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Session 1

P1. THERAPEUTIC POTENTIAL OF THE SPLICING INHIBITOR PLADIENOLIDE B IN ACUTE MYELOID LEUKEMIA - AN IN VITRO STUDY

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Introduction: The splicing of pre-mRNA into functional mRNA, carried out by the spliceosome, represents a crucial step for the cell's gene expression. Mutations in some spliceosome components have been identified in several hematological malignancies (HM), including myelodysplastic syndromes and acute myeloid leukemia (AML). The therapeutic potential of splicing inhibitors such as Pladienolide-B (Pla-B) has already been tested in several solid neoplasias. However, to our knowledge, it is not yet explored in HM, namely in AML.
Objective: To study the cytotoxic effect of Pla-B in AML cell lines with different genetic alterations.
Methods: We used 2 erithroleukemia cell lines: the K562 cells, with the *BCR-ABL1* fusion gene, and the HEL cells, with the JAK2 V617F mutation. Cells were incubated in the absence and presence of increasing concentrations of Pla-B (from 0.25 to 100 nM), in single dose (in all concentrations) and daily administration (for 0.5 nM). To evaluate cell viability and density, we used the trypan blue assay. Cell death was determined by microscopy (May-Grunwald Giemsa staining) and by flow cytometry (FC), using Annexin V and Propidium Iodide (PI) double staining and the Apostat Probe, to evaluate caspase expression levels. Cell cycle was evaluated by FC, using PI/RNase assay.DNA sequencing was performed to assess the presence of *SF3B1* mutations in exons 14 and 15. Results were considered significant when $p < 0.05$.
Results: Pla-B induces a decrease in cell proliferation and viability in a dose, time and cell type dependent manner. K562 cells are less sensitive to cell death induced by Pla-B than HEL cells, which may be due to their genetic differences. Pla-B inducing cell death preferentially by apoptosis and also showing a cytostatic effect, with a G_0/G_1 arrest. The mutations in *SF3B1* exon 14 and exon 15 are absent in both cell lines.
Conclusion: Our results suggest that Pla-B present a cytotoxic and cytostatic effect in both cell lines and may represent a new approach in the treatment of erythroleukemia. However, the therapeutic efficacy may be dependent on the cell genetic background.
This work was support by CIMAGO and R.A. was supported by FCT with a PhD grant (SFRH/BD/51994/2012).

P2. THE ROLE OF WNT AND HEDGEHOG SIGNALING PATHWAYS AS NOVEL PROGNOSTIC MARKERS AND THERAPEUTIC TARGETS IN PEDIATRIC B CELLS ACUTE LYMPHOBLASTIC LEUKEMIA - A PRELIMINARY STUDY

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Introduction: Deregulation of signaling pathways such as Wingless (Wnt) and Hedgheog (Hh) that participate in normal hematopoietic stem-cell self-renewal, differentiation and proliferation have been implied in the leukemogenic process.
Objective: Our goal was to establish the activation patterns of Wnt and Hh signaling pathways in pediatric B-ALL in order to

identify novel prognostic markers and new targets for therapeutic strategies.
Methods: We studied 9 bone marrow samples from pediatric patients with B-ALL, 8 at diagnosis and 1 at relapse (average age 10,2y, 4F:5M). Seven B-ALL had no recurrent genetic alterations (group 1, n = 6), including the relapse patient (group 2, n = 1), and 2 B-ALL had a t(4;11) (MLL-AF4) (group 3, n = 2). Three bone marrow samples without neoplastic disease were used as controls (average age 9y, 3F). All samples were submitted to a gene expression array that included 84 genes for each signaling pathway, Wnt (RT2 Profiler PCR Array, Qiagen) and Hh (Hedgehog Signaling Pathway, Biorad). Results were analyzed by RT2 Profiler PCR Array Data Analysis v3.5 (Qiagen) and considered statistically significant when $p \leq 0.05$.
Results: In B-ALL patients Wnt signaling pathway genes were tendentiously downregulated. Group 1, comparing with control group, showed downregulation of *WNT* (*WNT1/2B/5B/6/7A/7B/10A/10B/16*) ($p \leq 0.05$) and *FZD* (*FZD4/5/7/9*) ($p \leq 0.05$) family genes, Wnt negative regulators (*DKK3, KREMEN1, SFRP4*) ($p \leq 0.05$) and cell cycle target genes (*CCND1, RHOU*) ($p \leq 0.05$). Group 3, when compared with group 1 revealed upregulation of WNT (*WNT1, WNT10B*) ($p \leq 0.05$), FZD (*FZD3, FZD5, FZD9*) ($p \leq 0.05$), negatives regulators (*WIF1, KREMEN1*) ($p \leq 0.05$) and target genes *WISP1* and FOSL1 ($p \leq 0.05$). B-ALL patients at relapse, when compared with the other groups, showed a tendency for Wnt upregulation, including *LEF-1*. Hh signaling pathway results were more heterogeneous. Group 1, comparing to controls, presented a downregulation of some ligands (*SHH*) and receptors genes (*PTCHD1, BOC, LRP2*) ($p < 0.05$) and upregulation of others (*CDON, PTCHD2, RAB23)* ($p < 0.05$), with downregulation of some important Hh regulators and target genes (*GLI2, ZIC2, FGF9, OTX2, GREM1, SFRP1, VEGFA*) ($p < 0.05$) and upregulation of others (*CSNK1E, BCL2,* $p < 0.05$). Both, group 3 and the B-ALL patient at relapse, showed a tendency for downregulation of Hh ligands, target genes and transcription factors, when compared to group 1.
Conclusion: Our results, although very preliminary, suggest that B-ALL pediatric patients with no recurrent genetic alterations tend to present downregulation of Wnt signaling pathway genes and abnormal expression of Hh signaling pathway. Present results suggest that these pathways may provide novel prognostic markers and therapeutic targets, and are currently being validated in a larger patient cohort.
This work was supported by CIMAGO, GAI 2015, FMUC and Banco Santander Totta; Ana Pires by LPCC/Pfizer 2015.

P3. ANALYSIS OF DNA METHYLATION STATUS OF TUMOR SUPPRESSOR GENES IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Introduction: Hepatocellular carcinoma (HCC) is the second most frequent cause of cancer related deaths, with the heaviest burden on Southeast Asian and African countries, due to high rates of chronic Hepatitis B Virus infection. The incidence of this tumor on Occidental countries is rising, essentially related to chronic liver diseases as the alcoholic cirrhosis and the chronic Hepatitis C Virus infection. Epigenetics refers to heritable and reversible alterations on gene expression by regulatory mechanisms such as CpG island methylation, histone deacetylation and non-coding RNAs interference. Lately, epigenetic modifications have been pointed as being involved in HCC development through tumor suppressor gene silencing, oncogene activation and chromosomal instability.
Methods: The main objective of this work is to investigate the gene promoter methylation status of tumor suppressors genes involved in cell cycle, apoptosis and cell adhesion regulationusing a methylation-specific PCR protocol, in 12 patients with HCC and 5 patients with cholangiocarcinoma, and to correlate the methylation profile with clinical and pathological patient's characteristics.
Results: We observed that the hypermethylation frequency of *RASSF1A* and *p15* were significantly higher in HCCs compared with the corresponding non-neoplasic tissue, but differences in the hypermethylation status of *p21, PTEN, DAPK* and *GSTP1* were not statistically significant between both tissues. In cholangiocarcinoma, the level of methylation of *p15* was significantly higher in tumor tissue than in non-cancerous tissue, but no significant difference in methylation patterns was found between hepatocellular carcinoma and cholangiocarcinoma. No apparent correlation between the methylation level of the gene promoter in the HCC samples and the analyzed clinical parameters, including patient age, TNM stage, tumor differentiation, was observed. The methylation frequency of genes tested is higher in tumor and non-tumor samples from cirrhotic patient than from non-cirrhotic, being significant for *RASSF1A* in the tumor tissue, and for *p15* in non-neoplasic liver but, these significant differences were not observed in the plasma samples. In fact, the inconsistent results of MS-PCR for the paired samples of tissue (both tumoral and non-tumoral) and plasma suggested that plasma DNA could not stand for tissue DNA in our study.
Conclusion: In conclusion, our results suggest that methylation of *RASSF1A* and *p15* genes may have an important role in hepatocarcinogenesis. Aberrant methylation of genes was observed, not only in HCC, but can also be found in non-tumor tissue, mainly in the presence of cirrhosis, and examination of the methylation status in the plasma samples might have limited usage for HCC diagnosis.

P4. ASSOCIATION BETWEEN DIET-RELATED GENES POLYMORPHISMS AND COLORECTAL CANCER RISK

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Introduction: Association between colorectal cancer (CRC) and some diet patterns remain unclear. Some studies have proposed different adaptations to the same type of diet, suggesting that genetic factors could influence the individual answer to different types of diets. Diet and gene polymorphisms interaction may explain some of the inconsistencies related with diet and CRC association. This study aimed to investigate the profile of metabolic genes polymorphisms of lipid, folate and oxidative stress pathways in colorectal adenocarcinoma.
Methods: A total of 119 CRC biopsies and 100 blood samples from healthy subjects, used as control group, were included in this case-control study. APOA1; APOB; APOC3; APOE; CETP; LPL; PON1;

NPY; MTHFR; MTR; MTRR; MnSOD; SOD3; GSTP1; GSTT1 and GSTM1 common polymorphisms were genotyped using commercially available kits.
Results: Folate metabolism: MTHFR 677TT (p = 0.04; OR = 2.27; 95%CI 1.03 to 5.02), MTHFR 1298AC (p < 0.0001; OR = 3.20; 95%CI 1.80 to 5.68), MTR 2756GG (p = 0.0002; OR = 6.38; 95%CI 2.14 to 19.4) and MTRR 66GG (p = 0.046; OR = 1.88; 95%CI 1.01 to 3.50) mutated genotypes were prevalent among CRC subjects. Lipid metabolism: APOA1 -75AA (p = 0.001; OR = 3.51; 95%CI 1.59-7.72); APOC3 3175GG (p = 0.0002; OR = 11.58; 95%CI 2.52-53.22); CETP 279AA (p = 0.003; OR = 13.20; 95%CI 1.61-108.17), CETP 451AA (p < 0.0001) and NPY 7CC (15% vs 0%; p < 0.0001) mutant genotypes were found associated with CRC risk, while, APOE E4/E4 (0% vs 8%; p = 0.02) mutant haplotype seemed to have a protective effect. Regarding oxidative stress pathway genes: MNSOD 175CC (p < 0.0001; OR: 58.5; CI 13.3 to 256.7), SOD3 213GG (p < 0. 0001; OR: 21.89; CI 4.93 to 97.29), GSTP1 105GG (p < 0.0001; OR: 6.14; CI 2.85 to 13.26), GSTP1 114TT (p < 0.0001; OR: Infinity) and GSTT1 null (p < 0.0001; OR: 7.71; CI 3.83 to 15.56) mutated genotypes were correlated with CRC presence.
Conclusion: These findings suggest a positive association between CRC prevalence and common polymorphisms involved on the regulation of oxidative stress, folate and lipid metabolism. Dysregulation of APOA1, APOC3, CETP, NPY, MTHFR, MTR, MTRR, MnSOD, SOD3, GSTP1 and GSTT1 genes could be associated with p53 pathway deregulation, induced by metilation, oxidative stress and inflammatory status on colonic and rectal cells. The present study also provides preliminary evidence that mutations on diet-related genes may be understood as risk factors in this multifactorial disease.
This work was financed by GAI.

P5. DEVELOPMENT OF AN IN-HOUSE DATABASE FOR ARRAY COMPARATIVE GENOMIC HYBRIDIZATION RESULTS

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Introduction: The classification and interpretation of Copy Number Variants (CNVs) is performed by consulting available public databases and resources. In order to help this interpretation is also important to analyze CNVs already classified by the laboratory. We developed an in-house relational Database (DB) to store results and an application which allows to feed and query the DB.
Methods: A set of software requirements and functionalities was defined, using different techniques such as brainstorming, role playing and observation. Use Case Diagrams (UCD) were defined in order to model the dynamic behavior of the system and an Entity-Relationship Model (ERM) and a Relational Model (RM) were developed to model the database. When the software design was finished, a LAMP (Linux, Apache, MySQL and PHP) server was set up.
Results: An in-house database (DB) was modeled, developed and populated with 9910 CNVs records from several Clinic Cases from our laboratory. In order to validate the functionalities of the DB, several queries were made on the results stored in the database which allowed the evaluation of the CNVs distribution for several attributes, for example alteration type, size, classification and chromosome.
Conclusion: The arrayCGH DB proved to be an efficient way for uploading spreadsheets of arrayCGH results performed at our laboratory, and also for visualize CNV matches executed with CNVs from arrayCGH DB and/or UCSC Genome Browser. For reports management, the arrayCGH DB allows laboratory clinical geneticists to keep track and edit their arrayCGH results.

