



POSTER PRESENTATIONS

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The abstracts are the author's responsibility.

P1. E-CADHERIN AND SPHEROIDS FORMATION CONTRIBUTES TO THE OVARIAN CANCER METASTATIC SUCCESS

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Introduction: Ovarian cancer remains the gynaecological neoplasia with highest mortality rate, mainly due to late diagnosis and early intra-abdominal metastasis. From the solid tumour are formed multicellular aggregates, the spheroids, able to survive in suspension in the peritoneal fluid and establish distant metastasis in the abdominal mesothelial lining. The success of this depends on changes in cellular dynamics, namely the intercellular adhesion function of E-cadherin and the moderating role of the tumour's microenvironment.

Materials and methods: It was made a revision of scientific evidence from the last 10 years, obtained from "PubMed" and "Clinical Key", between March and September of 2017, using the key words: ovarian cancer, peritoneal metastasis, E-cadherin and adhesion molecule.

Results: The subexpression of E-cadherin allows the disintegration of the solid ovarian tumour by breaking down the adherens junctions, leading to the release of isolated cells into the peritoneal fluid. Paradoxically, this same molecule, or its fragments, seem to be vital to the formation of the spheroids, allowing intercellular adhesion of these aggregates and their survival to anoikis, also being verified the contribution of this molecule to their resistance to chemotherapy. The regulation of E-cadherin expression is simultaneously a conditioning factor and a consequence of tumour's microenvironment composition. The activation of cell pathways by Reactive Oxygen Species, ligands of the Epidermal Growth Factor Receptor and other processes of genetic subexpression, as well as the cleavage of the molecule by extracellular enzymes, apparently increase the dissemination capacity of tumour cells, by decreasing the levels of E-cadherin. On the other hand, soluble fragments of E-cadherin, resulting from its proteolytic cleavage, integrate the tumour's microenvironment and con-

tribute, alongside with some sustained expression of the molecule, to the tumour's dissemination process.

Conclusions: The role of E-cadherin in the metastatic process of ovarian cancer may justify the reserved prognosis associated with tumours with low phenotypic expression of the molecule and, at the same time, provides clues to an understanding of the metastatic pattern and therapeutic resistance of this neoplasia. In addition, the potential manipulation of E-cadherin and various components of the tumour's microenvironment may be useful to the development of targeted and more effective therapies.

P2. ETIOLOGY AND RISK FACTORS OF COLORECTAL CANCER - PREVENTION IS THE IDEAL STRATEGY FOR TREATMENT

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Introduction: Colorectal Cancer (CRC) is considered a major public health problem around the world as it is the second common cause of death. It is considered the fourth most common cancer diagnosed each year (the third in men after lung & prostate cancers and the second in women after breast cancer). In addition, it has large economic & emotional impacts by leaving victims with low survival rates & high recurrence rates especially in cases with late stages.

Objective: To study the etiology & risk factors (Modifiable & Non-Modifiable) of CRC to determine the ideal strategies for prevention of CRC in early diagnosis.

Materials and methods: By reviewing records of patients with CRC at "Tanta University Hospitals, Tanta, Egypt" from June 2016 until June 2017. Collected data included personal information, special habits, family history, past medical history & diagnostic workup by laboratory & radiologic examination.

Results: Age, the incidence of CRC increases with age as follow; patients over the age of 60 represent about 55%, patients between 50 to 60 represent about 25% & young patients group with age under 50 represent about 20%. Gender, men have a slightly higher risk of developing CRC than women. Race, Black people have the highest rates of CRC. Family History, if a person has a family history of colorectal cancer, his or her risk of developing the disease is nearly double especially if a first-degree relative was diagnosed at a younger age. Past history of

inflammatory bowel disease (IBD), adenomas or certain types of cancer like as ovarian cancer or uterine cancer increases the risk of CRC. We have other risk factors such as; Physical Inactivity & Obesity - Smoking - Heavy Alcohol Consumption - Unhealthy Diet as high red meat consumption, a cholesterol-rich diet with poor folic acid and vitamin B6 intake are associated with a high risk of colon cancer.

