, and is also associated with decreased overall survival ($p < 0.05$).

**Conclusion:** These results suggest the importance of IL-6 and TNF-$\alpha$ in distinguishing these two groups of stem cells, as well as the significance of these cells in the development of the disease.

## P26. ADIPOCYTOKINES GENE EXPRESSION IN COLORECTAL CANCER TISSUES – CORRELATION WITH PROTEIN SERUM LEVELS

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**Introduction:** Colorectal cancer (CRC) is one of the most frequent types of cancer in the western world. One mechanisms that could contribute to cancer pathogenesis and progression, namely in CRC, is the adipose tissue dysfunction, particularly alterations in adipocytokines levels. The aim of this study was to investigate the association between the adipocytokines gene expression in colorectal cancer and normal adjacent colon tissues, as well as in normal colon tissue (controls), the respective serum protein levels and the clinicopathological characteristics of CRC patients. We also aimed to explore it possible role in the development and progression of CRC.

**Methods:** Resistin, visfatin, leptin, adiponectin, TNF-$\alpha$ and MCP-1 gene expression in tumor and normal adjacent colon tissues was performed by RT-real time PCR (qPCR), and serum levels were measured by ELISA. Statistical analysis was carried out by variance analysis, ROC curves and a $p < 0.05$ was considered statistically significant.

**Results:** This study enrolled 52 CRC patients (17F/35M) with a median age of 72 (47 a 91) years, being 40% rectal adenocarcinoma and 60% colon adenocarcinoma. According to TMN staging, 9 (18%) of these patients were in stage I, 19 (38%) in stage II, 13 (26%) in stage III and 9 (18%) in stage IV. We also study 13 healthy controls (7F/6M) with a median age of 73 (36-91) years. The relative expression levels of adiponectin, resistin, visfatin, TNF-$\alpha$ and MCP-1 genes in normal and tumor colon tissue of patients with CRC were similar. Although the median of adiponectin and TNF-$\alpha$ gene expression levels were similar in normal and tumor tissues of patients and tissue of controls, we observe a tendential increase of gene expression in tissues of patients with CRC. Furthermore, in normal and tumor tissues of CCR patients a decrease in gene expression of resistin and MCP-1 is observed when compared with normal tissue of controls, but only for MCP-1 the differences were statistically significant ($p = 0.046$ and 0.03, respectively). The comparative analysis between serum levels of the adipocytokines with its gene expression in colon showed that tumor gene expression and serum levels of adiponectin, visfatin, and TNF-$\alpha$ have a similar profile, with a tendency to increase in cancer patients compared with controls. However, the values of serum levels and tissue gene expression of leptin, resistin and MCP-1 tended to be negatively correlated.

**Conclusion:** Adipocytokines gene expression has similar values in tumor tissues and morphologically normal adjacent colon tissues. Serum levels of adiponectin, visfatin and TNF-$\alpha$ are increased in CRC patients and seem to be correlated with their tissue gene expression.

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## P27. INFLUENCE OF THE ABC TRANSPORTERS' GENETIC PROFILES IN THE DEVELOPMENT OF MYELOID NEOPLASIAS

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**Introduction:** Genetic variability in xenobiotic transport related proteins can influence drugs' efficacy and could contribute to the susceptibility to hematological neoplasias, such as Acute Myeloid Leukemia (AML), Myelodysplastic Syndrome (MDS) or Chronic Myeloid Leukemia (CML). ABC (ATP-binding cassette) transporters superfamily includes membrane proteins which function is to transport substrates through intra- and extracellular membranes. MDR1 and MRP1 are membrane transporter proteins that, besides the above described functions, may play a role in the development, in the progression and in the therapeutic outcome in several neoplastic pathologies.

**Objectives:** In this context, we investigated the relevance of polymorphisms in *MDR1* (C3435T) and *MDP1* (G1666A) genes as risk factors for the development of myeloid neoplasias, such as MDS, AML and CML, as well as their possibility of being prognostic risk factors.

**Methods:** This study included 222 patients diagnosed with myeloid neoplasias (MN) (90 MDS, 48 AML, 71 CML, 13 MDS/MPN) and 119 healthy controls (CTL). The genetic profiles of *MDR1* (C3435T)

and *MRP1* (G1666A) were assessed by RFLP-PCR. The strength of association between polymorphisms and disease's development risk were estimated by odds ratio (OR) with 95% confidence interval (CI 95%). The influence of these polymorphisms in the patients overall survival was calculated by Kaplan Meier method.
**Results:** Our results show that allele C from *MDR1* is the most prevalent in all studied individuals (CTL = 52.1%; MDS = 53.0%; CML = 53.5%; MDS/MPN = 53.9%) except in AML patients (AML = 43.1%), and CT is the most common genotype in all groups (CTL = 47.1%; MDS = 43.4%; AML = 45.8%; CML = 36.6%; MDS/MPN = 46.2%). As to *MRP1* gene, we observed that allele A is the most frequent in all studied individuals (CTL = 72.0%; MDS = 76.7%; AML = 78.4%; CML = 68.6%; MDS/MPN = 61.5%) and we also perceived that the most common genotypes are AG in CTL (56.0%), CML (60.0%) and MDS/MPN (76.9%), and AA genotype in MDS and AML (54.4% and 56.8%, respectively). Moreover, GG genotype was only observed in MDS and CML patients (MDS = 1.1%; CML = 1.4%). Despite these polymorphisms don't individually contribute to the development of MN, we observed that the association between the genotype TT from *MDR1* with genotype AA from *MRP1* results in a decreased risk for the development of CML, with an odds ratio of 0.21-fold (CI95% 0.054-0.82; p = 0.03). Despite the association between genotypes TT from *MDR1* and AG from *MRP1* suggests an increased risk for the development of CML with an odds ratio of 3.5-fold (CI95% 0.97-12.9; p = 0.06). Moreover, none of these polymorphisms seem to influence the patients' overall survival.
**Conclusion:** These results suggest a contribution of the genetic polymorphisms of *MDR1* and *MRP1* genes, especially the genetic profiles, for the development of myeloid neoplasias. However, their role in progression and therapy response remain unknown. To clarify these issues a larger systematic and prospective studies will be needed.
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## P28. THE ROLE OF ADIPOCYTOKINES IN MONOCLONAL GAMMOPATHIES – A PRELIMINARY STUDY

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**Introduction:** Multiple myeloma (MM) is a neoplastic disease characterized by the growth of malignant plasma cells in the bone marrow (BM) with a subsequent overproduction of a monoclonal protein and development of bone lesions and soft-tissue masses. This malignancy starts with a monoclonal gammopathy of undetermined significance (MGUS). Adipocytokines are a group of hormones produced by the adipose tissue that play an important role in energy homeostasis, hematopoiesis, immunity, inflammation and angiogenesis. Moreover, adipocytokines can exhibit pro-inflammatory (e.g. leptin) or anti-inflammatory (e.g. adiponectin) properties. This work aim to explore the role of adipocytokines, namely adiponectin, leptin, resistin, MCP-1 and TNF-$\alpha$ in the pathogenesis of monoclonal gammopathies (MG) and correlate these adipocytokines levels with patient's clinical characteristics.
**Methods:** A total of 68 MG patients (31 MGUS and 37 MM patients, those 8 with smoldering MM – sMM – and 29 with symptomatic MM) and 35 healthy individuals were included in this study, after informed consent. Adiponectin, leptin, resistin, MCP-1 and TNF-$\alpha$ levels were quantified on the peripheral blood (PB) and BM using ELISA commercial kits.
**Results:** We observed significant higher levels of resistin and MCP-1 in PB of patients with MG (14.7 ± 13.0 ng/ml and 419 ± 344 pg/ml, respectively) compared to controls (9.2 ± 5.3 ng/ml and 277 ± 79 pg/ml, respectively). Besides that, resistin levels in PB are higher in symptomatic MM patients (18.4 ± 14.9 ng/mL) when compared with sMM (9.0 ± 2.2 ng/mL). Furthermore, symptomatic MM patients that exhibit an IgG monoclonal component had higher levels of MCP-1 in both PB (194.05 ± 93.36 ng/mL) and BM (484.35 ± 323.48 ng/mL). On the other hand, those who overproduce κ light chains show higher levels of MCP-1 in PB (242.25 ± 155.39 ng/mL). According to ISS classification, stage III patients, also show higher levels of MCP-1 in PB than those on stage I (stage III: 200.97 ± 88.18 ng/mL; stage I: 109.59 ± 3.97 ng/mL).
**Conclusion:** Our study suggests that adipocytokines may be envolved in the pathogenesis of MM by creating a pro-inflammatory state in the BM that contributes to the carcinogenesis process. These findings might contribute to a better understanding of this malignancy, may allow the development of new treatment approaches and to improve the prognosis classification.
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## P29. THE POTENTIAL THERAPEUTIC EFFECT OF SHIKONIN IN DIFFERENT HEMATOLOGICAL NEOPLASIAS