P6. GASTRIC CANCER AS A COMPLICATION OF COMMON VARIABLE IMMUNODEFICIENCY

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Introduction: Common variable immunodeficiency (CVID) is the most frequent symptomatic primary immune deficiency (PID) in adults. It is characterized by marked reduction in the blood levels of IgG and, at least, one of the isotypes IgM or IgA, rendering CVID patients susceptible to recurrent infections. Immunoglobulin replacement therapy greatly diminishes the incidence of infections. However, CVID patients are still at a higher risk of other serious complications, such as chronic lung disease, systemic granulomatous disease, autoimmunity, lymphoid hyperplasia and infiltrative disease, gastrointestinal disease, and malignancies. Cancer incidence is increased 5-fold in CVID patients compared to control populations, in particular gastric cancer (10 to 47-fold).
Methods: Thirty-four CVID patients are currently receiving immunoglobulin replacement therapyin the Service of Immunoallergology of CHUC. We reviewed the clinical evolution of 3 CVID patients who, in addition to the characteristic infectious complications, developed gastric cancer during the last 3 years (2012-2015).
Results: The key clinical data of patients are presented in the table.
Conclusion: Gastric malignancy is an ominous complication of CVID and regular surveillance in this high-risk population is essential to improve survival. Multidisciplinary follow-up is advisable in CVID patients. *Helicobacter pylori* infection is a modifiable risk factor for gastric cancer in the general population. Although antibodies were shown to contribute to the immune response against *Helicobacter pylori*, the significance of this infection for the overall increased risk of gastric malignancies in CVID is still controversial.

P7. LABORATORIAL MISTAKE OR BIOLOGICAL EXPLANATION? THE REASONS BEHIND UNEXPECTED RESULTS IN A LABORATORY DIAGNOSTIC SETTING

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One of the discoveries that marked the course of human cytogenetics resulted from a laboratory error. In 1952, Tsu reported in an addendum at the end of his paper that instead of using an isotonic saline solution to wash the cultures he used an hypotonic solution, resulting in well-spread metaphases. This error allowed Tijo and Levan to report, 4 years later, that humans had 46 instead of 48 chromosomes, a dogma for over 30 years. In a diagnostic laboratory setting, all the samples received are numbered and codified, so that when they are processed we deal with a code and not with patient names. In a cytogenetics and genetics laboratory we perform conventional cytogenetics analysis and genomic analyses, either in prenatal and postnatal samples. We establish the karyotype, we exclude maternal cells contamination in chorionic villus samples, we perform single-gene analyses, like *FMR1* gene associated with Fragile-X, and whole genome analyses like array-CGH. Despite coding the samples, we always know if they belong to males, females or if they are prenatal samples, we can determine the sex. Here we report representative samples whose

Table P6

Patient (initials)	MCJM	MLLMMF	AVR
Sex	Female	Female	Female
Age at diagnosis of CVID	31	41	46
Pretreatment antibody levels, peripheral blood (g/L)	IgG 2.47	IgG 4.97	IgG 1.2
	IgA < 0.06	IgA < 0.07	IgA < 0.06
	IgM 0.11	IgM 0.28	IgM < 0.18
Specific antibodies/Isohemagglutinins	Yes/Yes	Yes/Yes	Yes/Yes
Ig replacement (age at initiation)	IVIG (31)	IVIG (41)	SCIG (46)
Gastritis	Yes	Yes	Yes
Helicobacter pylori/Giardia infection	Yes/Yes	No/Yes	Yes/No
Gastric lymphoid hyperplasia/Intestinal metaplasia	Yes/Yes	No/Yes	Yes/Yes
Gastric cancer – age at diagnosis	39	63	62
Type of gastric cancer	Adenocarcinoma, intestinal type	Adenocarcinoma, intestinal type	Adenocarcinoma, intestinal type
HER2 expression	Negative	Positive	-
Tumour Staging (NCCN guidelines)	T1b/N1/M1	T4a/N3a/M0	T1a/N0/M0
Gastrectomy (age)/chemotherapy	Total (39)/Yes	Subtotal (63)/No	Subtotal (62)/No
Deceased (age)	Yes (40)	Yes (65)	No (62)
Cause of death	Cachexia, diarrhoea, hypokalemia	Surgical complications, abdominal abscesses, septic shock	-

IVIG: Intravenous immunoglobulin; SCIG: Subcutaneous immunoglobulin.

results could easily correspond to human laboratory errors, such as samples mismatch and contamination, but that turned out to be valid and correct results. One example is in *FMR1* gene analyses, when in a male sample we obtained 2 fragments when was only supposed to obtain 1, since males have only one X-chromosome, and we think of sample mismatch with a female, but in reality turned out to be an XXY male. Or when in a female, also for *FMR1* gene analyses, we obtained 3 fragments, posing the question of contamination with a second sample, when in reality was a triple X female. In prenatal samples, when prenatal diagnosis is performed in chorionic villus samples, maternal cell contamination has to be excluded, by comparing the genetic profiles of both fetus and mother, to assure we are analyzing only fetal material. In one of these cases, we had a fetal sample that did not correspond to the theoretical mother. We requested a second sample of the mother, again to exclude any sample mismatch, and the result was the same, only some markers, not the totality, were coincident between both samples. When we questioned the clinics, this woman had performed tubal ligation, and the hypothesis of being pregnant by oocyte donation was put into the table. When confronted with such results, that seem to be discrepant, we have to think of a biological mechanism that might explain it, but, as humans, and despite all the quality control and certainty in sample manipulation, we are confronted with the possibility of having committed a mistake. These situations are stressful, because they question the technician's performance and attention in the manipulation of the samples, and cause great anxiety while further tests are performed to explain and clarify the results obtained. Despite all the stress and anxiety, these situations create a constructive discussion among the work team, to try to explain the results and serve as lessons that teach us and give us experience, knowledge and maturity to deal with future cases.

P8. NON-RANDOM GENOMIC BREAKPOINTS IN ORAL CANCER: WHICH CHARACTERISTIC SEQUENCE MOTIFS ARE BEHIND THE BREAKS?

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Introduction: Oral Squamous Cell Carcinoma (OSCC) results from accumulation of several and complex chromosomal rearrangements. The precise assessment of these rearrangements that directs the formation of breakpoints will reveal which chromosomes breaks and reunites within genome, highlighting the genomic sequence lying in the vicinity of breakpoints (Daga et al. Open Med Inform J. 2015;9:1-8). This knowledge is crucial to shed light into the mechanism of OSCC carcinogenesis as well as the role of the genomic architecture. We aimed to investigate if there are some features of DNA sequence that may be involved in the breakpoints process and consequently if some sequence motifs may be directly involved in determining the location of theses breakpoints.
Methods: Genomic imbalances were analyzed through Agilent oligonucleotide microarray 4x180K in 100 biopsies of OSCC. The genomic sequences that are present in or surrounding the breakpoints of the imbalances identified were retrieved from UCSC genome browser.
Results: Our genomic studies in OSCC samples have revealed a complex pattern of non-random chromosome abnormalities. We

observed breaks in almost all chromosomes. The chromosomes 3, 6, 8, 11, 12, 14 and X presented breaks in a higher number of patients and the chromosomes 13, 21 and long arm of chromosome 2 and 19 did not present frequent breaks in our cohort. Regarding the chromosomal distribution, we observe several peri-centomeric and telomeric breakpoints in our samples. The great majority of the breakpoints identified in our cohort occurred in or around repeat sequences, such as LINES, SINES and LTR.
Conclusion: Characteristic sequence motifs at the breakpoints regions identified in this study support the involvement of the repetitive regions in the genomic imbalances of the OSCC. We highlight several chromosomal sites where are located specific repetitive sequences as predisposed to OSCC rearrangements.

P9. TESTICULAR GERM CELL TUMOUR – A PARADIGM IN THE SUCCESS OF ONCOLOGICAL TREATMENT

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Introduction: Testicular tumours constitute 5% of urological tumours. They are pathologically classified in germ cell tumours (GCT) – subdivided in seminomatous (SGCT) and nonseminomatous (NSGCT), sex cord stromal tumours and nonspecific miscellaneous stromal tumours. According to the TNM classification of 2009, GCT can be categorized in stages 0, I, II and III, with impact on the prognosis. Cure is expectable in most cases, even in metastasized patients.
Objective: To describe and classify testicular tumours; evaluate the impact of pathology data and initial staging of the disease on prognosis and overall survival of patients with GCT.
Methods: Retrospective analysis of the clinical files of all patients with testicular tumours treated in a tertiary hospital centre between 01-Jan-2009 and 30-Jun-2015. Description of the characteristics of the patients, tumours, tumour marker kinetics and treatments undertaken (primary and secondary). Analysis of pathological risk factors (lymphovascular, rete testis or albugineal invasion, presence of necrosis or intraepithelial neoplasia, predominant pathological type and tumour size) and their relationship with initial staging. Comparison of the results with a similar series from 1989 to 2003. Statistical analysis with SPSS®21: residual plot, chi-square and Kaplan Meier survival curves.
Results: 100 patients with testicular tumours were reviewed, with a mean follow-up of 35.3 ± 24.6 months. 84 were GCT (34 SGCT and 50 NSGCT), while the rest were mainly lymphomas (n = 13). Among SGCT, 29 were classified as stage I (85.3%), 3 as stage II (8.8%) and 2 as stage III (5.9%). Only 1 patient with stage III did not exhibit regression and died (mortality of 3.4%). All others are alive and recurrence-free. Regarding NSGCT, 27 (54.0%) were classified as stage I, 5 (10.0%) as stage II and 18 (36.0%) as stage III. There were no cases of recurrence, non-regression or death in stages I and II. There was no regression in 33.3% of stage III NSGCT, and there was recurrence of disease in 1 case (5.6%); these cases culminated in death (mortality of 38.9%). Of the pathological factors analysed, the presence of testicular necrosis is more commonly associated with advanced stages of disease (p = 0.03). No other pathological factors (including pT of the TNM classification) were related with initial staging of GCT. There were no differences in recurrence (p = 0.48) or mortality (p = 0.07) between SGCT and NSGCT. The global recurrence and mortality rates of GCT were 1.2% and 9.8%, respectively. Regarding the last study of GCT in this department (1989-2003) it was found that currently, more patients are diagnosed in stage I (46.3% vs 56.0%) and that mortality is far lower (19.5% vs 9.8%).
Conclusion: Most patients with GCT are diagnosed in stage I. The presence of necrosis in the pathological exam is more common in GCT stages II-III. The prognosis of NSGCT is worse than SGCT, although without statistical significance in this series. Globally, the prognosis of GCT is favourable.