Conclusions: We could classify the risk factors for modifiable & non-modifiable. Non-Modifiable risk factors are age, Gender, Race & family & history as well as past history of some diseases & tumors. Modifiable risk factors are Physical Inactivity, Obesity, Smoking, Heavy Alcohol Consumption, and unhealthy Diet. If we could control the modifiable risk factors via having a healthy lifestyle, practicing sports on regular basis, stop smoking & alcohol consumption and eating healthy food rich in vitamins (cooked green vegetables, fruits, fish) instead of high red meat & cholesterol diet.

P3. DIFFERENTIAL GENE EXPRESSION BETWEEN FLOOR OF MOUTH AND TONGUE CARCINOMAS IN THE TCGA DATABASE

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is a highly aggressive upper aerodigestive tract cancer that arises from a multistep process encompassing several cumulative molecular and genomic alterations. The clinical usage of a biomarker or set of biomarkers to predict the survival outcomes of HNSCC patients could affect both the patient's prognosis and the type and duration of the treatment. Among the different subtypes of HNSCC tongue and floor of the mouth tumors are the most common, with highly aggressive clinical courses as well as poor prognosis. Gene expression analysis seems to be a valuable method to categorize HNSCC into fairly homogeneous subtypes, with a prospective application in a clinical context. **Materials and methods:** RNA Sequencing (RNA Seq) data from HNSCC tumors was downloaded from The Cancer Genome Atlas (TCGA) and was selected according to location of the primary tumor (tongue and floor of mouth), resulting in 183 samples to study. The data was analyzed recurring to the LIMMA Bioconductor Package for R and IBM SPSS Statistics.

Results: In this cohort we found ten genes that were differentially expressed between tumors of the tongue and floor of mouth with significance ($p < 0.001$): *MYH3*, *DES*, *ANKRD1*, *MYLPP*, *SLN*, *ACTC1*, *CASQ2*, *TNNT3*, *MYBPH* and *CHRNA1*. In the studied cohort, all of these genes seem to be overexpressed in tumors of the tongue relatively to tumors of the floor of mouth. Some of these have already been associated with HNSCC, with at least two of them (*ACTC1*, *CASQ2*) being previously linked with an unfavorable prognostic. Kaplan-Meier survival analysis didn't show any significance when merely comparing the locations of the primary tumors, however the median survival time estimate in the case of patients that had tumors of the tongue is 30 months (approximately 2 years and 6 months) higher than the other type.

Conclusions: We were able to identify a set of genes that seem to differentiate tumors of the tongue and floor of mouth. Even though the survival analysis didn't result in significance, we were able to verify that 50% of the patients with tongue cancer survived two and a half years more than the other group. This approach has the potential to identify biomarkers with diagnostic and prognostic value leading the way to a new era of personalized treatments for tumors from different anatomic sites.

P4. DE NOVO UROLOGICAL MALIGNANCIES IN RENAL TRANSPLANT RECIPIENTS

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Introduction: Immunosuppressed transplant patients have a higher risk of malignancies development. The aim of this study was to determine the incidence of urological malignancies in renal transplant recipients, as well as to evaluate their monitoring, treatment and outcomes.

Materials and methods: We conducted a cross-sectional single-center study of 2897 patients who underwent renal transplantation between January 1987 and December 2016. Recipients presenting de novo urological malignancies were evaluated regarding type of cancer, treatments and their results posttransplant duration, immunosuppressive regimens, graft functional status. We retrospectively assessed the stage of the disease, treatment performed and consequent oncologic outcome.

Results: Sixty-one *de novo* urological malignancies were recorded in 58 patients. The overall incidence of urological malignancies was 2.1%. We identified 29 cases of prostate cancer, 23 of renal cell carcinoma, 6 of urothelial carcinoma and 1 case of penile cancer with incidence rates of 1.0%, 0.8%, 0.2% and 0.03%, respectively. No cases of testicular tumors were found. The mean age of neoplastic diagnosis was 58.7 ± 10.1 years. The mean time between renal transplantation and tumor development was 108.2 ± 90.9 months. Fifty-six patients received cadaveric kidneys. The remaining two recipients received a living donor kidney. The overall survival rate at 5 years after diagnosis of urologic tumor was 82.8%. Tumor-related death was found in 13.8% of patients. Four patients died with a functioning renal graft. The remaining six patients had no functional graft and were undergoing a dialysis program. Twenty-five (43.1%) cases had graft loss. Of these, 11 patients had a non functional graft when the diagnosis of urologic tumor was made. The therapeutic options did not differ from those used in non-transplanted patients. Patients with prostate carcinoma were mostly (65.5%) treated with radical prostatectomy. In cases of urothelial tumors, only one patient underwent radical cystoprostatectomy. All other cases were treated only with transurethral resection.