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**Introduction:** Several studies have demonstrated that β1 Integrin play an important role in adhesion of hematopoietic cells to the bone marrow microenvironment. Furthermore, β1 Integrin facilitates cancer cell adhesion, migration, invasion, and proliferation by activating intracellular signaling pathways, such as ERK/MAPK and PI3K/AKT. Shikonin (SHK) is a β1 Integrin inhibitor that seems to have anti-proliferative properties by inactivating these signaling pathways and others. However, the role of this drug in hematological neoplasia's is not yet clarified. Therefore, the aim of this study was to evaluate the potential therapeutic of SHK in *in vitro* models of different hematological neoplasias.
**Methods:** We used four hematological neoplasias cell lines: HEL cells, an Erythroleukemia cell line; NB-4 cells, an Acute Promyelocytic Leukemia cell line with the translocation t(15;17); F-36P cells, a Myelodysplastic Syndrome cell line; and H929 cells, a Multiple Myeloma cell line. The cells were cultured in absence and presence of different concentrations of SHK in a single dose and in daily dose. The effectiveness of SHK on cell viability were analyzed by the Rezasurin Assay. Cell death was determined by flow cytometry (FC), using the Annexin-V and propidium iodide double staining. It was also evaluated by FC the activation of caspases and cell cycle using the Apostat probe and IP/RNase, respectively.
**Results:** Our results showed that SHK reduce cell viability in a time, dose and cell type dependent manner, being the HEL cells the more sensitive and the H929 cells the lowest. The administration of SHK

in a daily dose seems to reduce more cell viability, especially in NB-4 cells, compared with cells treated with a same concentration in single dose. The antiproliferative effect was confirmed by the increase of the number of cells in G0/G1 and S phase. SHK induces cell death mainly by apoptosis, confirmed by FC, which may be related with the increase in activated caspases expression levels.
**Conclusion:** Our results suggest that SHK might be used as a new therapeutic approach in different hematological neoplasias. However the therapeutic efficacy may depend on the cell type and schedule of drug administration used.
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## P30. CHRONIC EXPOSURE TO OXIDATIVE STRESS INDUCERS INFLUENCE METHYLATION STATUS IN NORMAL AND NEOPLASTIC HEMATOLOGICAL CELLS

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**Introduction:** The pathogenesis of myeloid neoplasias is complex and involves multiple genetic and epigenetic events. The oxidative stress and abnormal DNA methylation have been implicated in some types of cancer, namely in myelodysplastic syndromes (MDS), chronic myeloid leukemia (CML) and acute promyelocytic leukemia (APL). Since both mechanisms are observed in some of these patients, we hypothesize that oxidative stress may influence DNA methylation. In this context, the present work aimed to analyze the influence of acute and chronic exposure to OS in global and localized methylation, as well as in the gene expression levels of epigenetic modulators and antioxidant enzymes.
**Methods:** In this study, normal lymphocytes (IMC cell line) and hematological neoplasia cell lines, the K-562 cells (CML), the F36P cells (MDS) and the NB-4 and HL-60 cells (APL with and without t(15;17), respectively), were exposed acutely (48h) and chronicle (± six months) to oxidative stress inducers (hydrogen peroxide and menadione). Cell proliferation and death were analyzed by trypan blue assay. ROS and GSH levels were analyzed using fluorescent probes (DCFH2DA, DHR123, DHE, DAF, and MO, respectively) by flow cytometry. DNA damage was analyzed through 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels (ELISA). The localized methylation status was analyzed through *p15*, *p16*, *DAPK* and *KEAP1* methylation profile (MSP). Global methylation status was assessed by 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC) levels and LINE-1 methylation. Epigenetic gene expression modulators, namely DNMT1, DNMT3a, DNMT3b, p300 and TET2, as well as oxidative stress genes asGPX1, GSTM1, NQO1, GSR, HMOX1, SOD1, KEAP1 and TXN, was analyzed by real time PCR. The statistical analysis was carried out by variance analysis ($p < 0.05$).
**Results:** Acute and chronic exposure to hydrogen peroxide ($H_2O_2$) and menadione (MND) increased intracellular ROS levels in all tested cells lines. However, acute exposure induced a decrease in GSH content while chronic exposure induced an increase, which may be reflect an adaptation to OS. OS exposure also induced an increase in 5-mC levels and a decrease in LINE-1 methylation. Moreover, OS also conduced to hypermethylation of *p15*, *p16* and *KEAP1* promoter genes in a cell type- and exposure-dependent manner. In all cells lines, we observed that chronic exposure to $H_2O_2$ and MND induced an increase in *DNMT1*, *DNMT3a*, *GSR*, *GSMT1* and *NQO1* gene expression and a decrease in *KEAP1*, *p15* and *p16* genes. In cells acutely exposed to these compounds, gene expression levels were cell line-dependent.
**Conclusion:** In summary, oxidative stress, mainly chronic exposure to hydrogen peroxide and menadione, leads to global hypomethylation and localized hypermethylation. These findings suggest a relationship between oxidative stress and aberrant methylation status, two common mechanisms involved in the development of hematological neoplasias.
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## P31. BUPARLISIB, ANOTHER STEP IN THE QUEST TO CURE HAEMATOLOGICAL MALIGNANCIES

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**Introduction:** PI3K, is an intermediate signalling molecule that acts by producing phosphorylated lipids that transduce signals from the cell surface to the cytoplasm. These signals are received by G-coupled proteins and receptor tyrosine kinases and are responsible for the activation of multiple effector pathways such as AKT, NF-KB and JNK/SAPK. Therefore, PI3K plays a very important role in key cellular processes like apoptosis, DNA repair, senescence, angiogenesis, motility and cell metabolism. This molecule is tightly regulated in normal cells; however, several studies show that the PI3K pathway is deregulated in several types of malignancies. Additionally, mutations in PI3K sub-units are not only associated with the tumorigenesis but also with the resistance to conventional anti-neoplasic therapies. The aim of this study is to evaluate the effect of Buparlisib (BKM), a Class I Phosphatidylinositol 3-kinase (PI3K) inhibitor, in *in vitro* models of myeloid haematological malignancies.
**Methods:** For this purpose, we used three *in vitro* models of haematological malignancies, Myelodysplastic Syndrome (F-36P cells), Erythroleukemia (HEL cells) and Acute Promyelocytic Leukemia (NB-4 cells). Cell lines were cultured in absence and presence of different concentrations of BKM that ranged from 50 nM to 10 μM. We used both daily (250 nM) and single dose administration schemes. In order to evaluate the BKM effect in these cell lines, we determined the cell viability at 24, 48 and 72 hours using a Resazurin Assay. Cell death was analysed using optical microscopy (May-Grunwald Giemsa staining), and by flow cytometry (FC) using the Annexin V and Propidium Iodide double staining. To analyse some mechanisms involved in cell death we used the ApoStat, a probe that identify and quantify caspase activity by FC. To analyse the cell cycle we used the Propidium Iodide/RNAse protocol by FC.
**Results:** Our preliminary results show that BKM reduces cell viability in time, dose and cell line dependent manner. The half maximal inhibitory concentration (IC50) at 72 hours of exposure