Session 2

P1. ASCORBIC ACID AND CONVENTIONAL CHEMOTHERAPEUTIC AGENTS SYNERGISTICALLY INHIBIT COLORECTAL CANCER CELLS PROLIFERATION AND TUMOR GROWTH

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Introduction: Colorectal cancer (CRC) is one of the most dangerous forms of cancer. Despite toxicity caused by conventional chemotherapy, namely 5-flourouracil (5-FU), oxaliplatin (Oxa) and irinotecan (Iri), these are currently used as first and second-line treatment for CRC. Several studies revealed that ascorbic acid (AA) at pharmacological concentrations can act as a pro-oxidant, promoting the formation of reactive oxygen species, such as hydrogen peroxide, which compromise cell viability. A positive feedback has been described in turn of the use of high doses of AA with reduced doses of chemotherapy. So, the aim of this study is to evaluate *in vitro* and *in vivo* the therapeutic potential of the combination of AA and conventional chemotherapeutic agents in CRC.
Methods: C2BBe1, LS1034 and WiDr cells were incubated with increasing concentrations of AA and 5-FU, Oxa or Iri, in monotherapy and in combination. Cell proliferation was evaluated through SRB assay after 24, 48, 72 and 96 hours of exposure. The half maximal inhibitory concentrations (IC_{50}) and the combination index were determined. Flow cytometry allowed to evaluate the influence of the treatment on cell viability and the induced types of cell death. For *in vivo* studies, WiDr cells were inoculated on the back of Balb/c nu/nu mice. AA and the three chemotherapeutic agents were intraperitoneal injected separately or in combination. During 14 days, body weight and tumor size were monitored.
Results: In all cell lines, it was observed that when AA concentration increases, cell proliferation decreases, being C2BBe1 cells the most sensitive to AA. In general, IC_{50} values of all chemotherapeutic agents significantly decreased when present in combination with AA, compared to monotherapy. However, the most promising results were obtained with AA and Oxa combination, being obtained a synergistic effect for all cell lines at 48h and 96h. Combined therapies also caused a decrease on cell viability and, consequently, cell death by apoptosis/necrosis increased. Moreover, the combination of AA and oxaliplatin or AA and irinotecan synergistically inhibited tumor growth.
Conclusion: Our study suggests that high doses of AA enhance chemosensitivity of CRC, even in the multidrug resistant cell line, LS1034. A synergistic effect between AA and conventional chemotherapeutic agents was also observed in a mice model of CRC. The data obtained could contribute to the development of a promising therapy for CRC with reduced doses of conventional chemotherapeutic drugs and consequently, a decrease in secondary effects.
The authors would like to thank Foundation for Science and Technology (FCT) (Strategic Project CNC.IBILI: UID/NEU/04539/2013), COMPETE-FEDER for financial support.

P2. COPY NUMBER ALTERATION ANALYSIS OF SNP ARRAY DATA IN HNSCC PATIENTS FROM TCGA DATABASE

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is a cancer that appears in regions of the upper aerodigestive tract and arises from cumulative genomic aberrations. These alterations normally occur prior to any phenotypic expression and pass unnoticed in most existing diagnosis tests. The early-on diagnosis, prediction of disease evolution and treatment outcome depend on the genomic profiling of the tumors of HNSCC patients and detection of the most prevalent altered regions. With this work we aimed to assess the most altered chromosomic regions in patients of HNSCC in order to identify biomarkers with diagnostic and prognostic value.
Methods: Single Nucleotide Polymorphisms (SNP) Array data from tumoral tissue of 526 patients with HNSCC was downloaded from The Cancer Genome Atlas (TCGA) database and analyzed using MATLAB.
Results: Using data analysis methods we detected genomic imbalances in all chromosomes and we noticed that the size of these alterations is quite variable amongst patients. Chromosomes 3, 5, 8, 9 and 11 were the ones that registered copy number alterations in a higher number of patients. In particular, the most frequently amplified regions were located at 8q24.21, 3q22.23, 5p15.33 and 11q13.3 and the most deleted regions were located at 3p21.2, 9p21.3, 8p22.3 and 11q23.2.
Conclusion: With this approach we were able to identify the most altered chromosomic regions in a broad heterogeneous population of HNSCC patients. Using these results we can then assess the genes encoded in these regions, and establish a correlation between genomic and clinical data, which has the potential to identify biomarkers for diagnostic and treatment outcome.

P3. LOCAL ANESTHETICS INDUCE CYTOTOSTATIC AND CYTOTOXIC EFFECTS AND INHIBIT CELL MIGRATION IN ORAL CANCER CELLS

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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. Besides the new and advanced therapeutic strategies, patients with OSCC show poor survival rates. Local anesthetics (LA) 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 LA, lidocaine and mepivacaine, 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 or mepivacaine 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-Giemsa 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. Cell migration was evaluated by the wound healing assay. Results were statistically analyzed.
Results: Our results showed that LA in monotherapy and in combination with conventional chemotherapy inhibited cell proliferation and migration, and induced cell death mainly by later apoptosis/necrosis in both cell lines in a dose, time, administration schedule and cell type dependent manner, being the HSC-3 the most sensitive relatively to BICR-10. The IC_{50} in HSC-3 cells was reached with 4.5 mM of LA while in BICR-10 cells the IC_{50} was reached with 6 mM of lidocaine and 9 mM of mepivaine. These results may be related with the increased in caspases and superoxide anion levels and decreased mitochondrial membrane potential. The high sensitivity of HSC-3 cells to LA may be related with the lowest GSH levels when compared to BICR-10 cells. Furthermore, the pre-G0/G1 peak observed in cell cycle analysis confirms apoptosis. LA also inhibits cell migration as we can observe a gap in wound healing assay after treatment comparing with the non-treated condition after 24h of scratch.
Conclusion: Our *in vitro* results showed that lidocaine and mepivacaine alone or in combination with conventional treatment may constitute a new complementary therapeutic approach in OSCC, namely in metastatic cancer.
This work is supported by CIMAGO, Gabinete de Apoio à Investigação (GAI)/Faculty of Medicine of University of Coimbra and Santander-Totta Bank.

P4. THE MTOR INHIBITOR EVEROLIMUS SENSITIZE ORAL CANCER CELLS TO CHEMOTHERAPEUTIC DRUGS INHIBITING CELL PROLIFERATION, MIGRATION AND INVASION

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Introduction: Oral squamous cell carcinoma (OSCC) represents the most common malignancy of the head and neck region, and over the last decade its incidence significantly increased. The limited

improvement in outcomes achieved by conventional therapies urges us to identify innovative molecular target strategies to treat OSCC. The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/ mammalian target of rapamycin (mTOR) signaling pathway plays a central role in the regulation of cell proliferation, migration, survival and angiogenesis. The aberrant activation of mTOR has been recently related with poor prognosis in various cancers including OSCC, becoming a promising molecular oriented anti-cancer target therapy not only for the cancer cell but also for the tumor microenvironment. In the present study, we evaluated the therapeutic efficacy of everolimus in monotherapy and in combination with cisplatin or 5-fluorouracil in two OSCC cell lines 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 everolimus in monotherapy or plus conventional chemotherapeutic drugs (cisplatin or 5-fluorouracil). Cell viability was assessed by the rezasurin assay and cell death by Optical Microscopy (May-Grünwald-Giemsa staining) and flow cytometry using the Annexin V/Propidium Iodide double staining. Cell cycle was assessed by flow cytometry using PIRNase kit. Cell migration was evaluated by the wound healing assay. Enzymatic activity of metalloproteinase 2 and 9 was assessed by gelatin zymography. Western Blot technic was used to evaluate the expression of total and phosphorylated AKT and P70S6K as well as the total expression of integrin beta 1 and beta catenin. Results were statistical analyzed.
Results: Everolimus alone and plus cisplatin or 5-fluorouracil significantly inhibited proliferation, migration and invasion, and induced apoptosis of the two cell lines in study, with more pronounced results in HSC-3 cells. In fact, after everolimus treatment (25 μM) alone and in combined regimen we observed a decrease in phosphorylated AKT and P70S6K expression as well as a decrease in total integrin beta 1 expression and in the enzymatic activity of MMP-2 and MMP-9. Wound healing assay showed a reduction on cell migration after treatment.
Conclusion: Taken together our *in vitro* results suggest that everolimus constitute a promising anti-tumor targeted therapy in OSCC treatment, namely in metastatic cancer, as alone as in combination with conventional chemotherapy, with highlight interest in the inhibition of cell migration, which is a major problem in this pathology.
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P5. IATROGENIC KAPOSI´S SARCOMA: CASE REPORT

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Introduction: Kaposi´s Sarcoma (KS) is a tumor caused by infection with human herpes virus 8 (HHV8). Four subtypes have been described: epidemic (AIDS related), endemic (previously typical African), classic (elderly men of Eastern European or Mediterranean ancestry) or iatrogenic (immunosuppressed patients). It is typically a systemic disease that can present with purplish lesions of the skin or mucosal surfaces with several morphologies: macular, patchy, plaque like, nodular and exophytic. At presentation, skin lesions can be either single or multiple and may cause pain, bleeding, or disfigurement. As involvement of lymph nodes and lymphatic spaces occurs, progressive edema can result. Visceral KS typically involves the aerodigestive tracts. Oropharyngeal and pulmonary lesions can result in life-threatening airway obstruction or respiratory failure. Diagnostic workup includes inspection of skin and mucosal surfaces, complete physical examination, blood count and, in case of HIV infection, CD4 lymphocyte count and viral load, chest X-ray, tuberculin test, anergy screen, and screen for sexually transmitted diseases. Definite diagnosis can be made only by biopsy and microscopic examination, which requires the identification of both spindle cells and vascular elements within the lesion. Treatment includes highly active antiretroviral therapy (HAART) in AIDS-related KS, systemic chemotherapy for patients with advanced disease and radiotherapy or cryosurgery for localized lesions. Radiation therapy can also be useful for palliation of pain, bleeding or edema. Typically, small fields that include only the distressing lesion and a small margin are treated, either with superficial quality x-rays or low-energy electrons (and bolus).
Methods: The authors present a case report of a 73 year old female gender patient with clinical history of rheumatoid arthritis undergoing corticotherapy and methotrexate for 25 years. She presents with moderate pain associated with purplish plaques and papules through the inferior third of the leg and feet with interdigital pappilary areas, both bilateral and symmetric. She also presents with lymphedema of both lower legs. She has negative serology for HIV 1 and 2 and biopsy of the described lesions confirmed the diagnostic of KS. The patient underwent radiotherapy for major feet and interdigital lesions. A radiation dose of 30 Gy in 10 fractions was delivered, with 4MV photons. Clinical evolution was assessed at the beginning, during and three months after the radiotherapy.
Results: A clinical regression of the tumor was observed with substantial pain and edema reduction. There were no treatment toxicities reported by the patient.
Conclusion: Radiotherapy is a common approach for localized KS, with approximately 70% of complete responses. One of the more commonly used dose-fractionation schemes is 30 Gy in 10 fractions delivered over 2 weeks, which has resulted in substantial benefit without significant toxicity for the typical patient.

P6. DOUBLE BALLOON ENTEROSCOPY IN SMALL BOWEL TUMORS: 11 YEARS' EXPERIENCE AT A TERTIARY-CARE HOSPITAL

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Introduction: The small bowel tumors prevalence is 3-6%, being 5.5% in Portugal. In our center, the prevalence of malignant small bowel tumors was 9.4% between 2005 and 2008. Double-balloon enteroscopy (DBE) is the gold standard in a suspicion of small bowel tumors.
Objective: To characterize the small bowel tumors detected by DBE and the diagnostic value of DBE in small bowel tumors.
Methods: Retrospective study of DBE performed between January 2005 and November 2015. Included all patients with a suspected small bowel pathology and negative previous conventional study. Collected clinical, endoscopic, imagiological and surgical data of diagnosed tumors. Evaluation of DBE diagnosis value comparing with Capsule endoscopy and CT enterography.
Results: Performed 267 DBE in 213 patients, 55.8% males and mean age of 60.4 ± 17.8yo. The main indication for DBE was obscure gastrointestinal bleeding in 62.5%, being occult in 71.9% of cases. Performed 187 (70.0%) exams by anterograde route, 73 (27.3%) by retrograde route and 7 (2.6%) by both routes. Lesions were detected by DBE in 66.3% (177/267) of cases, mainly angioectasias (67/177; 37.8%). Thirty two small bowel tumors in 30 patients were diagnosed. The diagnosis by DBE was 87.5%(28/32). Biopsies were obtained in 59.4% of tumors with 3 of them negative (15.8%). Most tumors were subepithelial lesions (17/32; 53.1%), malignant lesions

(19/32; 59.4%) and localized in jejunum (21/32; 65.6%). The main diagnosis of malignant tumors was gastrointestinal stromal tumors in 21.9% (7/32), adenocarcinoma in 15.6% (5/32), lymphoma in 5.8% (3/32) and metastatic tumors in 5.8% (3/32). According to the final diagnosis, DBE showed high diagnostic accuracy (93.4%; $p < 0.001$) comparing with Capsule endoscopy (86.1%; $p < 0.001$) and CT enterography (72.9%; $p = 0.032$).
Conclusion: Small bowel tumors diagnosis was 12.0% (32/267) by DBE and malignant in 7.1% (19/267) of exams. DBE is a useful tool with high diagnostic accuracy in the study of small bowel tumors.

P7. GASTRIC HELICOBACTER PYLORI INFECTION AND COLORECTAL CANCER: IS THERE A LINK?