Conclusions: Renal transplant patients have a higher incidence of urological tumors than the general population. Treatment of these tumors is not different from the rest of the patients. However, due to the increased incidence of tumors and possibly worse prognosis, renal transplant recipients should be screened more regularly.

P5. IMMATURE TERATOMA OF THE OVARY: A REMOTE POSSIBILITY

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Ovarian immature teratoma is a rare form of malignancy, representing 1% of all ovarian cancers, most frequent in the first decades of life. We report a case of a 15-years-old girl with complaints of lower abdominal pain, extending to the pelvic area. Imagiology revealed a cystic heterogeneous mass, with 105mm, in the right ovary, consistent with teratoma. Tumoural markers were negative. A laparoscopic excision was performed. Gross exam revealed a fragmented cystic mass containing hair and sebaceous material, with thin whitish wall. Microscopy showed mature teratomatous elements along with immature neuroectodermal tissue occupying > 3 LPF. Final diagnosis was grade 3 ovarian immature teratoma. Patient underwent active surveillance. Seven months later, imagiological control revealed multiple adenopathies, ascites, a cystic mass on the left

ovary, peritoneal implants and metastasis in pelvic bones. Ascitic cytology displayed malignant cells and the peritoneal biopsy confirmed the relapse, exclusively of immature elements, mainly immature neuroectodermal tissue. Analytically there was a slightly elevation of CA125 and NSE. Patient was, at the moment of submission, under chemotherapy treatment. Despite being a rare entity, ovarian immature teratoma should be considered in young patients with big ovarian masses, even with negative tumoural markers, and eventually discuss the preference for laparotomy.

P6. METASTATIC GASTRIC MELANOMA: A CASE REPORT

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The stomach is regarded as a rare site for metastatic disease. Although melanoma is the most common metastatic tumor of the gastrointestinal tract, the small, large bowels and rectum are the most commonly affected sites. Metastatic gastric melanoma is a rare phenomenon. Usually the endoscopic tumor appearance suggests a primary gastric carcinoma and microscopically may also be misdiagnosed as adenocarcinoma, therefore metastatic gastric melanoma is a challenging diagnosis. We report a 70 years old man with history of acral melanoma (right hallux) 2 years earlier. An upper endoscopy was performed and revealed a ulcerated lesion arising on the greater gastric curvature. Our patient presented a tumoral vegetant mass lesion with 4,5cm at the antrum. Furthermore we found two more lesions, one at the fundus and the other at the pyloric antrum. Microscopically, the cells of these lesions expressed HMB-45, S-100 and MelanA protein on immunohistochemical stains, all melanoma markers. Metastatic gastric melanoma isn't the first presumed diagnosis when studying a gastric mass because of its uncommon localization, non-specific clinical manifestations and macroscopical appearance. Although metastatic gastric melanoma is rare, it should be a possible diagnosis for any gastric mass lesion due to the increase in the melanoma incidence.

P7. LYNCH SYNDROME - 2 CASES: ONE WITH HETEROGENEOUS MLH1 EXPRESSION AND ONE PITFALL

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Lynch syndrome (LS), previously known as hereditary nonpolyposis colorectal cancer (HNPCC), is an autosomal dominant inherited cancer susceptibility syndrome caused by defects in the mismatch repair system (MMR). This system depends on a family of genes that are conserved across most living organisms and is responsible for repairing single-base mismatches that occur during DNA replication. In addition to colorectal cancer (CRC), hallmark diseases of LS include endometrial, ovarian cancer and other tumors. LS is the most frequent hereditary CRC accounting for 1-3% of all CRC. In our department we test defects in MMR with immunohistochemistry (IHC) by screening 4 proteins (MLH1/PMS2; MSH2/MSH6), for patients < 70 years and all patients covered by Bethesda criteria. A missing protein suggests a mutation in the gene that codes for that protein. A 64-year-old man with LS, previously submitted to colorectal surgery, was followed in Gastroenterology for 11 years. In a surveillance colonoscopy the patient presented a flat lesion and small polyps and was submitted to endoscopic resection. A 77-year-old woman with a neoplasia of the right colon was submitted to right hemicolectomy. The first case showed heterogeneous MLH1 expression (negative in solid areas of the flat lesion and positive in small villous lesions) and PMS2 loss, identified as flat lesion with superfi-