was 250 nM in the HEL cell line; 750 nM in the F-36P cell line and between 750 nM and 2500 nM in the NB-4 cell line. The daily administration scheme of a small dose (250 nM) of BKM reveals a positive effect when compared to the administration of the same dose in the single dose administration scheme, and this effect was more pronounced in the HEL cell line. This compound induces cell death by apoptosis, confirmed by morphological analysis, FC and by the increase of caspase levels and expression. The cell cycle analysis showed that the compound also induces cell cycle arrest in G2/M phase (HEL cells) and in S phase (NB-4 cells).
**Conclusion:** PI3K is a promising target for novel anti-cancer therapeutics, since BKM has the ability to induce cell death and cell cycle arrest. Our results suggest that BKM could be a new potential therapeutic approach in myeloid haematological malignancies.
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## P32. TARGETTING INTRACELLULAR SIGNALING IN MULTIPLE MYELOMA: THE ROLE OF NEW DRUGS AS THERAPEUTIC APPROACHES

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**Introduction:** Multiple myeloma (MM) is a plasma cell neoplasia characterized by clonal proliferation of malignant plasma cells in the bone marrow microenvironment. Currently, Bortezomib (a proteosome inhibitor) is used as a first line treatment for MM. A complex signalling network is activated in plasma cells, allowing the survival, proliferation and avoiding apoptosis. The knowledge of these changes would enable the use of new drugs in MM. PI3K/AKT/mTOR pathway is one of deregulated pathways that contributes to survival and cell proliferation of cancer cells. Another strategy of cancer cells is the escape to apoptosis, mediated by deregulation of the levels of IAPs proteins and the NF-KB/proteasome pathways. Since many patients seems to be resistant to therapy is important to explore new therapeutic targets. The aim of this study is to evaluate the potential therapeutic of Everolimus (an mTOR inhibitor), Silibinin (a survivin inhibitor), Gambogic Acid (a proteasome inhibitor) and Parthenolide (an IkB inhibitor) in MM.
**Methods:** For this purpose, we used a Multiple Myeloma cell line, the H929 cells. Cells were cultured in absence and presence of different concentrations of Everolimus, Silibinin, Gambogic Acid and Parthenolide alone, and in association with Bortezomib. To evaluate the effect of these inhibitors on cell viability we used the Resazurin Assay. Cell death was determined by optical microscopy (May-Grunwald Giemsa staining), by flow cytometry (FC) using the Annexin V and Propidium Iodide double staining, and by the expression levels of the activated caspases, using the Apostat probe. The effect of the drugs in cell cycle was determined by flow cytometry using Propidium Iodide incorporation.
**Results:** Our results show that all of the tested compounds induced cell death in a time- and dose-dependent manner, with IC50 values of 17.5 μM for Everolimus, approximately 150 μM for Silibinin, less than 150 μM for Gambogic Acid and 12.5 μM for Parthenolide, after 72h of treatment. These compounds induced cell dead mainly by apoptosis, confirmed by the increase of activated caspases expression and morphological aspects. These drugs also induce cell cycle arrest in G0/G1 phase, mainly the mTOR inhibitor. The association of lower doses of these inhibitors with a small dose of Bortezomib shows an additive cytotoxic effect.
**Conclusion:** In conclusion, our results suggest that inhibition of multiple signalling pathways could be used as a new potential approach in the treatment of MM.
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## P33. LUNG CANCER WITH FIVE YEARS SURVIVAL

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**Introduction:** Lung cancer is the leading cause of cancer-related death in the world and is still associated with a poor prognosis. The five year survival in stage IV is less than 5%. In Portugal, the estimated incidence is 29/100,000 inhabitants and lung adenocarcinoma is now the most common cancer (50-60%). The incidence has been increasing, not only by the increase of smokers among women, but also by environmental changes not related to tobacco.
**Case report:** The authors report a case of a 49-year-old female patient, ex-smoker, with a history of migraine, who refers occipital-cervical headache, with a month of evolution, with nausea and vomiting. A CE CT scan revealed a unique right parietal lesion, confirmed by MRI, compatible with brain metastasis. Subsequently a craniotomy was performed with excision of the lesion. Histologic examination revealed a high suspicion for metastatic adenocarcinoma of pulmonary origin. Chest radiograph showed a nodular opacity in the right upper lobe (RUL) and chest CT confirmed the nodule in the anterior segment of RUL, with 2.4 × 1.7 cm, which contacted with the visceral pleura. Bronchoscopy showed no change. Transthoracic biopsy was performed and the histological result was acinar adenocarcinoma apparently primitive of the lung. For staging the disease a PET/CT was done, whose study was consistent with active neoplasia in the RUL without other hipercaptant lesions. Then the patient was submitted to right upper lobectomy, holocranial radiotherapy and cytostatic chemotherapy. Currently the patient presents no signs of recurrence or metastasis, after five and a half years of follow up.
**Discussion:** The authors present this case report due to its rarity, since the prognosis would be quite reserved, which was not verified. Such cases justify the use of all therapeutic weapons available, addressed case by case.

## P34. CYSTIC ADENOID CARCINOMA OF THE BRONCHUS - A CLINICAL CASE

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**Introduction:** Adenoid cystic carcinoma (ACC) of the tracheobronchial tree is rare, it represents 1% of all respiratory tract cancers. Symptoms are related to airway obstruction and therefore mimic those of asthma or chronic bronchitis which may delay diagnosis. ACC usually arise in the lower trachea and less commonly they are found in the bronchial and extrathoracic trachea.
**Case report:** A non- smoking 36-year-old male was referred in January 2012 complaining in the last two months of a persistent

cough, occasionally productive. He also reported more recently haemoptysis. Chest CT demonstrated a 6 × 4 cm homogeneous soft tissue mass encircling the left pulmonary hilum causing complete atelectasis of the left lung. Bronchoscopic examination showed a circumferential submucosal mass starting 2 cm below the main carina that protruded and obstructed the main left bronchus. Biopsies of the lesion revealed an ACC. Surgical tumour resectability proved to be difficult due to invasion of adjacent critical tissues and vessels. Therefore, a left pneumonectomy had to be carried out. Histopathology confirmed a cystic adenoid carcinoma infiltrating respiratory mucosa of the left bronchus with invasion of hilar lymph nodes. Surgery was followed by adjuvant chemotherapy. The patient has remained well, asymptomatic with no sign of recurrence.
**Discussion:** Although cystic adenoid carcinoma is considered a type of low grade tumour, when it is located in the bronchus it behaves more aggressively than its tracheal counterpart. Tumour growth may be life threatening and surgery is the mainstay of treatment often combined to adjuvant treatment to provide the best chance of prolonged survival.