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Introduction: Helicobacter pylori (Hp) is the only bacterium with a potential carcinogen in humans, already established for gastric cancer. However, systemic effects have been proposed, including colorectal carcinogenesis. The involved mechanisms remain unknown, being gastrin a candidate trophic factor.
Objective: To determine the Hp role in the development of adenomatous polyps and colorectal cancer (CRC).
Methods: Retrospective case-control study of a total of 1,271 routine upper GI endoscopies, performed during 6 months. Selected 306 patients with additional evaluation of gastric Hp infection and 116 with at least one total colonoscopy. There were evaluated demographic variables, classic risk factors of CRC and conditions associated with hypergastrinemia (CAH).
Results: Hp gastric infection occurred in 37.9% (n = 44), being moderate to severe in 61.4% (n = 27). The development of adenomatous polyps/CRC occurred in 25.9% (n = 30), being more frequent in Hp-positive patients (45.5% vs 13.9%; $p < 0.001$), with a 5-fold increased risk (OR 5.167; $p < 0.001$). The moderate to severe Hp infection was associated with an 11-fold increased risk (OR 11.188; $p < 0.001$). The CAH were present in 62.1% (n = 72). The development of adenomatous polyps/CRC was more frequent in CAH (22.4% vs 3.4%; $p = 0.001$), showing a 5-fold higher risk (OR 5.652; $p = 0.001$). After multivariate analysis, the only significant risk factors for the development of adenomatous polyps/CRC were moderate to severe Hp infection (OR 17.618; $p = 0.024$) and long-term proton pump inhibitors (PPI) use (OR 31.289; $p = 0.010$). There was no association with the classic risk factors of CRC.
Conclusion: Hp gastric infection was associated with a higher risk of adenomatous polyps/CRC development. Hp eradication (especially in moderate to severe infection) and the rational use of PPI may reduce colorectal cancer risk. In patients with indication for long-term PPI use, a more aggressive colorectal cancer surveillance could be considered. Further studies are needed to evaluate the real impact of Hp/gastrin in colorectal carcinogenesis.

P8. NEW CONTRIBUTIONS TO THE HNSCC CARCINOGENESIS MODEL: PIECING TOGETHER GENOMICS AND EPIGENETICS DATA

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Introduction: Nowadays, Head and Neck Squamous Cell Carcinoma (HNSCC) is considered the sixth most common malignant tumour worldwide. The 5-year survival rate for the patients diagnosed with this type of cancer is one of the lowest and, regardless technological and clinical advances, it does not have improved much in the last years. Late diagnosis, high rates of regional metastasis and locoregional recurrence are among the reasons behind this huge problem. Histological evolution of HNSCC is well established, starting from benign epithelium, through hyperplastic and dysplastic alterations, until *in situ* carcinoma and metastasis development. In spite the fact that several progression models have been proposed, the genetic and epigenetic aberrations underlying each stage are not fully understood. Besides, HNSCC can progress quickly, hampering the detection of the earlier alterations. Moreover, the only specific prognostic factors that are routinely taken in account are the presence of nodal and distant metastasis and the stage and site of the primary tumour, disregarding the molecular heterogeneity characteristics of this type of cancer, which often leads to distinct biological behaviours. Therefore, it is crucial to identify the sequential alterations that happen in the carcinogenesis process of this type of cancer. Our aim was to identify new alterations that could be associated to different stages of the HNSCC carcinogenesis.
Methods: Two commercial HNSCC cell lines were used: a nodal metastatic cell line and a dysplastic cell line. Both cell lines were cultured in DMEM supplemented with 10% FBS and 1% of penicillin and streptomycin. For the dysplastic cell line, 1% of hydrocortisone was also added. The genomic analysis was performed by array Comparative Genomic Hybridization (aCGH), whereas the methylation profile was assessed through Methylation Specific-Multiplex ligation-dependent probe amplification (MS-MLPA).
Results: In the dysplastic cell line we could observe 3p loss; 7p gain; 9p21-p22 loss; 11q distal loss without 11q13 amplification, 13q losses and 17p deletion. These alterations have already been associated with the earlier stages of HNSCC carcinogenesis. The metastatic cell line showed 1q gains; 3q gains; 5p gain; 5q11 loss; 7q11.2 gain and 8q24-qter gain. Additionally, 12p and 12q gains were observed in the metastatic cell line, being these aberrations already associated to invasiveness in germ cell tumours. Furthermore, 20q gain, which is associated to metastasis in several cancers, is also observed in the metastatic cell line. MS-MLPA revealed that co-methylation of *TP73* and *ESR1* could represent a biomarker for the development of metastasis, especially when the lymph nodes are involved, and that methylation of *CHFR* and *CDH13* could be representative of tumours in earlier stages of development.
Conclusion: This study emphasise some genomic and epigenetic aberrations associated to the early stages of HNSCC carcinogenesis and to the involvement of nodal metastasis. These markers could be helpful for the early detection of potential malignant lesions, for prognostic and for therapy targets development and should be tested in HNSCC biopsies.

Session 3

P1. RADIUM-223 IN THE TREATMENT OF METASTATIC PROSTATE CANCER

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Introduction: Metastatic castration-resistant prostate cancer (mCRPC) is in therapeutic terms the biggest challenge of prostate

cancer (PCa). The radiopharmaceutical Radium-223 (^{223}Ra) acts as a calcium mimetic by forming complexes with the bone mineral hydroxyapatite in areas of high bone turnover, thereby directly targeting the areas of bone metastases. It is considered the best treatment for patients with mCRPC. The radiopharmaceutical ^{99m}Tc- HMDP is also an analog of calcium used in bone scintigraphy. Given the relative lack of information of the molecular pathways responsible for the effects of ^{223}Ra in cells of mCRPC, this project aims to study the effects of ^{223}Ra in cell lines of PCa, using as controls one osteosarcoma cell line and the uptake profile of ^{99m}Tc-HMDP.

Methods: Three tumor cell lines PC3 (metastatic PCa), LNCaP (no metastatic PCa) and MNNG-HOS (osteosarcoma) were incubated with the ^{223}Ra radiopharmaceutical (0.5 µCi/mL) or ^{99m}Tc-HMDP (25 µCi/mL). For uptake studies samples of 200 µL were taken at 5, 30, 60, 90, 120, 150 and 180 minutes. These were centrifuged to separate the supernatant and the pellet. The uptake percentage of ^{223}Ra and ^{99m}Tc-HMDP was determined after measuring the radioactivity of both fractions in a well counter, in counts per minute.

Results: Preliminary results show that, at 120 minutes, the percentage of ^{223}Ra uptake by PC3 is twice higher (1.6 ± 0.1%) than by MNNG-HOS (0.8 ± 0.13%). A similar uptake profile was obtained for LNCaP cells (1.26 ± 0.16%). The uptake of ^{223}Ra by MNNG-HOS is five times higher (1.0 ± 0.1%) than the uptake of ^{99m}Tc-HMDP (0.2 ± 0.09%). In PC3 cells line the same is verified. By the other side, in LNCaP cell line the uptake profile is similar between the two radiopharmaceuticals.

Conclusion: Preliminary results suggest that, although both radiopharmaceuticals are analogues of calcium, the uptake mechanisms may be different. This study also enhances the therapeutic potential of ^{223}Ra in the treatment of metastatic PCa.

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P2. A MULTIRESIDUE APPROACH FOR THE SIMULTANEOUS QUANTIFICATION OF 38 ANTIBIOTICS IN MACROALGAE BY ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Introduction: Together with fish, algae reared in aquaculture systems have gained importance in the last years, for many purposes. Besides their use as biofilters of effluents, macroalgae's rich nutritional profiles have increased their inclusion in human diets but also in animal feeds as sources of fatty acids, especially important for the fish industry. Nonetheless, algae are continuously exposed to environmental contaminants including antibiotics and possess the ability for bioaccumulation of such compounds. Thus, to prevent unintentional contamination of feeds and subsequently fish and other organisms, algae should be analyzed to check for antibiotics before incorporation. Therefore, the present work describes the development and validation of a sensitive screening and confirmatory multi-residue analytical method to be applied in routine analyses on macroalgae samples (*Ulva lactuca*). The method developed allows the simultaneous detection of an extended list of 38 antibiotics from tetracyclines, macrolides, sulfonamides, benzenoids, quinolones and penicillins. The compounds selected cover the most prescribed compounds in veterinary medicine but also banned substances such as chloramphenicol.

Methods: Green macroalgae (*Ulva lactuca*) samples were collected at a local beach during low tide. To optimize the method four different extracting solvents were tested and the quantification of the 38 antibiotics was performed by UOPLC-MS/MS using internal standards for each class of antibiotics.

Results and conclusion: Until now, few methods were available for the quantification of antibiotics in macroalgae and none with the multi-residue and multi-class approach described in the present work. The method presented was fully validated for the simultaneous quantification of 38 antibiotics used in veterinary medicine and covering the classes most used (tetracyclines, penicillins, sulfonamides, quinolones, macrolides, benzenoids and chloramphenicol). This fact allied to the absence of solid phase extraction, low run chromatographic time and sample volume and combined with LOQ as low as 0.02 µg kg^{-1} is translated in low costs and a fast method. Its application in routine analyses of algae reared in aquaculture systems is therefore recommended for the majority of the compounds investigated here to assure animal and human welfare.

P3. TGF-β CANONICAL SIGNALLING PATHWAY IN ORTHODONTIC TOOTH MOVEMENT

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Introduction: Following application of orthodontic forces, the cellular responses comprise the synthesis of cytokines, prostaglandins and growth factors that play a crucial role in the biochemical processes underlying tooth movement. Knowledge on the role of TGF-β signaling pathway on orthodontic tooth movement is scarce, being still unknown whether this multifunctional cytokine canonical pathway mediated by Smads is involved in the changes observed in the periodontal tissues remodelling. Therefore, the propose of the present study was to evaluate the expression of TGF-β in the periodontal tissues under the stress of orthodontic forces, as well as the expression of the R-Smads, aiming to assess the role of the canonical TGF-β pathway on the remodeling, adaptation and repair processes occurring along the orthodontic movement.

Methods: 70 male albino *Wistar* rats were divided in 5 groups enclosing 15 animals each except the control group with 10 rats. Excluding the control, all the animals were submitted to an orthodontic force according to the method of Waldo and Rothblatt for 24, 48, 72 and 96 hours. Periodontal tissues histological analysis was performed and TGF-β, Smad2 and Smad3 expression assessed immunohistochemically (IHC).

Results: Histological analysis revealed morphologic changes, e.g., mesenchymal cell proliferation (hipercellularity) and angiogenesis in the periodontal ligament within the first 24 hours following the application of orthodontic forces. Even though, at 72 hours morphologic changes could still be observed, at 96 hours most of the periodontal ligament repair and remodelling process was over. Immunohistochemical analysis revealed that neither TGF-β nor the R-Smads, Smad2 and Smad3, were expressed in the control group. As to the treatment groups, TGF-β expression could be observed in the compression side being particularly intense in osteoblasts and osteoblast-like cells at the alveolar surface at 72 and 96 hours. As to Smad2, its expression could be observed in all the periodontal tissue along the treatment at both the compression and tension sides but, was particularly

intense in osteoblasts at 72 and 96 hours. Conversely, Smad3 expression was absent.
Conclusion: TGF-β expression in the compression side of treated animals showed that its signaling pathway is activated where osteogenesis were occurring. The absence of coincidental expression of TGF-β, Smad2 and Smad3 suggests that TGF-β did not signal through the canonical signaling pathway and that both R-Smads act independently of each another. Smad3 expression absence also suggests that periodontal tissue repair did not involve pathologic fibrotic tissue production. Increased expression of TGF-β at the peak of the osteogenesis process indicates that the growth factor may be used as a biological marker of osteogenesis of the pressure side during the orthodontic tooth movement.