cial submucosal invasive carcinoma, in the context of surveillance colonoscopy, in a patient with an MLH1 germline mutation (262delATC). The second case exhibited an undifferentiated carcinoma of the right colon with a solid pattern, with invasion of the serosa and mesocolon, vascular embolization and without metastatic disease in 15 regional lymph nodes. It also showed loss of MLH1/PMS2, due to BRAF V600E mutation. In a pathologic department is important to have a protocol deciding which patients should be screened for LS, and if necessary proceed with genetic test. After the diagnosis an adequate surveillance is mandatory, because in LS the progression adenoma-carcinoma is fast. Endoscopic resection in LS may also have an important role also, as in the first case. However we should be aware that not always the loss of MMR means LS, and that there are pitfalls in the interpretation of MMR proteins in IHC. BRAF V600E mutation is strongly associated with MLH1 inactivation secondary to promoter hypermethylation. It has been used to distinguish LS-associated from sporadic MSI-positive tumors.

P8. GENOMIC AND EPIGENETIC CHARACTERIZATION OF CHOLANGIOCARCINOMA WITH METHYLATION-SPECIFIC MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION

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Introduction: Cholangiocarcinoma (CCA) is a rare malignant tumor originating from the epithelial cells of the biliary tree and is commonly classified as intrahepatic (ICC) and extrahepatic (ECC), based on anatomical location. It represents less than 2% of all human malignant neoplasms and about 3% of all gastrointestinal tumors but it is the second most common primary liver cancer (10-25%). The diagnosis of CCA is not easy to obtain so the prognosis is usually poor, with an incidence and mortality rate very close to each other. The aim of this study was to perform a genetic and epigenetic characterization of ICC and ECC patients, in order to identify the most frequently altered genes in this type of tumors.

Materials and methods: The genetic and epigenetic characterization was performed by Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) in samples of 5 ECC and 8 ICC patients, of which three types of tissues were analyzed: tumoral, non-tumoral and peri-tumoral.

Results and conclusions: The results obtained revealed common alterations between some of the patients and in the three types of tissues. Regarding the analysis of copy number variation, the genes that were found to be most frequently altered in all of the samples were *CHFR*, *GSTP1*, *PYCARD*, *TP53*, *BRCA1*, *STK11* and *GATA5* and these samples presented more copy number gains than losses. However, 3 of the patients studied did not present any type of copy number alteration. It is also worth mentioning that 3 of the patients presented similar results in the three tissues studied, namely gain of *MSH6*, *VHL*, *GSTP1*, *CHFR*, *PYCARD*, *TP53*, *BRCA1*, *STK11* and *GATA5* and one of the patients only presented alterations in the tumor tissue, namely losses in *ESR1*, *PAX5*, *PAX6*, *WT1* and *BRCA2* and gains in *GSTP1*, *ATM*, *RB1*, *PYCARD* and *BRCA1*. On the other hand, one patient only revealed alterations in the non-tumoral and peri-tumoral tissues, namely gains in *MSH6*, *VHL*, *CHFR*, *PYCARD*, *BRCA1*, *STK11* and *GATA5*. Concerning the analysis of the methylation profile, the key genes which were shown to be methylated were *MSH6*, *ESR1*, *PAX5*, *KLLN*, *PAX6*, *WT1*, *GSTP1*, *CDH13* and

GATA5. These results are important to better characterize this neoplasm and to design different study approaches to identify potential genetic and epigenetic biomarkers.