## P35. SUBTYPES OF SQUAMOUS CELL CARCINOMA WITH EGFR MUTATIONS - IMMUNOHISTOCHEMISTRY INTERPRETATION

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**Introduction:** Squamous cell carcinoma (SCC) of the lung develops in the bronchial epithelium according to the sequence squamous metaplasia/dysplasia and are characterized by keratinization and/or presence of intercellular bridges. The presence of at least 10% of tumor differentiation exhibiting these features is required for diagnosis. EGFR mutations are most commonly reported in lung adenocarcinomas. Typically activating mutations of the EGFR gene are not present in the SCC. Supported on a small immunohistochemistry panel we examined the diagnostic accuracy, relating to the mutational analysis of the EGFR gene in SCC.
**Methods:** We studied 47 SCC.surgical specimens. A panel of immunohistochemical markers (TTF1, P63 and CK5/6, CD56, VIM) were used to confirm the diagnosis, to assess the proliferative index and the EMT differentiation. EGFR was studied by immunohistochemistry (protein expression), FISH (number of copies) and direct sequencing (mutational status).
**Results:** We identified three SCC cases with mutated EGFR (deletion in exon 19), one diagnosed as clear cell variant, and all with negative TTF1 expression and with expression of high molecular weight cytokeratins. The proliferative index varied between 10% and 50% of these cases. These cases showed expression of EGFR protein increased EGFR copy number, either by amplification (1 case) or by high polysomy (2 cases).
**Conclusion:** Mutations in the EGFR gene occur rarely in the SCC, with a frequency described below 5%; so the routine molecular testing is not recommended for SCC histology. Although therapy with tyrosine kinase inhibitors of the EGFR gene appear to be less effective in SCC with EGFR mutation than in patients with adenocarcinoma with EGFR mutation, some SCC patients with EGFR mutation can obtain clinical benefit from tyrosine kinase inhibitors therapy. To better identify these patients the following parameters should be taken into account: no formation of keratin pearls and clear/large cell, where the CK7 and Vim are relevant; p63 as a single marker leads to the interpretation of myoepithelial carcinoma.

## P36. BRONCO-PULMONARY CARCINOMA WITH TTF1 POSITIVITY PRESENT EGFR MUTATIONS

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**Introduction:** The molecular characterization of broncho-pulmonary adenocarcinomas allowed the development of new therapies directed to EGFR. TTF-1 expression is a prognostic factor for improved survival in lung adenocarcinomas and mutations in the epidermal growth factor receptor (EGFR) are associated with this expression. Correlating the TTF-1 expression with mutations in exons 19 and 21 of the EGFR gene in lung adenocarcinomas was established.
**Methods:** We evaluated EGFR gene mutations in 66 FFPE adenocarcinoma biopsies; 53 cases expressed TTF-1 and 10 did not express. Additionally we considered 3 TTF-1 negative cases because of their acinar differentiation, although classified as pleomorphic carcinoma (1) and adenosquamous (2). After DNA extraction and polymerase chain reaction (PCR) determined EGFR gene mutations of exons 19 and 21 of the by Sanger sequencing.
**Results:** We identified 9 (3 in exon 19and 6 in exon 21) mutations in the EGFR gene in TTF-1 positive group. Only a single TTF-1 negative case had mutation in exon 19 of the EGFR gene. The remaining 3 complex carcinomas do not have mutations of the EGFR gene.
**Conclusion:** The TTF-1 positive adenocarcinomas, defining Adenocarcinomas Respiratory Terminal Unit (TRU), had higher percentage (17%) of mutations in the EGFR gene when compared with bronchial adenocarcinoma/complex carcinomas (pleomorphic and adenosquamous carcinomas) TTF-1 negative, where mutations can be considered more rare.

## P37. VIMENTIN AND HIGH WEIGHT MOLECULAR CYTOKERATINS EXPRESSION IN BRONCHIOLITIS BASAL CELLS

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**Introduction:** Terminal Respiratory Unit (TRU) inflammation has been recognized as a carcinogenic field for bronchial-pulmonary carcinomas. An immunohistochemical study was developed to verify the behavior of TRU – basal cells in bronchiolitis.
**Methods:** A series of surgical biopsies concerning broncholitis classified as Chronic bronchiolitis (CB) 11 cases, considering lymphocytic infiltrate along with smooth muscle cells hyperplasia; 15 cases of Respiratory Broncholites (RB) and 14 cases of Broncholitis obliterans (BO) was compared with bronchiolitis with preserved morphology (BPM)- 5 cases, obtained after surgical drainage of spontaneous pneumothorax due to persistent infant pleural scars. The immunohistochemical panel considered TTF1, CK 5.6.18, and vimentin applied to all cases.
**Results:** The BPM cases presented TTF1 expression and negativity for vimentin and CK5.6.18 (1 positive case). Vimentin and CK 5.6.18 basal cells expression was correlated progressively with BO, CB and RB, representing 8/14, 4/11 and 2/15 cases for vimentin and 8/4, 5/11, and 7/15 cases for CK5.6. 18 positive cases, respectively.
**Conclusion:** These results are important to understand TRU carcinogenesis potential where adenosquamous carcinomas and pleomorphic carcinomas may develop. Basal cell metaplasia with

CK5.6.18 represents bronchial basal cell phenotype, maintaining TTF1 expression and epithelial-mesenchimal transition is represented by vimentin expression.

## P38. ATYPICAL ALK REARRANGMENTS IN PULMONARY LUNG CANCER WITH ATYPICAL ALK REARRANGEMENTS

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**Introduction:** Patients with bronchial-pulmonary carcinomas (BPC) who harbor anaplastic lymphoma kinase (*ALK*) gene rearrangements can derive significant clinical benefit from crizotinib, a small molecule inhibitor of the *ALK* tyrosine kinase. Rearrangement of *ALK* gene is the best predictor of response to crizotinib.
**Objectives:** To identify and select patients who may benefit from this therapy, evaluating *ALK* gene rearrangement, by FISH, in BPC.
**Methods:** In total 185 biopsies of BPC were tested for *ALK* rearrangement and copy number by fluorescence in situ hybridization (FISH) with ZytoLight SPEC ALK/EML4 Tricheck. The pattern was considered positive for cells exhibiting break-apart or isolated signals. The presence of > 15% cells with rearrangements identifed by FISH classify tumours as *ALK*-positive.
**Results:** FISH-positive results were demonstrated in 16 of 185 cases (8.6%). One of these FISH-positive tumours does not meet the current criteria break-apart positivity, but presents 3' *ALK* doublets and triplets. We also found *ALK* copy number gain (> 3 copies per cell in 40% of cells) in *ALK*-negative tumors. Three cases were considered borderline.
**Conclusion:** The clinical behavior of BPC cases with variant *ALK* signal patterns is less clear. Amplification of the *ALK* fusion has been seen in patients undergoing crizotinib therapy and may be a sign of developing resistance. Given the large number of signaling pathways that are influenced by *ALK* fusion products, the behavior and appropriate treatment of NSCLC cases that have variant *ALK* signal patterns require further studies. Atypical patterns may represent direct or inverse duplications or more complex rearrangements specifically involving the region of interest, although being less frequent they may harbor an *ALK*-positive rearrangement. These cases should be studied by additional testing with a second detection method.

## P39. THE BEAS-2B CELL LINE AS A MODEL SYSTEM FOR THE STUDY OF LUNG CARCINOGENESIS: EFFECTS OF CULTURE CONDITIONS AND CULTURE LENGTH

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**Introduction:** Choosing the right experimental model for the study of lung carcinogenesis is not straightforward. The tracheobronchial tree of animal models is not readily accessible and, in addition, these models are expensive. To this end, the use of primary cell cultures has an important limitation: cells cultured directly after extraction from a donor suffer from senescence, precluding long-term studies. The time non-transformed bronchial cell lines can be maintained in culture is also relatively short. Thus, researchers often employ immortalized cell lines. The BEAS-2B cell (ECCAC; Cat.No. 95102433), established Curtis by Harris and collaborators in 1988 (Reddel et al. Cancer Res. 1988;48:1904-9) has been broadly used as a model of airway epithelium, namely in studies of lung carcinogenesis. Although immortalized through viral infection, BEAS-2B cells retain features of human bronchial epithelial cells and only became tumorigenic (very weakly) at very high passages (Reddel et al. Cancer Res. 1993;53:985-91). In this study, we extended its characterization and assessed the effects of culture conditions and length of culture on different parameters, namely proliferation and intermediary metabolism.
**Methods:** BEAS-2B cells were grown in LHC-9 medium. Culture flasks and multiwell plates were pre-coated with gelatin, except when otherwise stated. Doubling times were calculated by monitoring total cell numbers (determined by Trypan Blue dye exclusion) in cultures in the exponential phase of growth. Glucose and lactate levels were determined using commercial kits (from bioMérieux and Randox, respectively). GTG banded chromosomes were prepared according to standard procedures.
**Results and conclusion:** Growth conditions: In the absence of coating, cell adhesion to the substrate was significantly reduced. As a consequence, cultures lost the typical cobblestone growth of epithelial cells and formed foci. Nonetheless, the duplication rate of the "foci cultures" was identical to that of control cultures (i.e., those grown in coated vessels). Rates of glucose uptake and lactate production were unaffected. Culture stability: In this study, cultures could be maintained for over 100 passages/600 doublings, but, over time, several alterations became evident. Specifically, cells became bigger, lost their characteristic diamond shape and crisscrossing became more frequent. In addition, the majority of chromosomal alterations (structural and numeric) present at early passages disappeared, while a new set emerged. A slightly augmented growth rate was also observed in aged cultures.
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## P40. NANODIRECT-PDT: EGFR TARGETED NANOPARTICLES AND PHOTODYNAMIC THERAPY FOR LUNG CANCER