P4. BUPARLISIB, A NEW THERAPEUTIC APPROACH IN MYELOID MALIGNANCIES

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Introduction: The Phosphoinositide 3-kinase (PI3K), is an intermediate signaling molecule that is involved in the activation of multiple effector pathways such as PI3K/AKT/mTOR. PI3K plays a very important role in key cellular processes like cell growth, survival, proliferation, metabolism, motility and DNA transcription. This pathway is deregulated in several types of malignancies including hematological malignancies. Additionally, deregulation of this pathway is associated with the resistance to conventional anti-neoplasic therapies.
Objective: To evaluate the effect of Buparlisib (BKM120) a pan-Class I PI3K inhibitor on in vitro models of myeloid malignancies.
Methods: For this purpose, we used three in vitro models of myeloid malignancies, F-36P cells (Erythroleukemia secondary to Myelodysplastic Syndrome), HEL cells (Erythroleukemia) and NB-4 cells (Acute Promyelocytic Leukemia). Cell lines were cultured in absence and presence of different concentrations of BKM120 administered in daily and single dose schemes. Cell viability was determined at 24, 48 and 72 hours using the Resazurin Assay. Cell death was analyzed by optical microscopy (May-Grunwald Giemsa staining), and by Flow Cytometry (FC) using the *Annexin V* and *Propidium Iodide* double staining. FC was also used to analyze the cell cycle (*Propidium Iodide/RNAse protocol*) and to access caspases activity (*ApoStat* probe). We also used *Western Blot* to quantify the expression and phosphorylation of proteins involved in the PI3K/AKT pathway.
Results: BKM120 induces a decrease in cell proliferation in a dose, time and cell type dependent manner. In fact, the HEL cells are the most sensitive and the NB-4 cells the lowest (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 of 2,500 nM in the NB-4 cell line). Moreover, the daily administration scheme of a small dose of BKM120 reveals a positive effect when compared to the administration of the same dose in the single dose administration scheme, being this effect more pronounced in the HEL cell line. This compound induces cell death predominantly by apoptosis, confirmed by morphological analysis, FC and by the increase of cells expressing *Caspases* and also its absolute expression levels. The cell cycle analysis showed that BKM120 induces cell cycle arrests in the tested cell lines.
Conclusion: Our results show that the PI3K inhibition by BKM120 has the ability to induce anti-proliferative and cytotoxic effects in acute myeloid leukemia cell lines, suggesting that PI3K could be a promising therapeutic target for novel anti-cancer therapeutics in patients with acute myeloid leukemias.
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P5. THE EMBRIONARY SIGNALING PATHWAYS AS THERAPEUTIC TARGETS IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: Embrionary signaling pathways, such as Wingless (WNT) and Hedgehog (Hh) pathways, are essential for differentiation and self-regulation of stem cells, and their deregulation have been associated to several hematological neoplasias. Acute lymphoblastic leukemia (ALL) is characterized by the abnormal proliferation and accumulation of immature lymphoid cells within the bone marrow and lymphoid tissues, which can arise from the aberrant activation of embryonic signaling pathways. Therefore, these pathways may constitute a new therapeutic target for ALL treatment.
Objective: To evaluate the therapeutic potential of IWR-1 and GDC-0449, inhibitors of WNT and Hh signaling pathways, respectively, in ALL *in vitro* models.
Methods: For this purpose, we used two ALL cell lines, CEM as a model for T cell ALL, and KOPN-8 as a B cell ALL model. Both cell lines were cultured in absence and presence of different concentrations of IWR-1 and GDC-0449. Trypan blue assay was used to evaluate the effect of these inhibitors on cell viability and cell density. Cell death was determined by optical microscopy (May-Grunwald Giemsa staining) and by flow cytometry (FC) using the Annexin V and Propidium Iodide double staining. FC was also used to measuring levels of apoptosis protein modulators, BAX and BCL-2, cell cycle (PI/RNase assay) and mitochondrial membrane potential (through the fluorescent probe JC1).
Results: Our results showed that IWR-1 reduces only the viability and proliferation of KOPN-8 cells in a time and dose dependent manner (being the maximal inhibitory concentration, IC50, at 48 hours of exposure was, approximately 50 μM), having no effect in CEM cells. On the other hand, GDC-0449 reduces cell viability and proliferation in a time, dose and cell line dependent manner, being the KOPN-8 cells the most sensitive. We found that the IC50 of GDC-0449 at 48 hours of exposure was, approximately of 75 μM and above 200 μM for KOPN-8 and CEM cells, respectively. Both compounds, IWR-1 and GDC-0449, induced cell death by apoptosis, confirmed by the BAX/BCL-2 ratio and mitochondrial membrane depolarization. The analysis of cell cycle progression revealed that GDC induces cell cycle arrest in G_2/M phase in KOPN-8 cells.
Conclusion: Our results suggest that IWR-1 and GDC-0449 could be a potential new targeted therapy in acute lymphoblastic leukemia. However, therapeutic efficacy may be cell type-dependent.
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P6. MERKEL CELL CARCINOMA – ELDERLY PATIENT: CASE REPORT

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Introduction: Merkel cell carcinoma (MCC) is an uncommon and aggressive primary cutaneous neuroendocrine (small-cell) carcinoma originating from the basal epidermis and was first reported by Toker in 1972. MCC incidence increases progressively with age. There are few cases in patients younger than 50 years, and the median age at diagnosis is about 65 years. Risk factors include male sex, increased age, fair skin, previous malignancies, UV light exposure, and immunosuppression (specifically, HIV or organ transplantation). There is a tendency for early and frequent loco regional metastases and recurrence, and the majority of patients die from distant metastases. Non-sun-exposed MCC variants have been described, and they tend to be associated with even worse survival. MCC usually presents as a painless, indurated, solitary dermal nodule with a slightly erythematous to deeply violaceous color, and rarely, an ulcer. MCC can infiltrate locally via dermal lymphatics, resulting in multiple satellite lesions. Because of its nonspecific clinical appearance, MCC is rarely suspected prior to biopsy. The acronym AEIOU is used to summarize the classic clinical characteristics of MCC (Asymptomatic, Expanding rapidly, Immunosuppression, Older than 50 years of age, UV exposure on fair skin). The clinical presentation is frequently mistaken for basal cell carcinoma, amelanotic melanoma, or cutaneous malignancies.
Methods: The authors describe a clinical case of a 93 years old white male that presented with a Merkel cell carcinoma on the right malar region of the face. The lesion was exophytic, ulcerated, friable and with rough dimensions of 8 × 7,5 × 6 cm. A parotid adenopathy was also present. The patient underwent radiotherapy (RT) treatments with a total dose of 60 Gray/30 fractions over 6 weeks concerning the tumor mass and the right parotid adenopathy under Intensity-modulated radiotherapy (IMRT) technique.
Results: The side effects of the treatments carried out were thoroughly monitored and were exceptionally well tolerated. The patient presented an excellent response with an almost total eradication of the tumor. Quality of life was significantly improved.
Conclusion: MCC is a relatively uncommon, neuroendocrine, cutaneous malignancy. Traditional therapy involves surgical resection with negative margins, when feasible, with or without adjuvant radiation depending on the size of the primary lesion, surgical margins and stage of disease. In the presented case report due to the massive extension, age and comorbidities surgery was not an option. The patient presented a surprising tumor response with good tolerance to the exclusive RT treatments performed under IMRT technique.

P7. VEGF EXPRESSION IN CD138+/CD19- AND CD138+/CD19+ PLASMA CELLS FROM PATIENTS WITH MONOCLONAL GAMMOPATHIES

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Introduction: Vascular endothelial growth factor (VEGF) is a potent angiogenic peptide with biologic effects that include regulation of extracellular matrix remodeling and inflammatory cytokine generation with an important role in the bone marrow microenvironment of multiple myeloma (MM). Angiogenesis is enhanced in the bone marrow of MM patients in parallel with tumor progression. Myeloma and stromal cells secrete angiogenic factors that include VEGF. Previous studies showed heterogeneity in the expression of VEGF between plasma cells (PCs) from the same MM patient. However, no clear association with expression levels, phenotypic subtypes of PCs and prognosis was demonstrated.
Methods: Bone marrow PCs from 128 patients with monoclonal gammopathies, 60 patients with newly diagnosed symptomatic MM and 68 with monoclonal gammopathy of uncertain significance (MGUS) and also from 11 non-neoplastic controls (Ctr) were analysed between April 2010 and July 2013. We evaluated the expression of cytoplasmic VEGF with monoclonal antibodies by flow cytometry in the two populations of PCs, identified by gating CD138+/CD19- (clonal PCs) and CD138+/CD19+ (non-clonal PCs). The results are presented as percentage of PCs expressing VEGF and as expression levels of VEGF in mean intensity of fluorescence (MIF). The effects of VEGF expression on progression-free survival (PFS) and overall survival (OS) were analysed. For statistical analysis, software IBM SPSS Statistics v22 was used. Survival was estimated according to the Kaplan-Meier method.
Results: In our cohort of patients, median age was 70 (39-86) years, 52% were male. We found increased expression levels of VEGF in CD138+/CD19- PCs from MM (80 ± 7.5 MIF) compared to MGUS patients (61.7 ± 6.2 MIF) ($p = 0.011$), as well as superior to CD138+/CD19+ PCs expression (39.92 ± 1.74 MIF) in both populations of patients ($p < 0.001$ and $p = 0.02$, respectively). No differences were observed in the expression levels of VEGF in CD138+/CD19+ PCs from MM (39.92 ± 1.74 MIF), MGUS patients (41.18 ± 1.92 MIF) and controls (32.8 ± 1.5 MIF). However, the percentage of CD138+/CD19+ expressing VEGF was significantly higher in MGUS (39.4 ± 4%) and in MM patients (46.7 ± 4.5%) compared to Ctr (13.5 ± 0.5%) ($p = 0.019$ and $p = 0.003$, respectively). In MM patients, we also found an association between increased VEGF expression levels in CD138+/CD19- PCs (superior or equal to 175 MIF) and inferior PFS ($p = 0.002$) and OS ($p = 0.003$), irrespective of first line therapy (bortezomib-based regimens for fit patients or alkylating-based treatments for unfit patients). Interestingly, we also observed an incresed percentage of CD138+/CD19+ PCs (higher or equal to 21%) expressing VEGF in MM patients with a more favorable PFS ($p = 0.04$) and OS ($p = 0.008$).
Conclusion: The results of our investigation showed that CD138+/CD19- and CD138+/CD19+ PCs have diferences in what concerns VEGF expression, not only in MM patients, but also in MGUS patients. The increased expression of VEGF in clonal PCs from MM compared to MGUS patients evidences the relevance of VEGF in myelomagenesis. We also demonstrated a negative prognostic impact of an increased VEGF expression in CD138+/CD19- PCs, highlighting the role of VEGF in the survival and maintenance of clonal PCs and as a predictor of outcome in MM progression. The association between the percentage of CD138+/CD19+ PCs and survival supports the suggestion that these cells may not be neutral players in the complex pathogenesis of MM. The results of our study should be further investigated in larger series of patients.
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P8. IL1 PATHWAY ASSOCIATED GENES AND RISK PREDICTION OF EARR

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Introduction: Orthodontic-induced external apical root resorption (EARR) is caused by a local inflammatory response triggered

by mechanical stress. It is a complex phenotype, depending on multiple low penetrance genetic and non-genetic variables. Interleukin-1 beta (IL1*B)* pathway is a key player, influencing the two major interdependent and opposing factors, alveolar bone remodeling and root resorption.
Methods: Using a stepwise multiple linear regression model, 195 patients submitted to orthodontic treatment were searched for clinical and genetic factors associated with %EARRmax (maximum %EARR value obtained in each patient).The four maxillary incisors and the two maxillary canines were assessed. Three functional SNPs were genotyped.
Results: Four of the nine explored clinical variables showed to be significantly associated with %EARRmax ($p < 0.01$). Only homozygosity/hemizygosity for variant C from *IRAK1* gene showed a significant association ($p = 0.018$), revealing to be a protective factor. The model showed that clinical and genetic variables explained about 30% (determination coefficient =.285) of the %EARRmax variability. Each additional month of treatment represented an average increase of 0.3% (B = 0.003), use of Hyrax appliance increased the amount of %EARRmax by 10% (B = 0.097), patients with premolar extractions had about 4% higher values (B = 0.041), on average, female values were 4% below male values (B = - 0.043) and the presence of CC genotype of *IRAK1* gene was associated with an average decrease of 6% (B = -0.056). The contribution of duration of treatment was relatively constant over time.
Conclusion: A new IL1 pathway gene is proposed as a susceptibility gene for EARR; the level of risk prediction achieved is not clinically relevant.