P9. MOLECULAR KARYOTYPE OF HEPATIC NEOPLASMS

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Introduction: Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) are the two most common primary hepatic neoplasms. While HCC arises from hepatocytes, the CC is a rare malignant tumor originating from the epithelial cells of the biliary tree and is commonly classified as intrahepatic and extrahepatic, based on anatomical location. The HCC is the most common primary hepatic tumor, representing 85-90% of all cases. CC represents less than 2% of all human malignant neoplasms and about 3% of all gastrointestinal tumors but it is the second most common primary liver cancer representing 10-25% of all cases. The prognosis of these two types of tumors is usually poor since the diagnosis is not easy to obtain. The aim of this study was to perform a genomic characterization of HCC and intrahepatic (ICC) and extrahepatic (ECC) CC patients.

Materials and methods: The genomic characterization was performed by Array Comparative Genomic Hybridization (aCGH) in 11 HCC, 6 ECC and 7 ICC patients.

Results and conclusions: The results obtained revealed some common alterations between the patients of each group. Several HCC patients revealed gain of 1q, 2q37.2, 8q, 14q32.33 and 17q21.31 and loss of 3q26.1, 6p22.2 and 12p13.31. Regarding the ICC patients, the most common alterations observed were gain of 2q37.3 and Xp and loss of 3p, 6p25.3, 11q11, 14q, 16q, Yp and Yq. The patients of ECC also revealed some common alterations namely gain of 2q37.3, 6p25.3 and 16p25.3 and loss of 3q26.1, 6p25.3-22.3, 12p13.31, 17p, 18q, Yp. Some of these alterations are also common between patients of these three different groups. These regions contain genes whose alteration may be related to the development of these tumors. The genomic characterization of these patients is important to the study of such tumors since it allows to find potential biomarkers of both diagnosis and prognosis which is essential for achieving an earlier diagnosis and improving treatments.

P10. BASAL CELL CARCINOMA GENOMIC CHARACTERIZATION IN INDIVIDUALS TREATED BY RADIOTHERAPY FOR TINEA CAPITIS

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Introduction: Basal Cell Carcinoma (BCC) is the most common form of non-melanoma skin cancer (NMSC), representing 80% of the ca-

ses. Its histogenesis is not yet fully defined, but it is thought to originate from the basal cells of the epidermis and/or hair follicles. The development of BCC is associated with several risk factors, the main one being exposure to radiation, such as UV radiation or ionizing radiation (radiotherapy). In the first half of the twentieth century, most children who contracted *tinea capitis* were treated with low-dose X-ray therapy and several decades after the treatment, in adulthood, began to develop scalp BCC's, which are thought to be related with the exposure to ionizing radiation. The main goal of this study is to characterize, from the genomic point of view, biopsies of BCC's from individuals who were treated with radiotherapy for tinea capitis in their childhood.

Materials and methods: Genomic characterization was performed in 13 samples, using Array Comparative Genomic Hybridization (aCGH) technique.

Results: We found some common alterations in the patients of our cohort. There are gains in the number of copies in the following regions: 2q37.3 (38% of the cases), 9p21.3 (54% of the cases), 12p13.31 (54% of the cases), 15q11.2 (38% of the cases), 15q26.1 (38% of the cases); there are also copy number losses in the following regions: 3q26.1 (54% of the cases), 4q13.2 (31% of the cases), 5q11.1 (38% of the cases), 6p25.3 (31% of the cases), 9q21.11-q22.3 (62% of the cases), 16p11.2-11.1 (38% of the cases).

Conclusions: Since the reported results are only preliminary, further studies will have to be carried out in order to better understand how altered regions affect the development of BCCs. Genomic characterization studies may allow the discovery of biomarkers that, in the future, may be an important tool in the diagnosis and prognosis of basal cell carcinomas.

P11. SYNTHESIS OF CU(II) COMPLEXES DERIVED FROM IMIDAZOLE AND CYTOTOXIC ACTIVITY EVALUATION AGAINST BREAST CANCER

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Introduction: Despite the existence of new therapeutic options, breast cancer (BC) remains the leading cause of cancer death and the most commonly diagnosed among females worldwide. Studies have reported that imidazole derivatives show anticancer, antimicrobial, antibacterial, antifungal and antioxidant activities. Furthermore, recently it has been found that the association between imidazole ligands and copper increases their DNA binding affinity giving potential anticancer activity. So, we synthesized three novel Cu(II) complexes using heterocyclic nitroimidazole derivatives as ligands. The aim of this study is to evaluate the cytotoxic activity of these complexes in two BC cell lines.