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**Introduction:** Lung cancer (LC) is leading death cause of cancer worldwide being expected almost 2 million deaths in 2020. Following diagnosis, only 15% survive ≥ 5 years. Photodynamic therapy (PDT) relies on administration of a molecule, the photosensitizer (PS), whose effect is triggered only after light activation. PDT was already applied to LC, and although its use is limited, it preserves lung function, can be repeated and combined with other therapies, being useful in palliation and in the eradicated of stages 0/1 centrally located LC. Due to their encapsulation ability, liposomes are efficient drug carriers. Previously we developed liposomes appropriate to use in the pulmonary network. Moreover, nanobodies are the smallest functional antigen binding fragments derived from naturally occurring heavy chain only antibodies. They bind with great specificity and high affinity, to their antigens. Therefore, the aim of this project is to develop and evaluate a novel transport system to carry photosensitizers (PS) to treat lung cancer (LC): endothelial growth factor receptor (EGFR) targeted nanobodies

coupled to lipossomes, which were developed on purpose to apply in pulmonary network and can be administered as aerosols.
**Methods:** The liposome formulation was composed of disteroylphosphatidylcholine, phosphatidylglicerol, N-acetyl muramic acid, cholesterol and maleimide-polyethyleneglycol 2000 disterylphosphatidylethanolamine. The lipidic mixture was evaporated to form a thin lipid film. The PS, previously synthetized, was added during hydration of the film. The liposomes were then extruded through 100 nm pore size filters. The nanobody Ega1, previously activated, was conjugated to the liposomes overnight. The conjugation was evaluated in SDS-PAGE. In order to verify the outcome of the formulation the lung cancer cell lines H1299 and A549 were incubated with the formulation for 2 hours and light activation was performed with 10J. The metabolic activity was evaluated through the MTT assay, cell viability was evaluated by flow cytometry. EGFR expression of the cell lines was evaluated by flow cytometry and western blot. Fluorescence microscopy studies to evaluated cell nanoparticle interaction were also performed.
**Results:** It was possible to successfully conjugate the nanobody to the liposomes, and with a ratio of 1.6 nmol nanobody per 1μmol total lipid is possible to obtain a conjugation yield of 40%. Metabolic activity results show that cell proliferation is inhibited proportionally to the concentration of the formulation. Photodynamic therapy based on the formulation prepared led to a decrease of viability of H1299 and of A549 cells to approximately 40%. Concomitantly cells in death by apoptosis and necrosis were augmented. The nanoparticles interaction with the cell lines was verified. Both H1299 and A459 cells express EGFR.
**Conclusion:** Preliminary data show that it was possible to obtain a formulation able to interact with lung cancer cells and to induce cell death therefore further studies are encouraged.
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## P41. HEPATOCELLULAR CARCINOMA THERAPY BASED ON HUMAN AMNIOTIC MEMBRANE: INHIBITION OF GLUT AND PGP EXPRESSION

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**Introduction:** Hepatocellular carcinoma (HCC) has a high incidence and mortality throughout the world. This is due, at least in part, to the lack of effective therapies for this type of cancer. Multiple studies indicate that hepatocellular carcinoma have increased levels of glucose transporter (GLUT), increasing its tumorigenic potential. In addition, there is still a link between the expression of GLUT and resistance to chemotherapy. Our research group already showed that hAM protein extracts (hAMPE) are able to inhibit the metabolic activity of hepatocellular carcinoma (HCC) cell lines. Thus, the aim of this study was to evaluate *in vitro* the effect of protein extracts of hAM (hAMPE) in membrane expression of GLUT and glycoprotein-1 expression (Pgp) in human HCC.
**Methods:** After reception, hAM were subjected to mechanical actions in order to extract the proteins subsequently quantified in Nanodrop®. *In vitro* studies were performed in two HCC cell lines: HuH7 (mP53) and Hep3B2.1-7 (nP53). Cells were incubated with 1 μg/μL of hAMPE for 72 hours. GLUT-1, 2, 3, 5, 12 and Pgp expression were evaluated by fluorescence, using a plate reader.
**Results:** hAMPE decreases the expression of GLUT3 (14%) on HuH7 cells when compared to control. Regarding the Hep3B2.1-7 cells, it was demonstrated that treatment with hAMPE significantly decreases the expression of GLUT2 (13%) and GLUT5 (29%) relative to control. However, the GLUT1 expression increases 13% in this cell line. In what concerns to Pgp expression, it was observed an inhibition in Hep3B2.1-7 cells (32%), but not in the HuH7 cells.
**Conclusion:** hAMPE was capable to inhibit the transport of glucose and tumor resistance, suggesting its usefulness for the treatment of HCC, particularly for HCC which do not express p53. In the future, further studies are required in order to better clarify the relationship between treatment with hAMPE and the glycolytic metabolism and tumor resistance.

## P42. CANCER CELL DEATH PROFILE INDUCED BY HUMAN AMNIOTIC MEMBRANE EXTRACTS TREATMENT

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**Introduction:** Recently, some studies have revealed the anticancer effect of human amniotic membrane (hAM). As a new research area, the cellular mechanisms responsible for anti-cancer properties of hAM are poorly understood. Previously, our research group showed that hAM protein extracts (hAMPE) are able to inhibit the metabolic activity of hepatocellular carcinoma (HCC) cell lines. This carcinoma has a high worldwide incidence and mortality. Due to the lack of effective therapies for HCC, it is important to discover new treatments for this type of cancer. Therefore, the aim of this study was to unravel the cell death profile induced by hAMPE in a human HCC cell line.
**Methods:** hAM were obtained from healthy women with informed consent according to the guidelines of Ethical Committee of Coimbra Hospital and University Centre (Coimbra, Portugal). hAM were washed with phosphate buffered solution and subjected to mechanical actions in order to extract proteins, which were quantified using Nanodrop®. *In vitro* studies were performed in a human HCC cell line: Hep3B2.1-7 (nP53). To evaluate the existence and type of cell death, annexin V (AV) and propidium iodide (PI) were used. The results were obtained using a fluorescence plate reader. BAX/BCL2 ratio and cytochrome C expression were also determined using the same methodology. The expression of caspase 3, 8 and 9 was determined by western blot.
**Results:** Through our results, we can see that hAMPE induces cell death by apoptosis and necrosis in the cell line under study. We found that there is no alteration of BAX/BCL2 ratio or cytochrome C expression after treatment. Regarding the caspases expression, our results show an increase of caspase 3 and caspase 8 after hAMPE treatment. The expression of caspase 9 was not altered by the treatment.
**Conclusion:** hAMPE induces cell death through apoptosis and necrosis in the Hep3B2.1-7 cells. In addition, it was demonstrated that the effect of hAMPE treatment is through the extrinsic pathway of apoptosis.