P9. GENOMIC CHARACTERIZATION OF PRIMARY CULTURE CELLS OF ORAL SQUAMOUS CELL CARCINOMA – PRELIMINARY DATA

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignant tumour in the world, having a worldwide incidence of 600,000 new cases per year. HNSCC can arise from ten different sub-anatomic sites, being oral cavity the most frequently affected one. Despite technological advances and refinement of multimodal therapies, oral squamous cell carcinomas (OSCC) are still diagnosed in a late stage and the 5-year survival rate has not improved much in the past decades. It is generally accepted that solid tumours, such as OSCC, result from a multistep process in which genomic alterations play a major role. One of the main challenges is to identify specific cancer biomarkers that will help to improve early diagnosis and survival rates. Conventional and molecular cytogenetic analysis of tumour cell lines have been helpful to find genetic alterations that seem to have a major role in cancer development and progression, thus having the potential to identify biomarkers with clinical and therapeutic value. With this study we aimed to characterize the relationship between DNA copy number aberrations and chromosomal rearrangements in three OSCC primary culture cells.
Methods: Primary culture cells were established from surgically resected samples obtained from three different patients diagnosed with OSCC. The cells were cultured in DMEM supplemented with FBS, penicillin and streptomycin. The characterization was assessed by karyotype and array comparative genomic hybridization (aCGH) using sex-matched healthy controls.
Results: The cytogenetic results showed complex karyotypes with several numeric and structural alterations. These cells seem to be near-triploid, with at least 63 chromosomes each. We identified several chromosomal rearrangements, including i(3)(q10), i(5)(p10) and i(9)(q10). Cells with such alteration showed, by aCGH, gains of the entire chromosome arm of 3q, 5p and 9q respectively. In addition, through aCGH, other genomic imbalances were detected in almost all chromosomes, being the size of these imbalances often variable between the three samples. In general, the most common copy number alterations were observed in chromosomes 3, 5, 8, 9, 18 and X, where are mapped important genes for oral carcinogenesis process.
Conclusion: The cytogenetic characterization and aCGH analysis revealed the most frequent genomic imbalances described for OSCC. We report the relationship between genomic imbalances and cytogenetic rearrangements. These preliminary findings will be useful to understand the different clinical outcome of these patients and for designing further basic and clinical research.

Session 4

P1. EVEROLIMUS AS A NEW THERAPEUTIC OPTION IN CML RESISTANT TO TKI – A PRELIMINARY STUDY

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Introduction: *BCR-ABL1* fusion gene, which encodes an oncoprotein with a deregulated tyrosine kinase activity, is the hallmark of chronic myeloid leukemia (CML) – a myeloproliferative disorder. Despite the high rate of response to treatment with Imatinib and other tyrosine kinase inhibitors (TKI), the number of patients with suboptimal response or resistance to TKI has been increased. The BCR-ABL oncoprotein activates multiples signaling pathways responsible for tumor cells characteristic, namely the high cellular proliferation and resistance to apoptosis. One of these pathways is PI3K/AKT/mTOR pathway, correlated with increase of survival and resistance to apoptosis. Leukemic stem cells use these pathways as mechanism of defense against treatment and as tumor maintenance.
Objective: To evaluate the therapeutic potential of Everolimus, a mTOR inhibitor in CML cell lines sensitive and resistant to TKI and in primary cultures of CML patients.
Methods: For this purpose, we used a CML cell line sensitive to Imatinib, the K562 cells, and established two sub-cell lines resistant to Imatinib, the K562-RC and K562-RD cells. Cell lines were treated in the absence and presence of different concentrations of Everolimus and the effect in cell viability was analyzed by the resazurin assay. Cell death was determined by flow cytometry (FC) using the Annexin V/Propidium Iodide staining. The cell cycle analysis was accessed using Propidium Iodide incorporation by FC. *Ex-vivo* studies were performed in bone marrow and in peripheral blood samples of 8 patients with suboptimal response to TKI treatment. Patients' samples were culture with increasing

concentrations of Everolimus during 48h. Cytotoxic effect was evaluated by FC in different cell populations using Annexin V staining.
Results: Our results show that Everolimus induced a reduction in cell lines viability, with an IC50 of 20 μM for sensitive cells and 25 μM for Imatinib resistant cell lines. The cell death was induced by apoptosis and this drug has also an antiproliferative effect through an arrest in cell cycle progression in G_0/G_1. In *ex-vivo* studies, Everolimus reduced cell viability and increase apoptosis of hematopoietic stem cells ($CD34^+$ cells) without cytotoxicity to lymphocytes.
Conclusion: Our preliminary results reveal the efficacy of Everolimus in inducing cell death in cancer CML cells, without cytotoxicity to normal cells, suggesting that Everolimus could be an alternative targeted therapeutic approach in patients with suboptimal response to TKI treatment.
This work was support by CIMAGO (Project 18/12) and R.A. was supported by FCT with a PhD grant (SFRH/BD/51994/2012).

P2. IMMUNE MONITORING OF TYROSINE KINASE INHIBITOR THERAPY IN CHRONIC MYELOID LEUKEMIA

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Introduction: Different effects on the immune system are reported at the diagnosis of Chronic Myeloid Leukemia (CML) and during therapy depending on Tyrosine Kinase Inhibitors (TKI) and interferon-alpha protocols. Some of these are dose-dependent effects. At present time, several clinical trials are establishing also the safety parameters for cessation of therapy in a selected group of Chronic Myeloid Leukemia (CML) patients. Although there is no consensus for laboratory evaluation of immune response for clinical trials, the Human Immunophenotyping Consortium (HIPC) aims at the standardization of protocols based on multicolor flow cytometry (Maecker *et al*. Nat Rev Immunol 2012). The current study aimed at the evaluation of the immune status in treated CML patients to establish additional efficacy parameters of different therapeutic strategies.
Methods: We used an extended 10-parameter panel for flow cytometry analysis of T cells (Th1, Th2, Th17, Tregs), B cells (including plasmablasts), monocytes (classical and non-classical), dendritic cells (myeloid and plasmacytoid) and NK cells ($CD56^{bright}$ and $CD56^{dim}$) in CML immune monitoring. Additionally, activation and maturation profile, as well as memory status was investigated for T and B cells. Intracellular cytokine staining, phosphorylation studies, response to multiple stimuli and gene expression profiling of relevant sorted cells was also performed for evaluation of immune response in these patients. Deep analysis of specific subsets (NK, NKT and γδ T cells) and pathways [CD137/CD137L, PD-1/PD-L1/PD-L2, TACTILE (CD96), DNAM-1 (CD226) and TIGIT] were explored.
Results: Significant impact on NK cells, NKT and Th17 cells was observed in CML patients undergoing therapy with TKI. Maturation and activation status of different lymphocyte subsets was found affected by therapy. Optimal response was found associated to immune response robustness of CML patients.
Conclusion: CML successful treatment is highly influenced by the immune response and immune-based monitoring could be an important tool in the near future.

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P3. SILIBININ AND GAMBOGIC ACID INDUCE REACTIVE OXYGEN SPECIES PRODUCTION AND CELL DEATH IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

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Introduction: T-cell acute lymphoblastic leukemia (T-ALL) accounts for about 15% of acute lymphoblastic leukemias in children. Besides the new and advanced therapeutic strategies chemotherapy is the most common treatment of T-ALL, but is associated with systemic toxicity, poor survival rate and relapse requiring the identification of new target therapies. Silibinin (SLB) and gambogic acid (GA) are natural products isolated from Chinese herbs that have been found to inhibit proliferation, induce apoptosis, suppress angiogenesis, delay metastasis and enhance chemotherapy, exhibiting anti-cancer potential. However, the role of these natural compounds is still unclear in hematologic neoplasias. In this work, we evaluated the therapeutic efficacy of SLB and GA in a T-ALL cell line alone and in combination with each other and studied the underlying mechanism.
Methods: The T-ALL cell line, CCRF-CEM/CEM, was maintained in culture in absence and in presence of increasing concentration of SLB and/or GA in a daily or single dose administration. Cell viability was assessed by the trypan blue assay and cell death by Optical Microscopy (May-Grünwald-Giemsa 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), reactive oxygen species production (hydrogen peroxide, H_2O_2; superoxide anion, $O2^{\cdot-}$, 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. The expression of activated caspase 3 and survivin were also evaluated by flow cytometry using specific monoclonal antibodies. Results were statistical analyzed.
Results: SLB and GA induced cytostatic and cytotoxic effects in CEM cells, in a dose, time and administration schedule dependent manner. Cell death occurs mainly by apoptosis with mechanisms related to the increase of reactive oxygen species production; decrease in GSH levels and in the mitochondrial membrane potential. We also observed a pre-G0/G1 peack and morphologic characteristics, such as the presence of blebbing, which confirms apoptosis. These compounds also induced significant increase in caspase 3 and a significant decrease in survivin. When we associate SLB with GA in doses inferior of IC50 we observed a synergistic cytotoxic effect.
Conclusion: Taken together our results suggest that the natural compounds SLB and GA may constitute a promising anti-tumor targeted therapy in T-ALL cells, alone and in combination with each other, which may reduce the secondary effects.

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P4. TTF1 IS EXPRESSED BY BRONCHIAL BASAL CELLS IN PULMONARY ADENOCARCINOMAS

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Introduction: Bronchial and bronchiolar epithelium adaptation to tobacco can be an outstanding screening method of carcinoma development/carcinogenesis after basal cells, cylindrical and mucous cells hyperplasia/metaplasia with intermingled immunophenotype in between bronchioles and bronchial expression. Also it is known that positive TTF-1 staining is useful for pulmonary adenocarcinomas differential diagnosis. The aim of this work was to evaluate whether variations in thyroid transcription factor 1 (TTF-1) staining can be found in different subtypes of pulmonary carcinomas.
Methods: Ten pulmonary carcinomas were used for TTF-1 immunoexpression evaluation, being: 3 well differentiated epidermoid carcinomas and 7 adenocarcinomas (acinar 3; solid 2; mucinous 1; and papillary/micropapillary 1). All samples were compared with either bronchial or bronchiolar cells and following immunostain panel was applied: CK7, TTF1, Ck8.18, and CD10 for adenocarcinomas, VIM for EMT, CK5.6 and p63 for epidermoid differentiation, CD56 for NE cell type, and Ki67.
Results: In this study we found that bronchial epithelium had TTF1 expression in adenocarcinomas and negativity in epidermoid carcinomas. Furthermore, in epidermoid carcinomas, bronchial epithelium metaplasia had high CK5.6 and low p63 expression with vimentin negativity; one case had bronchial TTF1 basal cell positivity; in bronchioles, CK5.6 was relevant with lower expression of p63. In adenocarcinomas, vimentin was expressed in all cases, but not in one acinar case with desmoplastic stroma, where bronchioles expressed vimentin.
Conclusion: Although there was not a specific immunophenotype for adenocarcinomas, it seems that vimentin expression is relevant in adenocarcinoma carcinogenesis with TTF1 bronchial expression while in epidermoid carcinomas, CK5.6 keeps being a confident marker, relevant in bronchioles where it is usually absent.

P5. BRONCHIAL CARCINOGENESIS SEEMS TO BE RELATED WITH VIMENTIN AND HIGH WEIGHT MOLECULAR CYTOKERATIN EXPRESSION

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Introduction: Two compartments have been characterized for lung carcinogenesis; one concerns bronchial epithelium and the other refers to peripheral bronchioles and alveoli, the so-called terminal respiratory unit (TRU). TRU inflammation has also been recognized as a carcinogenic pond for bronchial-pulmonary carcinomas after adult stem cells remodeling/alterations. This work aimed to develop an immunohistochemical procedure to verify the behavior of TRU epithelial cells in bronchiolitis.
Methods: A total of 40 broncholitis surgical biopsies, 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), were compared with preserved morphology (BPM) - 5 cases, obtained after surgical drainage of spontaneous pneumothorax due to infant pleural scars pneumothorax related. All samples were immunohistochemical characterized for TTF1, CK5.6.18, and vimentin expression.
Results: BPM cases presented 1 positive case with positivity expression of TTF1 and negativity for vimentin and CK5.6.18. Vimentin and CK5.6.18 basal epithelial cells expression was correlated progressively with BO, CB and RB. Vimentin registered 8 positives in 14 cases (BO), 4 positives in 11 cases (CB) and 2 positives in 15 (RB). Regarding CK5.6.18 results were registered 8 positives in 14 cases (BO), 5 positives in 11 cases (CB), and 7 positives in 15 (RB).
Conclusion: These results suggests that TRU carcinogenesis potentiality relates with bronchialization of bronchiolar epithelium, as a pre-neoplasic condition for adenosquamous carcinomas and pleomorphic carcinomas: basal cell metaplasia with CK5.6.18 expression represents bronchial basal cell phenotype, maintaining TTF1 expression and epithelial-mesenchimal transition is represented by vimentin overexpression.