Materials and methods: Nitroimidazole derived ligands containing cyclohexylamine, morpholine and piperidine and the respective Cu(II) complexes were synthesized. MCF-7 and HCC1806 (multidrug resistant) cell lines were cultured and grown in proper conditions. To evaluate the cytotoxic activity of these Cu(II) complexes on both cell lines, MTT colorimetric assay was used. MCF-7 and HCC1806 cells were seeded in 48 well-plates at a density of 50×10^5 cells per well for MCF-7 and 60×10^5 for HCC1806. Then, cells were treated with increasing concentrations of the complexes, from 0.5 to 200 μ M. After 48h of incubation, medium was removed and MTT was added. Two hours later, isopropanol was added in

order to dissolve formazan crystals. The absorbance was read at 570 and 620 nm.

Results: Preliminary results show that Cu(II) complexes exhibit anticancer activity in both cell lines and the respective IC₅₀ (half maximal inhibitory concentration) was calculated. When MCF-7 cells were treated with a 50 µM concentration of the complexes, cell proliferation was 11.0%, 92.0% and 81.7% for nitroimidazole derived complexes containing cyclohexylamine, morpholine and piperidine, respectively. For HCC1806 cells, a 50 µM concentration of the complexes containing cyclohexylamine, morpholine and piperidine show a cell proliferation of 0.5%, 65.7% and 7.9%, respectively. The complex containing cyclohexylamine presented the best anticancer activity for both cell lines (MCF-7: IC₅₀ = 22.2; HCC1806: IC₅₀ = 2.9).

Conclusions: Cu(II) complexes derived from nitroimidazole presented anticancer activity against two BC cell lines. The complex containing cyclohexylamine revealed to be the most promising compound in both cell lines, especially in HCC1806, basaloid triple-negative breast cancer, known as therapy-resistant.

P12. FADU, A PHARYNGEAL TUMOR CELL LINE: CYTOGENETIC AND GENOMIC CHARACTERIZATION

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Introduction: Pharynx cancer lies within the head and neck malignancies. It tends to grow silently, being the patients usually asymptomatic. Therefore, the diagnosis is frequently made in a late stage, when the disease has a poor prognosis. Pharyngeal carcinoma may cause pain, bleeding and impairment of vital functions such as swallowing, breathing and speaking. The characterization of tumor cell lines has 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 work, we aimed to perform the characterization of the FaDu cell line, a human epithelial cell line derived from a squamous cell carcinoma of the hypopharynx.

Materials and methods: The cell line was cultured in DMEM supplemented with FBS. The characterization was assessed by karyotype and array comparative genomic hybridization (aCGH) using a sex-matched healthy control.

Results: The cytogenetic results showed complex karyotypes with several structural alterations. This cell line is near-triploid, with an average number of 69 chromosomes. We identified several chromosomal rearrangements, including mainly the formation of isochromosomes and whole-arm translocations. aCGH showed several genomic imbalances, being the chromosomes 1, 4, 5, 8, 11, 12, 13, X and Y the most altered ones. In addition, through karyotype, three copies of apparently normal chromosomes were frequently observed, such as 16, 17 and 18. These results are in agreement with aCGH.

Conclusions: We observed a relationship between genomic imbalances and cytogenetic rearrangements. These findings are useful to establish the genetic profile of hypopharynx carcinoma. The use of well-characterized cancer cell lines constitutes a powerful tool to improve our understanding of the molecular mechanisms underlying pharyngeal tumors, as well as to provide a research basis for pharmacological studies.

P13. CYTOTOXICITY OF RU(II) AND RU(III) SALEN COMPLEXES AGAINST BREAST CANCER CELL LINES

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Introduction: Breast cancer in women has a very high incidence rate, and is thus the main cause of death by this disease worldwide. The search for new anticancer drugs has increased in the last decades since chemotherapeutic drugs used nowadays show many adverse effects and cancer resistance. Previous studies have shown that metallic salen complexes exhibit antitumor activity and therefore their potential as possible chemotherapeutic agents has been analyzed. Additionally, Ru complexes have revealed cytotoxic activity, proving greater selectivity for tumor cells compared to normal ones. They present different mechanisms of action and are less toxic relatively to Pt complexes, being for this reason pointed out in the literature as a credible alternative to current drugs used in chemotherapy. The aim of this study is to synthesize four novel Ru(III) and Ru(II) chlorinated salen complexes and test their cytotoxicity on two breast cancer cell lines, MCF-7 and HCC1806.