## P43. MAY OUR DIET INTERFERE WITH COLON CANCER AGGRESSIVENESS PROVOKED BY WARBURG EFFECT?

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**Introduction:** During tumorigenesis, tumor cells develop metabolic alterations to support the energetic requirements provoked by the higher proliferation. Thus, tumor cells intensify glycolytic metabolism and decrease the oxidative metabolism (Warburg effect). Butyrate is produced by decomposition of dietary fiber by intestine's bacteria and it is described to have an important role on the colon homeostasis. The aim of this study is to evaluate whether butyrate (obtained from diet) interferes with the aggressiveness provoked by Warburg effect in colon cancer cells.
**Methods:** WiDr and C2BBe1 cell lines were cultured with low glucose content (5 mM). To perform the uptake studies, cells were incubated with or without butyrate before the incubation with $^{18}$F-FDG (25 μCi/ml). At different times, samples of cell suspension were collected to evaluate $^{18}$F-FDG uptake percentage. To evaluate the membrane expression of GLUT-1, -3, -5 and -12 after butyrate exposure for 1 and 24 hours, flow cytometry was used. To evaluate the lactate production, the coupling between glycolysis and the Krebs cycle, and the turnover of Krebs cycle with or without the presence of butyrate, glucose uniformly labeled with carbon-13 was added to the medium without glucose. NMR technique allowed us to evaluate different parameters, including butyrate uptake by tumor cells.
**Results:** In WiDr and C2BBe1 cell line we observed that incubation with butyrate decreases $^{18}$F-FDG uptake. Taking into account GLUTs expression, in WiDr cells we observed a higher expression of GLUT-12 at the membrane. With butyrate exposure, a decrease in GLUT-12 membrane expression was observed. In C2BBe1 cell line butyrate, in some cases promoted an increase in GLUTs expression. With NMR technique it was possible to confirm the uptake studies, in that butyrate induced a decrease in lactate production in both cell lines. When WiDr cells are exposed to butyrate, the coupling between glycolysis and Krebs cycle increased. In both cell lines it was possible to observe that butyrate interferes with glucose consumption and that oxidative metabolism was more pronounced.
**Conclusion:** The results obtained suggest that butyrate (obtained from diet) interferes and in some cases attenuate the Warburg effect, decreasing tumor aggressiveness. With these studies is indeed marked the importance of a balanced/personalized diet in colon cancer prevention and treatment.

## P44. PRIMARY HEPATIC GIST: AN UNCOMMON FORM OF MESENCHIMAL TUMOR

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**Introduction:** The authors will present the clinical case of an atypical form of hepatic gastrointestinal stromal tumour (GIST).
**Case report:** The patient is a male, 60 years old, referred to the hospital consult due to abdominal pain, distension and a voluminous, tumorous formation on the left liver lobe (ultrasound). For better characterization an abdominal tomography was performed confirming a lesion with 14.9 cm of diameter, which was later biopsied with ecographic guidance showing a hepatic sarcoma/GIST. The complementary tests (gastro-intestinal endoscopic study and PET scan) did not find any other lesions. The patient was subject to left hepatectomy and hepatic hilum lymphadenectomy in November 2007, and histology confirmed neuro and angioinvasive GIST with a mitotic rate of 5/10CGA. During the follow-up he revealed ahipercation of 18F-FDG in the anterior gastric wall and duodenum. The patient performed upper endoscopy with ecography and colonoscopy that excluded a malignant lesion. In 2009, a pulmonary mass with 13.3 cm of diameter in the left inferior lobe (diagnosed with TC and confirmed with PET scan) was found. These exams also showed a heterogenic caption of 18F-FDG by the liver, with two hepatic lesions with hypermetabolism. The lesions were maintained under surveillance, with imagiologic and clinic revaluation every 3 months. In 2013, the pulmonary mass showed an enlargement, measuring 28 cm of diameter. A transthoracic biopsy was performed revealing a secondary location. He was submitted to thoracotomy to exscind the mass. No complications occurred. The patient started tyrosine kinase inhibitors and is currently alive and well.
**Discussion:** GISTs are neoplasms of the gastrointestinal tract, mesentery, or omentum that express the protein-tyrosine kinase cKIT (CD117) and are the most common mesenchymal tumours arising at these sites. The importance of this case lies on its rareness. With this report, our main aim isto alert physicians to the possibility of a GIST being, in fact, a primary tumour arising from the liver and showing up, years later, in the lung.

## P45. ROLE OF OXIDATIVE STRESS AND DETOX GENES IN COLORECTAL CANCER DEVELOPMENT

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**Introduction:** Reactive oxygen species (ROS) is a consequence of aerobic respiration. Oxidative stress induces high levels of ROS that damage DNA, producing mutations. Superoxide dismutases (SOD) are enzymes responsible for the detoxification of superoxide ROS. Given the potential role of oxidative stress in colorectal cancer (CRC) etiology, it is reasonable that colorectal cancer risk might be modified by SOD2 and SOD3 polymorphisms. This study aimed to determinate the association between common polymorphisms of SOD2 and SOD3 genes and CRC.
**Methods:** SOD2 V16A, SOD2 -28C > T, SOD2 T175C and SOD3 R213G polymorphisms were determined by PCR-SSP techniques in 50 CRC patients and 50 healthy controls.
**Results:** Our results showed that the polymorphisms studied seem to influence CRC prevalence since their mutated alleles had a high frequency in the CRC patients when compared with controls (SOD2 16A: 58% vs 40%, $p < 0.05$; SOD2 -28T: 58% vs 42%, $p < 0.05$; SOD3 213G: 58% vs 40%, $p < 0.01$).
**Conclusion:** These findings provide insight into the potential pathogenesis of oxidative stress in CRC. SOD2 and SOD3 polymorphisms seem to be associated with a decrease of enzyme activity and accumulation of ROS. Therefore, this may be an important biomarker of disease risk, progression and survival for CRC patients thus it is very important to clarify the true impact of these genes in CRC pathogenesis.

## P46. LIPID METABOLISM GENES POLYMORPHISMS ROLE IN COLORECTAL CANCER DEVELOPMENT

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**Introduction:** Some polymorphism LPL, APOE and NPY have been associated with the decrease of the colon absorption cholesterol, and increase of excretion secondary bile acids that seems to be a major risk of development of colorectal. Lipoprotein Lipase (LPL) is a key enzyme in lipid metabolism and responsible for catalyzing lipolysis of triglycerides in lipoproteins VLDL and chylomicrons. Alternatively, Apoliprotein E (APOE) variant influences the enterohepatic metabolism of cholesterol and bile acids. Moreover, Neuropeptide Y (NPY) is a neurotransmitter expressed in the central nervous system and involved in appetite satisfaction levels. This study aimed to determinate the association between common polymorphisms of genes involved in lipid metabolism (APOE, LPL, NPY) and CRC.
**Methods:** NPY L7P, NPY 7PP, APOE E2E2, APOE E4E4 and LPL C1595G genotypes was determined by PCR-SSP technics in 50 CRC patients and 50 healthy controls.
**Results:** In this study APOE E2E2, NPY L7P and LPL C1595G polymorphisms seemed to be associated with CRC prevalence, since their mutated alleles had a high frequency in the CRC patients (NPY 7P: 40% vs 6%; $p < 0.0001$; APOE E2E2: 6% vs 0%; $p < 0.05$; LPL1595G: 40% vs 20%; $p < 0.005$ ). We also detected a high frequency of NPY 7PP mutated genotype in the CRC group (15% vs 1%, $p < 0.0001$) while APOE E4E4 genotype showed a protective effect in CRC when compared with control subjects (0% vs 8%; $p < 0.05$).
**Conclusion:** These findings provide insight into the potential pathogenesis of NPY, APOE and LPL polymorphisms in CRC predisposition and progression. Dysregulation of NPY, LPL and APOE proteins are associated with increased levels of cholesterol/triglycerides and bile acids that are involved with increased risk of CRC predisposal. In this sense it is very important to clarify the true impact of these genes in CRC pathogenesis.