P6. ALK/ROS1 EVALUATION RESULT ON PREVIOUSLY IMMUNOSTAINED SLIDES

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Introduction: EML4-ALK together with ROS1 mutation gene defines a tumor group of pulmonary adenocarcinomas responsive to targeted crizotinib anti-ALK treatment. FISH is currently considered to be the gold standard for therapy selection. FFPE samples manipulation still the most common biological sample among molecular pathology Lab. Lab confrontation with scarce material ends with immunostained slides to perform other techniques such as FISH. This work aimed to describe a unique technique for ALK/ROS1 FISH performance in previous immunostained slides.
Methods: Immunostained negative slides were chosen to perform ALK and ROS1 FISH techniques. Cover slip was removed in xylene for 24-48 hours. FISH protocol as currently described was then applied, followed by a break-apart probe specific to the ALK locus according to manufacturer's instructions. FISH-positive cases were defined as > 15% split signals in tumor cells (At Shaw et al).
Results: Selecting immunostained negative slide reveled to be essential for good visualization of FISH probes signals. The slides visualized presented little or no autofluorecence in immunostained negative slides. One hundred neoplasic cells were counted in each one; with over 15% presenting spliced signals, to be considered positive.
Conclusion: Although Labs normally use intact FFPE samples to perform FISH techniques, routine negative immunostained slides of this study case could be considered as a rigorous quality testing. By this method, FISH ALK, MET and ROS1 are feasible on previously immunostained slides.

P7. NARROWING 12Q24.33 DELETION CRITICAL REGION: P2RX2 GENE INVOLVEMENT IN INTELLECTUAL DISABILITY

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With the widespread analysis of patients with intellectual disability, multiple congenital anomalies and autism spectrum disorders by array-Comparative Genomic Hybridization (array-CGH), new causative imbalances and new genomic syndromes have been identified. Whereas some chromosomes are more prone to suffer genomic imbalances, due to the presence of low copy repeats that mediate non-allelic homologous recombination, chromosome 12 is not a particularly affected chromosome. Deletions at the terminal band of chromosome 12 are rare, and to date only three patients with intellectual disability are reported, with deletions of 4.5 Mb, 1.6 Mb and 660 Kb in size. Here we report a 16 years old male patient with global developmental delay, dysmorphisms and behavioral disturbances that was studied by Agilent 180K oligonucleotide array-CGH and revealed an 83Kb interstitial deletion, containing only 5 genes. The *P2RX2* geneencodes a subunit of the synaptic purinergic receptor P2X and was considered responsible for the intellectual disability in the previously described patients. As the described patient narrows the minimal critical region between the four patients, reinforces the role of this gene as the responsible for the intellectual disability, which all patients present. The two patients with larger deletions present more manifestations, such as obesity, short stature and renal problems, which might be justified by the involvement of further genes. In the previously reported patients the deletions were *de novo*, while in our patient parents samples have not been received yet do determine the origin of the imbalance, but there is a positive familial history of behavioral disturbances. The reported patient has the smallest 12q24.33 deletion reported involving *P2RX2* gene, and provides further evidence for the involvement of this gene in intellectual disability.

P8. BLADDER CANCER AFTER KIDNEY TRANSPLANTATION

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Introduction: The bladder cancer in kidney transplant patients is challenging due to their state of immunosuppression and frequent existence of major comorbidities. The best treatment for this disease and the clinical results are not yet fully defined. The objective was to determine if there is a higher incidence rate of bladder cancers in kidney transplant patients, to analyze the therapeutic strategy in our department and evaluate the outcomes.
Methods: Retrospective study including the 2,514 patients who underwent kidney transplantation in our department between June 1990 and February 2015. Of these, we identified 7 patients with de novo diagnosis of bladder urothelial carcinoma. We compared the incidence rate of this disease in renal transplant patients to the general population in Portugal. Demographics, clinical and pathological data, management and oncological outcomes were collected and analyzed.
Results: 7 of 2,514 transplanted patients developed de novo bladder urothelial cancer (0.28%) against an overall incidence rate in Portugal of 0.012%. Four were smokers (57.1%). Diagnosis occurred at a median of 2.9 years after renal transplantation. Median age at the time of transplantation was 57 ± 5 years and 71.4% of patients were men. Diagnosis was incidental in 3 (42.8%) and the remaining manifested as hematuria. In 57.1% (4) tumors were single and 42.8% (3) had tumors with diameters ranging between 1.5 and 3cm. The pathological examination revealed: 2 TaG1, 1 T1G3, 2 T1G2 (one with associated Cis) and 2 T2G3. Three patients underwent intravesical therapy on usual doses with Mitomycin C (42.8%) and one with BCG (14.2%). The patient on BCG had to stop treatment after 12 instillations due to UTI. No patient has made re- transurethral resection. The two patients with muscle-invasive carcinoma underwent radical cystectomy without major complications. The TaG1 patients didn't relapse. Two of the patients with non-muscle invasive tumor (T1G3 and T1G2) relapsed at a median of 22 months and one of them was submitted to radical cystectomy due to recurrent T1G3. Two patients died due to progression of the disease at a median of 18 months from the time of diagnosis. For a median follow-up time of 28 months overall mortality was 28.5%.
Conclusion: Kidney transplant patients exhibit a higher incidence of bladder cancer compared to the general population. Although patients with TaG1 tumors do not appear to show increased recurrence and progression rates, in the others the disease appears to be more aggressive in terms of risk of recurrence and specific mortality, justifying therapeutic management equally aggressive in these patients.

P9. THE CASE OF THE MYSTERIOUS CHROMOSOME(S)

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Introduction: Supernumerary marker chromosomes (SMC) are relatively common in prenatal diagnosis, being its identification a challenging for diagnosis and genetic counselling. Males with a 47,XXY karyotype have the clinical phenotype of Klinefelter syndrome. A few 47,XXY cases with a female phenotype have been reported. Females with XY karyotype are usually sterile, due to degeneration of the initially present ovaries into non-functional gonads. Some of these sex-reversal cases can be attributed to mutation or deletion of the SRY gene.
Methods: A 39 year-old woman was referred to amniocentesis due to advanced maternal age. Conventional and Molecular Cytogenetic analysis were performed in culture amniocytes.
Results: Three different lines were found: one in most of the cells with an extra chromosome that looks like a Y chromosome, another line with two similar markers chromosomes and one normal female line - 46,XX, in only a few cells. These markers chromosomes were all C-positive. The analysis by fluorescence in-situ hybridization (FISH) with probes for the Y-centromere (DYZ3) and SRY showed no hybridization for the centromeric probe and SRY gene. Nevertheless, the hybridization with whole Y chromosome painting probe (WCP-Y) was positive. Ultrasound revealed a female fetus with no alterations. Karyotype of the father was normal and cytogenetics analysis in mother lymphocytes revealed the same three lines found in the fetus.
Conclusion: We describe an unusual supernumerary marker chromosome derived from Y in a fertile woman and her daughter. To our knowledge, this is the first reported case of a mosaicism involving three cell lines with one or two markers derived from Y chromosome in a fertile female. This is relevant when we discuss the importance of the Y chromosome in sex determination and the impact in the fertility of XY females.

Session 5

P1. NFE2L2 AND KEAP1 GENE EXPRESSION ANALYSIS IN HEMATOLOGICAL MALIGNANCIES – CLINICAL IMPLICATIONS

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Introduction: Oxidative stress (OS) as the result of an imbalance between reactive oxygen species (ROS) and antioxidant defense mechanisms has been observed in almost all cancers, including leukemia, where it contributes to disease development and progression. NRF2 (*Nuclear factor erythroid-2-related factor 2*) is a transcription factor codified by *NFE2L2* gene (*Nuclearfactorerythroid2-like 2*) that activate many antioxidant and detoxification genes, and is strongly regulated by KEAP1 (*Kelch-like ECH-associated protein 1*). NRF2-KEAP1 system seems to have an extremely important role in OS regulation, contributing for disease development and/or influence therapy response.
Objective: To evaluate the expression levels of *NFE2L2* and *KEAP1* genes in Myelodysplastic Syndrome (MDS) and Monoclonal Gammopathies (MG) patients, as well as its correlation with clinical and laboratorial data, in order to identify potential biomarkers of diagnosis and/or prognosis.
Methods: We evaluated 113 patients, 49 MDS (24 refractory cytopenia with multilineage dysplasia; 7 chronic myelomonocytic leukemia; 6 refractory cytopenia with unilineage dysplasia; 5 refractory anemia with excess blasts-1; 4 refractory anemia with ringed sideroblasts; 2 refractory anemia with excess blasts-2; 1 5q-), 34 MG (13 monoclonal gammopathy of undetermined significance; 21 multiple myeloma) and 30 controls (CTL). Samples were collected after informed consent obtained in accordance with the Helsinki Declaration. Real-time PCR was used to evaluate the gene expression level of *NFE2L2*, *KEAP1* and *GUS* (control gene). Results were considered statistically significant when $p < 0.05$.
Results: Our preliminary results showed that patient´s group presents higher *KEAP1* expression levels comparing to controls (patients: 0.175; CTL: 0.097; $p = 0.04$), while *NFE2L2* did not presented differences between any groups. When we analyzed by pathology we observe that *KEAP1* gene expression levels was higher in GM patients compared with controls (GM: 0.2059; CTL: 0.09717; $p = 0.009$). No association was observed between MDS and CTL; however, RCDM patients have lower levels of *NFE2L2* (RCMD: 1.754; CTL: 5.033; $p < 0.05$) and higher levels of *KEAP1* (RCMD: 0.185; CTL: 0.097; $p = 0.005$). Through ROC analysis we observed that *NFE2L2* (cut off < 2.044; sensitivity: 75%; specificity: 83.3%) and *KEAP1* (cut off > 0.1627; sensitivity: 54.17%; specificity: 86.67%) levels might be diagnostic biomarkers for RCMD patients, as well as *KEAP1* levels (cut off > 0.1645; sensitivity: 52.38%; specificity: 86.67%) for MM patients. Survival analysis showed that RCMD patients with *NFE2L2* expression levels over 2.044 and *KEAP1* level under 0.1645 present a tendency for lower survival, as well as MM patients with *KEAP1* level under 0.1645.
Conclusion: Our results suggest that the expression pattern of *NFE2L2* and *KEAP1* genes might be associated with the development of hematological malignancies, and may have a great potential as diagnostic biomarkers for myelodysplastic syndrome and multiple myeloma.

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P2. SCRAPING CELLS OF THE ORAL CAVITY: A VIABLE METHOD FOR OSCC DETECTION?

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Introduction: Oral squamous cell carcinoma (OSCC) results from accumulation of numerous genetic and epigenetic changes, followed by clonal expansion. Despite of technological progress and improvement in therapeutic approaches, the five-year survival rate remains low, mainly due to diagnosis in advanced stage and frequent development of loco-regional recurrences. Until now, biopsy is the main and more accurate technique used to detect OSSC. However, as biopsy is an expensive and also invasive method, it is mostly used when already exists a suspicion of malignancy. Therefore, improved diagnostic methods should be developed for early detection and to follow up patients after treatment. Taking this into account, the main goal of this work was to evaluate the reliability in the detection of genetic and epigenetic alterations present in samples collected by scraping the OSCC surface, in order to validate this non-invasive approach to early detect these tumours and their relapses.
Methods: Tumour cells were acquired by scraping the tumour surface from 27 OSSC patients. Biopsies of the tumour were also collected from the same patients. Samples from healthy donors were used as controls. Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) was conducted to screen copy number alterations and DNA methylation patterns in 41 and 27 tumour suppressor genes, respectively. The MS-MLPA results from biopsies were compared with results acquired from scraped cells.
Results: Our results from scraped cells showed that, between the genes analysed, the most frequently hypermethylated were *WT1* (21/27), *PAX5* (10/27), *RARB* (9/27) and *GATA5* (9/27). The results obtained from biopsies are in agreement with those from scraped cells *WT1* (23/27), *PAX5* (10/27), *RARB* (10/27) *GATA5* (9/27). Regarding copy number variation, in both cases (biopsies and scraped cells), the most frequent alterations were localized at chromosomes 3, 9, 10 and 11. Additionally, in biopsies we also found alterations at chromosomes 19 and 20. In both types of tumour samples, the most frequent imbalance detected was loss of *CDKN2A* gene (10/27-scraped cells; 8/27- biopsies). The most frequent gains observed in scraped cells were *CASR* (8/27) and *CD44* (7/27) gene. However, the most frequent gains observed in biopsies were *STK11* (10/27) and *GATA5* (9/27).
Conclusion: Several studies have been showing that analysis of genetic and epigenetic aberrations can be important to establish biomarkers in order to identify tumours in early stages. Considering this, we try to validate a non-invasive alternative to biopsies in order to detect OSCC. Our preliminary data demonstrated that several genetic and epigenetic alterations identified in tumour biopsies are concordant with those identified in scraped cells of the tumour surface. In this pilot cohort, the agreement between both types of samples seems to be higher for the results of methylation. Considering that methylation is acknowledged as an initial alteration in OSCC development and progression model, we can suggest that

scraped cells of oral surface can be used as a powerful and reliable tool to identify biomarkers to early diagnosis OSCC primary tumours and also recurrences during and after the treatment.