Materials and methods: Ru salen complexes were synthesized from camphoric acid derivatives, MCF-7 and HCC1806 breast cancer cell lines were cultured in appropriate culture medium. The effect of the compounds on cell metabolic activity was evaluated by colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test. For this study, 50000 MCF-7 cells and 60000 HCC1806 cells were seeded per well in 48-well plates and after 24h were incubated with increasing concentrations of the complexes (0.5 to 200 µM). After 48h, cell proliferation was evaluated through MTT assay. Dose response curves were plotted and IC₅₀ (half maximal inhibitory concentration) values for each ruthenium complex were determined.

Results: All compounds induced a decrease in cell viability in a dose-dependent way. Preliminary studies show that for the MCF-7 cell line the tetrachlorinated Ru(III) complex presents greater cytotoxicity (IC₅₀ < 5 µM) than the other compounds which have IC₅₀ values of 10-20 µM. Similar results were observed in the HCC1806 cell line in the presence of the same complex (IC₅₀ < 5 µM) and for the remaining compounds, IC₅₀ values were 10-15 µM. The tetrachlorinated Ru(III) complex is, consequently, the most promising showing similar cytotoxic activity in both cell lines.

Conclusions: All compounds revealed dose-dependent cytotoxic effects. The tetrachlorinated Ru(III) complex was found to be the most promising, exhibiting high anticancer activity in both cell lines, namely in the HCC1806 chemoresistant cell line.

P14. PROGNOSTIC BIOMARKERS FROM GENOMIC, EPIGENETIC AND TRANSCRIPTOMIC DATA OF HEAD AND NECK CANCER

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is an emergent health problem worldwide. These tumors present heterogeneity at phenotypical, aetiological, biological and clinical level. A significant percentage of HNSCC patients develop loco-regional and distant recurrences. Even with progresses in surgery, radiation and chemotherapy, approximately half of all patients die of the disease. Risk stratification for HNSCC is essential to decrease mortality and improve quality of life of the patients. The present work aimed to perform a molecular characterization of HNSCC to predict recurrence/metastasis development using genomic, epigenetic and transcriptomic approaches.

Materials and methods: We analyzed the same HNSCC patients through different molecular technologies, namely Multiplex Ligation-dependent Probe Amplification (MLPA), Methylation Specific MLPA (MS-MLPA) and microarray approaches. The identified biomarkers and molecular signatures were validated with TCGA (The Cancer Genome Atlas) data.

Results: We report different genetic and epigenetic signatures related to tumor stage and anatomic site as well as tobacco use, such as gains at *MYC* and *WISP1* genes, losses at *MLH1* and *ATM* genes and gene promoter methylation of *WT1*, *MSH6*, *GATA5* and *PAX5*. The genetic analysis of non-tumor samples (from surgical margins) revealed some imbalances similar to those observed in the tumor samples, which reinforce the importance of molecularly analyze the high-risk patients even before the visible morphological changes and also the suspicious lesions in order to early diagnose these tumors and their recurrences. We also identified molecular signatures with capability to predict the recurrent/metastatic disease development and clinical outcome that comprise chromosomal regions of 5p, 6p, 8p, 9p, 11q, 12q, 15q and 17p, where are mapped important genes for the carcinogenesis process.

Conclusions: In this study, using either direct probe panels or genome-wide approaches we identified the most common chromosomal regions with imbalances and altered genes. As expected, whole-genome techniques revealed new chromosomal regions and genes that seem to have a role in HNSCC development and behavior. Overall, through these comprehensive genomic, epigenetic and transcriptomic characterization we identified potential biomarkers and molecular signatures of prognosis and survival, which may open the door for personalized medicine in HNSCC patients.