## P47. FOLATE METABOLISM GENES POLYMORPHISMS ROLE IN COLORECTAL CANCER DEVELOPMENT

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**Introduction:** Folate pathway directly influences the synthesis of purins and indirectly the hypometylation of the global DNA and hypermethylation of tumour suppressor genes. This molecule can modulate the risk of developing the colorectal cancer (CRC). The intracellular folate metabolism is regulated by several enzymes including methylenetetrahydrofolate reductase (MTHFR) methionine synthase (MTR) and methionine synthase reductase (MTRR). Thus, genetic changes in *MTHFR* gene can influence the development/predisposal of CRC. The aim of the study was to explore and describe the effect of polymorphisms in folate-associated genes (*MTHFR C677T* and *A1298C*) in colorectal cancer predisposal.
**Methods:** Paraffin-embedded tissues from 50 healthy patients and 50 patients with colorectal cancer were analyzed. Genotyping of polymorphisms in the folate-associated gene *MTHFR* (*C677T* and *A1298C*), *MTR 2756 A > G and MTRR A66G* were done by PCR-SSP technique.
**Results:** The MTHFR *677TT* (46% vs 25%; $p < 0.001$) and MTHFR *1298CC* (45% vs 21%; $p < 0.001$) genotype variants were associated to low activity of the enzyme, consequently decreasing the efficiency of the metabolism of folate. Both variants appear to be linked to the prevalence of colorectal cancer.
**Conclusion:** These findings provided insight into the potential pathogenesis of polymorphisms in CRC predisposal and prognostic. Dysregulation of *MTHFR* gene is associated to decreased levels of the MTHFR enzyme, a key enzyme in the folate metabolism, correlated to increased CRC predisposal. Thus, this study contributes to the development of new prevention, diagnostic and therapeutic strategies.

## P48. IODINE-131: COULD BE AN OPTION FOR CHOLANGIOCARCINOMA TREATMENT?

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**Introduction:** Cholangiocarcinoma (CC) is a malignancy with poor prognosis and a reduced survival rate. It was shown that CC, has increased sodium-iodide symporter (NIS) expression. This molecule is responsible for cellular iodine uptake and is a key molecule in the metabolic radiotherapy with iodine-131 ($^{131}$I).This study aims to evaluate therapeutic efficacy of $^{131}$I metabolic radiotherapy in human CC as well as in human cholangiocytes.
**Methods:** One CC cell line (TFK-1) and another of normal cholangiocytes (H69) were used. It was evaluated the NIS expression. Subsequently, the cells were irradiated with $^{131}$I increasing doses (0.35-60 Gy), in order to evaluate and characterize metabolic radiotherapy effects using different molecular biology techniques.
**Results:** It was observed that both cell lines express NIS. $^{131}$I treatment induced cell viability decrease in a dose dependent manner in TFK-1. The predominant cell death type was apoptosis, followed by BAX/BCL-2 ratio increase. There was also the cytochrome C release and mitochondrial membrane depolarization. Concerning cell cycle, were not detected differences between conditions and control cells. Two hours after the irradiation, it was observed an intracellular peroxides production increase, but there were no differences in superoxide anion production. Forty-eight hours after the irradiation were not detected differences in both intracellular peroxides and superoxide anion production. It was observed differences in superoxide dismutase production, 2 and 48 hours after irradiation. Concerning glutathione, it was observed a decreased production only 48 hours after irradiation, and for higher doses. It was verified that $^{131}$I induce DNA breaks in TFK-1 cells in a dose dependent manner. Concerning H69 cell line, $^{131}$I irradiation doesn't affect cell survival.
**Conclusion:** $^{131}$I treatment induces cell viability decrease in TFK-1 by apoptosis. Intracellular peroxides seem to be involved. Metabolic radiotherapy with $^{131}$I may be a promising option for the treatment of CC.

## P49. PHOTOSENSITIZATION OF HT29 COLON CANCER CELLS BY TWO MESO-SUBSTITUTED PORPHYRINS: 5,10,15,20-TETRAKIS(QUINOLIN-2-YL)PORPHYRIN (2-TQP) AND 5,10,15,20-TETRAKIS(4-CARBOXYPHENYL) PORPHYRIN (TCPP)

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**Introduction:** Photodynamic therapy (PDT) is a selective and minimally invasive therapeutic approach that combines the action of a photosensitizer agent (PS), light and molecular oxygen to produce reactive oxygen species, responsible for the selective destruction of harmful cells. The present study describes the chemical and biological characterization of 5, 10, 15, 20-tetrakis(quinolin-2-yl) porphyrin (2-TQP) and 5, 10, 15, 20-tetrakis (4-carboxyphenyl) porphyrin (TCPP) as PDT agents, aiming to establish correlations between their chemical properties and their photodynamic efficiency. Photodynamic activity was assessed in the HT29 cell line, a potential model system for colorectal cancer, which is one of the most common causes of cancer death worldwide.
**Methods:** 2-TQP and TCPP were synthesized following the *Adler-Longo* method, by condensation of 2-quinolinecarboxaldehyde or 4-carboxybenzaldehyde, respectively, with pyrrole in acid media. After synthesis, chemical and photo-physical characterization, the photodynamic activity of both porphyrins was evaluated in the HT29 cell line. HT-29 cells were grown as monolayers in RPMI medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum, in a humidified atmosphere of 95% air and 5% $CO_2$, at 37 $^oC$. Cells were allowed to attach before treatment with several concentrations of each porphyrin in DMSO solutions (0.5% v/v) and irradiated 24h later with a fluence rate of 10.8 $mW/cm^2$. Cell viability was determined 24 h late by the MTT assay. For each experiment, control cultures were established and processed in parallel. The statistical comparisons between treated and control groups were performed using ANOVA followed by Dunnett's multiple comparison *post hoc* test.
**Results:** Both porphyrins were obtained as pure compounds. They exhibited high singlet oxygen quantum yields and strong absorbances. Moreover, they were harmless to HT29 cells in the dark, but highly cytotoxic upon light activation. As expected, the photodynamic activity depended on both porphyrin concentration and light dose. For the same concentration and light dose, 2-TQP was more effective than TCPP. This effect might be due to the higher hydrophobic character of 2TQP, which favours its accumulation within cancer cells.
**Conclusion:** Our results suggest that both porphyrins meet essential requirements of an ideal photosensitizer. Further studies will be performed to assess the selectivity of both porphyrins to cancer cells and to determine their intracellular localization.
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## P50. PREDICTIVE FACTORS FOR LIVER FAILURE AFTER HEPATECTOMY IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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**Introduction:** Posthepatectomy liver failure (PHLF) is a severe and the most serious complication after hepatectomy (LR); particularly in patients with chronic hepatic disease. The predictive factors (PF) of PHLF in patients with hepatocellular carcinoma (HCC) in our department were evaluated.
**Methods:** One-hundred and five patients (121 LR) with a medium age of 64 ± 11.7 years (34-85) underwent LR for HCC in our department. Eighty-five percent were male, 69% had chronic hepatic disease (cirrhosis in 56%) and 90% were classified as Child-Pugh A. Forty-nine major LR were made (minor in 72). PHLF was defined using the International Study Group of Liver Surgery definition. Thirteen PF of PHLF were evaluated using univariate and multivariate analyses. Differences were considered to be significant when $p < 0.05$.
**Results:** PHLF incidence was 20% (Grade A: 47.8%; B: 43.5%; C: 8.7%). On univariate analysis six PF of PHLF were found: cirrhosis ($p = 0.012$; OR 4.07), number of nodules ($p = 0.024$; OR 4.5), platelet count < 120 ($p < 0.001$; OR 6.67), INR > 1.2 ($p < 0.001$; OR 12.06), Prothrombin time activity (PRT) < 75% ($p < 0.001$; OR 8.23), Serum albumin < 3.5 g/dL ($p < 0.001$; OR 6.48); in multivariate analysis age > 70 years ($p = 0.023$; HR 5.56), number of nodules ($p = 0.024$; HR 5.53), INR > 1.2 ($p = 0.009$; HR 10.28) and PRT < 75% ($p = 0.03$; HR 6.22) were identified as PF of PHLF.
**Conclusion:** The PF of PHLF should be considered in the selection of patients with HCC for LR to decide if they can benefit of pre-operative strategies (chemoembolization or portal vein embolization) and/or of other surgical or nonsurgical treatments.