P3. C-REACTIVE PROTEIN IN PATIENTS WITH HEAD AND NECK CANCER: IMPLICATIONS FOR CANCER CACHEXIA AND QUALITY OF LIFE

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Introduction: Cachexia is associated to decreased physical functioning, quality of life, and worse overall prognosis, being the main cause of death in 20-40% of Head and Neck Cancer (HNC) patients. Cancer cachexia is strongly associated to the inflammatory response, which can be positively modified by physical activity. This study aimed to investigate association of C-reactive protein (CRP), a marker of systemic inflammation, with body composition, quality of life and physical activity in HNC patients.
Methods: Seventeen (14 males and 3 female; age, 59.8 ± 3.1 years old; weigh, 62.0 ± 2.9 kg; height, 1.66 ± 0.02 m; body mass index, 22.7 ± 1.3 kg/m^2) consecutive HNC patients were recruited from Maxillofacial Surgery Department, Coimbra Hospital and University Centre before any treatment. Outcome measures included demographic and medical variables, body composition (assessed by bioimpedance), daily physical activity (International physical activity questionnaire), quality of life (QLQ30–H&N35 module), and plasma levels of C-reactive protein. Correlation analysis was conducted to assess the association between the assessed variables. Results are given as mean ± standard error of mean.
Results: Patients presented 21.5 ± 3.4% of fat mass, 79.1 ± 3.1% of fat free mass and 22.4 ± 1.3 kg of muscle mass in the appendicular skeleton. In general patients spent a total of 247.5 ± 203.9 min/day in sitting activities, and performed a total of 5,758.9 ± 1,984.3 MET-min/week of physical activity; being 1,049.4 ± 683.2 MET-min/week spent in moderate activities. The global heath quality of life of the patients was 45.1 ± 7.1 points, scoring 80.2 ± 4.3 on functional scales and 16.0 ± 4.3 on symptom scales. A significant inverse correlation was found between levels of C-reactive protein and body mass index (r = -0.520, p = 0.047), body weight (r = -0.546, p = 0.035) and lean body mass index (r = -0.533, p = 0.05). Higher levels of C-reactive protein were also associated with lower daily time spent in moderate intensity physical activity (r = -0.648, p = 0.009), and lower quality of life (r = -0.602, p = 0.018). Those patients with higher appendicular skeletal muscle mass also showed higher quality of life (r = 0.521, p = 0.039). A significant correlation was found between sedentary time and symptom scales (r = 0.499, p = 0.042), i.e. more time spent in sitting activities is associated with higher symptom scales score.
Conclusion: C-reactive protein, a marker of systemic inflammation, is associated with lower body weight, lean body mass, quality of life and moderate-intensity physical activity in HNC patients. These preliminary findings highlight the relevance of strategies, including physical activity, to reduce inflammatory status.
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P4. HEAD AND NECK SQUAMOUS CELL CARCINOMA: GENOMIC IMBALANCES REVEALED IN X AND Y CHROMOSOMES

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Introduction: Head and Neck Squamous Cell Carcinoma (HNSCC) arises from gradual accumulation of somatic genomic instability and alterations that leads to carcinogenesis and cancer progression. Imbalances in X and Y chromosomes have been associated with different types of human tumors. This study aimed to identify the most frequent chromosomal regions with genomic imbalances in X and Y chromosomes associated to HNSCC.
Methods: Biopsies of HNSCC were acquired from 104 patients and array-Comparative Genomic Hybridization (aCGH) was performed using an Agilent oligonucleotide microarray 4x180K. Healthy donors were used as controls.
Results: Our HNSCC cohort presented numerous genomic imbalances in the sex chromosomes. We observed several recurrent minimal regions of gain in X chromosome, including Xp22.33, Xq25, Xq21.1, Xp11.22 and Xq24-q25 and of loss, including Xp11.23, Xp11.3-p11.23 and Xq26. In Y chromosome we observed gain at Yp11.32 and loss at Yp11.32-p11.31 and Yp11.2. In these regions several genes associated to cancer, apoptosis, proliferation, differentiation and angiogenesis are mapped. In X chromosome the gain of Xp22.33 was the alteration observed in a higher number of patients. In Y chromosome the most common aberration observed was loss at Yp11.32-p11.31.
Conclusion: Our results showed several genomic alterations in sex chromosomes, allowing the delimitation of the minimal most common chromosomal regions of gains and losses in these chromosomes. We identified in these chromosomes several genes associated to biological processes related to cancer development. Further studies are ongoing to verify the prognostic and diagnostic value of these genes in HNSCC.

P5. SHIKONIN INDUCE APOPTOSIS THROUGH OXIDATIVE STRESS IN DIFFERENT HEMATOLOGICAL NEOPLASIAS

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Introduction: Shikonin (SHK) is the most important component of *Lithospermum erythrorhizon* and it has been described as having multiple biological functions, such as anti-inflammatory and antitumor effects. Several studies have showed the potential therapeutic in innumerous types of solid cancers, including lung adenocarcinoma, colorectal cancer and glioma. It has been demonstrated that SHK decreases tumor cells proliferation and induces apoptosis. Many molecular mechanisms have been discussed. It seems that SHK increases reactive oxygen species (ROS) production which lead to oxidative damage and, consequently, to apoptosis. However, the molecular mechanisms of action have not been completely defined, mainly in hematological neoplasias.

Objective: To evaluate the potential therapeutic of SHK in *in vitro* models of different hematological neoplasias and to evaluate if it cytotoxic effects are mediated by oxidative stress.
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 two different administration schemes: in single dose and in daily dose. Cell viability was analyzed by the Rezasurin Assay and daily dose scheme was compared with the single dose scheme. Cell death was determined by flow cytometry (FC), using the Annexin-V and propidium iodide (PI) double staining. It was also evaluated by FC the activation of caspases and cell cycle using the Apostat probe and PI/RNase, respectively. The oxidative stress was evaluated by FC using the dies dihydrorhodamine 123 to measure peroxides, mercury orange to measure GSH concentration and JC-1 to evaluate mitochondrial membrane potential.
Results: Our results showed that SHK reduce cell viability in a time, dose, administration scheme and cell type dependent manner, being the HEL cells the more sensitive. The administration of SHK in a daily dose seems to reduce more accentuated the cell viability, especially in NB-4 cells. The antiproliferative effect was confirmed by the increase of the number of cells in S phase. SHK induces cell death mainly by apoptosis, confirmed by FC, which may be related with the increase in activated caspases expression levels and the decrease of mitochondrial membrane potential in treated cells. These results may also be related with the oxidative stress induced by SHK which is related with the increase in peroxides levels in all cell lines and the decrease of antioxidant defenses, measured by GSH concentration.
Conclusion: Our results suggest that SHK induce apoptosis through oxidative stress and it 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.
This study is supported by the CIMAGO and R. Alves is supported by Portuguese Foundation to Science (FCT) grant (SFRH/BD/51994/2012).

P6. BLADDER CANCER – CLINICAL AND HISTOLOGICAL RESULTS OF SECOND TRANSURETHRAL RESECTION

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Introduction: About 80% of bladder cancer cases are not muscle-invasive, being these cases treated with transurethral resection of bladder (TURB). However, part of these patients presents high rates of recurrence and progression of disease as well residual tumor in some cases, leading to a second TURB.
Objective: Evaluate clinical relevance of second TURB in non muscle-invasive bladder cancer.
Methods: We evaluated patients undergoing TURB between 2009 and 2013. The clinical course for each patient, the recurrence and progression rates and the residual tumor presence were evaluated.
Results: During the study period, 47 patients underwent second TURB and 16 patients (34%) showed residual tumor. The mean age was 73 ± 1.5 years. The average time between first and second TURB was 41.1 ± 6.3 days. Histological results were: no tumor, 31 cases (66%); pTa, 7 cases (14.9%); pT1, 6 cases (12.8%); ≥ pT2, 3 cases (6.4%). The recurrence rate in patients with tumor in second TURB was 46.2% at 18 months of follow-up. On the other hand, the group with no tumor in second TURB showed a recurrence rate of 33.3%. This difference was not statistically significant.
Conclusion: In accordance with current guidelines, this study showed that only one TURB will be insufficient in many patients with bladder cancer. In some cases a second TURB can identify a muscle-invasive disease omitted at first surgery. Thus, the second resection should be recommended in those patients with high risk of bladder cancer in order to ensure complete resection and set patients who require other therapeutic approaches.

P7. OMEGA-3 FATTY ACIDS – AN OPTION IN CHEMOPREVENTION OF BLADDER CANCER?

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Introduction and objective: Omega-3 (ω-3) fatty acids have been tested on prevention and treatment of several cancer types, but the efficacy on "in vivo" bladder cancer has not been analyzed yet. This study aimed at evaluating the chemopreventive efficacy of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) mixture in an animal model of bladder cancer.
Methods: Forty-four male Wistar rats were divided into 4 groups during a 20-week protocol: control; carcinogen—N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN); ω-3 (DHA + EPA); and ω-3 + BBN. BBN and ω-3 were given during the initial 8 weeks. At week 20 blood and bladder were collected and checked for the presence of urothelium lesions and tumors, markers of inflammation, proliferation, and redox status.
Results: Incidence of bladder carcinoma was, control (0%), ω-3 (0%), BBN (65%), and ω-3 + BBN (62.5%). The ω-3 + BBN group had no infiltrative tumors or carcinoma in situ, and tumor volume was significantly reduced compared to the BBN (0.9 ± 0.1 mm^3 vs 112.5 ± 6.4 mm^3). Also, it showed a reduced MDA/TAS ratio and BBN-induced serum CRP, TGF-β1, and CD31 were prevented.
Conclusion: Omega-3 fatty acids inhibit the development of premalignant and malignant lesions in a rat model of bladder cancer, which might be due to anti-inflammatory, antioxidant, anti-proliferative, and anti-angiogenic properties.

P8. THE ROLE OF ATORVASTATIN IN BLADDER CANCER PREVENTION

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Objective: To evaluate the anticarcinogenic effects of Atorvastatin (Atorva) on a rat model with urinary bladder carcinogenesis induced by 0.05% of N-butyl-N-(4-hydroxibutil)nitrosamine (BBN), and assess the relevance of inflammation, proliferation and oxidative stress in tumor growth and its prevention.
Methods: Forty-four male Wistar rats were divided into four groups: (1) Control: vehicle; (2) Atorva: atorvastatin 3 mg/kg bw/day; (3) Carcinogen: BBN (0.05%); (4) Preventive: atorvastatin 3 mg/kg bw/day + BBN. A two phase protocol was used, in which the drug and the carcinogen were given between week 1 and 8 and tumor development or chemoprevention were expressed between week 9 and 20. At this phase, the urinary bladders were collected for macroscopic, histological and immunohistochemical (p53, ki67, CD31) evaluation. Serum was assessed for markers of inflammation, proliferation and redox status.
Results: The incidence of vesical carcinoma was: Control 0/8 (0%); Atorva 0/8 (0%); Carcinogen 13/20 (65%) and Preventive 1/8 (12.5%). The number and volume of tumors were significantly lower in the Preventive group, with a marked reduction in hyperplasia,

dysplasia and carcinoma in situ lesions. An anti-proliferative, anti-inflammatory and antioxidant profile was also observed in the Preventive group. p53 and ki67 immunostaining were significantly increased in the BBN-treated rats, which was prevented in the Preventive group. No differences were found for CD31 expression.

Conclusion: Atorvastatin demonstrated an inhibitory effect on urinary bladder cancer development, probably due to its antioxidant, anti-proliferative and anti-inflammatory properties. This data supports a role of cancer chemoprevention strategies based on Atorvastatin.