## P51. ASCORBIC ACID-INDUCED CYTOTOXICITY IN THREE COLON CANCER CELL LINES

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**Introduction:** Colon cancer (CC) is a major health problem with more than one million new cases diagnosed worldwide every year. Studies developed in the last decades revealed that ascorbic acid can be a potential anticancer agent, as well as, it can contribute to the development of a promising therapy with reduced doses of conventional chemotherapeutic drugs and consequently, a decrease in secondary effects. The cytotoxic effect of pharmacological concentrations of AA seems to be based on the generation of hydrogen peroxide. The increased production of hydrogen peroxide, coupled with the breakdown of the activity of antioxidant enzymes and the presence of transition metals in cancer cells, may result in the selective cytotoxicity of AA and the subsequent revelation of its therapeutic potential. The aim of this study is to evaluate the mechanism of action of AA in three human colon cancer cell lines.
**Methods:** WiDr, C2BBe1 and LS1034 (multidrug resistant) cell lines were cultured in appropriate culture medium and incubated in absence and presence of AA during 24 hours. Flow cytometry was performed to evaluate cell viability and types of death, BAX and BCL-2 expression, alterations on cell cycle and mitochondrial membrane potential (MMP) and the redox intracellular environment through determination of ROS production (superoxide radical and peroxides) and expression of reduced glutathione (GSH). Comet assay was used in order to assess DNA damage induced by different concentrations of AA.

**Results:** AA induced a decrease on cell viability in a dose-dependent way, being C2BBe1 cells the most sensitive to AA. As AA concentration increased, BAX/BCL-2 ratio increased and MMP decreased, for all cell lines under study. With higher concentrations, AA induced cell cycle alterations, especially on C2BBe1 and LS1034 cells. Regarding redox intracellular environment, it was noticed that with increasing AA concentrations, superoxide radical levels increased and peroxides levels decreased on C2BBe1 and WiDr cells. The antioxidant defense GSH expression also decreased. In LS1034 cells there were no changes in ROS or GSH production. In all cell lines, AA caused DNA damage in a dose-dependent manner.
**Conclusion:** High doses of AA induce a cytotoxic effect through the mitochondria dependent pathway, with evidences of DNA damage. Results obtained in LS1034 cells point to a ROS non-mediated cytotoxicity in this cell line, indicating that the AA mechanism of action depends on the genetic background of cells. The data obtained bring new insights regarding the applicability of AA in CC disease.

## P52. COMBINATION OF BUTYRATE AND IRINOTECAN IN COLON CANCER TREATMENT

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**Introduction:** High levels of dietary fiber are related with a lower risk for developing colon cancer (CC). Microbial fermentation of fiber by the gut produces short chain fatty acids, such as butyrate. Butyrate is an important energy source for colonocytes and it plays an important role in maintenance of the colon homeostasis. It was reported that butyrate may be a chemopreventive agent. Irinotecan is used as second-line treatment, but, there remains the doubt about the benefits and risks, namely, the large interindividual variability in pharmacogenetic behavior. The use of natural compounds to turn the resistant cells more sensitive to chemotherapy seems to be a possible solution. The aim of this study is to evaluate the therapeutic potential of the combination of butyrate and irinotecan.
**Methods:** C2BBe1, WiDr and LS1034 cells were incubated with increasing sodium butyrate (1-50 mM) and irinotecan (0.1-100 μM) concentrations, separately and in combination. In order to obtain the $IC_{50}$ (half maximal inhibitory concentration) after 48, 72 and 96h, cell proliferation was evaluated through MTT assay. Flow cytometry was performed to study the combination effect on cell viability and types of death, BAX and BCL-2 expression and alterations on mitochondrial membrane potential (MMP). It was also determined VEGF expression using western blot. *In vivo* studies with Balb/c nu/nu mice were conducted through inoculation of WiDr cells on their back. During several days the body weight and tumor size were monitorized, in order to ascertain the effect of the combination on tumor growth.
**Results:** It was observed that as butyrate incubation time increases, cell proliferation decreases, being obtained lower $IC_{50}$ values. The combination of butyrate and irinotecan significantly decreased cell proliferation compared to monotherapy, in all cell lines, being LS1034 cells the most sensitive to the combination at longer incubation times. In all cell lines, combination of butyrate and irinotecan also decreased cell viability. Moreover, it was observed an increase of BAX/BCL-2 ratio and a decrease of MMP, most evident on LS1034 cells, that can be correlated with the apoptosis results. Preliminary results showed that VEGF expression decreased with butyrate treatment in all cell lines. The data obtained *in vivo* suggest that butyrate and irinotecan combination synergistically inhibit tumor growth.
**Conclusion:** Our study suggests that butyrate and irinotecan combination has a significant cytotoxic effect on the three CC cell lines and inhibit tumor growth. The use of natural compounds as butyrate in combination with chemotherapeutic agents can be a new solution for CC treatment.

## P53. PREVENTING GASTRIC CANCER - IS THE BASAL VALUE OF UREA BREATH TEST A PREDICTIVE FACTOR FOR SUCCESSFUL HELICOBACTER PYLORI ERADICATION?

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**Introduction:** Urea breath test (UBT) is a highly sensitive and specific non-invasive method for *Helicobacter pylori* (Hp) detection. Its' basal value, expressed as delta over baseline (DOB), may be a predictive factor of eradication success and antibiotic resistance.
**Objectives:** Evaluate if pretreatment DOB values may predict a successful Hp eradication and Hp resistance to antibiotics.
**Methods:** One hundred and ninety-seven consecutive patients (females – 66.5%; mean age – 43.9 ± 14.5 years; residence in urban area – 44.7%; chronic alcohol consumption – 27.9%; smokers – 11.2%), who received first-line eradication treatment with amoxicillin, clarithromycin and pantoprazole for 14 days, were included. For one hundred of them Hp resistance patterns to clarithromycin, levofloxacin and metronidazole were available.
**Results:** Successful eradication was achieved in 72.6% of cases. Therapeutic compliance was high (95.9%) and did not interfere with treatment success. Males had higher Hp eradication rates (81.8% *vs* 67.9%; OR = 2.13; CI95% 1.03-4.35). The other epidemiologic variables didn't affect eradication. The following resistance patterns were detected: clarithromycin – 22%, levofloxacin – 27% and metronidazole – 30%. Pretreatment DOB values did not differ between patients with eradication success and failure (41.2 ± 22.2 *vs* 44.1 ± 25; p = 0.673), resistance or susceptibility to clarithromycin (44.8 ± 27.6 *vs* 39.8 ± 22.8; p = 0.567) and metronidazole (36.7 ± 23.2 *vs* 42.8 ± 24.1; p = 0.266). However, DOB values were significantly higher in patients with levofloxacin-resistant Hp (48.8 ± 25.6 *vs* 38 ± 22.7; p = 0.043).
**Conclusion:** Pretreatment DOB values didn't show to be predictive of Hp eradication success with first-line triple therapy and didn't predict clarithromycin or metronidazole Hp resistance. However, higher initial values were observed in patients with levofloxacin resistant Hp, and that can help us to choose first-line therapy, by avoiding empiric use of this antibiotic.