P15. ANTICANCER ACTIVITY OF RNA ISOLATED FROM THE *CANTHARELLUS CIBARIUS* MUSHROOM

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Cantharellus cibarius is an edible mushroom of ample consumption due to its properties. Previous studies revealed that this mushroom has a high antiproliferative activity against several cancer cell lines. The main responsible for this action is a fraction, isolated by anion exchange chromatography, which was thought to be mainly composed of ribonucleic acid (RNA). In this study, we aim to confirm the nature of the molecule carrying antitumor activity and observe its action. The fraction with anticancer activity was characterized by evaluating

its composition in sugars and nucleic acids (UV-vis spectrophotometry, agarose gel electrophoresis and FTIR). Detected RNA was sequenced, followed by comparison to existing databases. Moreover, the isolated fraction was placed in contact with cancer cell lines, namely colorectal cancer cells (Caco-2) followed by RNA-FISH methodology in order to detect and localize the RNAs within cells. The results achieved in this work suggest that the triggering components of this anticancer activity are the small RNAs: (1) typical UV-vis spectrum of nucleic acids, (2) small RNAs presence in the agarose gel, (3) FTIR spectrum typical of nucleic acids, namely RNA and (4) ribose as the main sugar constituent of the isolated fraction, with no indication of deoxyribose. It was observed that the fraction under study shows a high antitumor activity, even at low concentrations, since cell death of Caco-2 cells occurred in contact with it after 48h. This molecule showed to be stable due to its ability to survive all extraction processes from the mushroom (insofar as no kit was used) and to integrate inside the Caco-2 cells crossing both cellular and nuclear membranes (observed by RNA-FISH). Through bioinformatic analysis we conclude that the sequence responsible for this anticancer activity is a new sequence that belongs to the *C. cibarius* genome and that does not exist in the Human genome. It is an RNA of small size, with a high percentage of guanines and cytosines and capable of forming stable secondary structures. Thus, we assume that it is a regulatory small non-coding RNA (sncRNA). *C. cibarius* has been shown to be a mushroom with high anticancer activity. Once the target RNA sequence and its interactions with the cell have been discovered, it becomes promising for future artificial synthesis of this RNA (mimics). Also, we may be in the presence of a new therapeutic target that may be used in the future against certain types of cancer.

P16. METHYLATION AND COPY NUMBER ALTERATIONS IN LARYNGEAL CANCER

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Introduction: Laryngeal cancer is the second most common malignancy of the head and neck, accounting for approximately 20% of cases. In Portugal, the 3rd European country with the highest incidence of laryngeal cancer, approximately 600 new cases are diagnosed per year. With a low 5 year-survival rate, mainly explained by a late diagnosis, tumour aggressiveness and rapid metastatic process, it is essential to identify biomarkers to anticipate cancer detection in an early stage. Previous studies reported multiple chromosomal regions amplified or deleted in Laryngeal squamous cell carcinoma (LSCC). Moreover, epigenetic modifications especially affecting tumour suppressor genes seem to be involved in the pathogenesis and progression of LSCC. The main goal of this study was the cytogenomic evaluation and DNA methylation patterns characterization of laryngeal cancer in order to identify putative diagnostic and prognostic biomarkers.

Materials and methods: Tumour and non-tumour laryngeal tissue samples obtained from twenty one patients diagnosed with laryngeal cancer were used. Detection of copy number variations (CNVs) was performed using array Comparative Genomic Hybridization (aCGH) and Methylation Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA). Furthermore, methylation patterns of target genes were assessed by MS-MLPA analysis.

Results: aCGH revealed frequent gains of chromosomes 3q, 7p, 8, 9q, 11q, 12p, 17q and 18p while losses were frequently found in chromosome 3p, 9p, 11p and Y. Amplifications of *GATA5* and *CDK6* were the most common events among tumour samples while *VHL*,

CDKN2A, *ATM* and *CADM* genes were found to be frequently deleted. Methylation of *GATA5* was frequent in tumour samples being associated with late stages while *WT1* was highly methylated in non-tumour samples, being an early epigenetic event in laryngeal cancer. **Conclusions:** This study confirmed some cytogenomic alterations associated with laryngeal carcinoma that have already been reported. Additionally new alterations have been identified. Further studies are required in order to validate the role of these genes and cytogenetic regions as diagnostic and prognostic biomarkers in laryngeal cancer.

P17. CAP-INDEPENDENT TRANSLATION OF MTOR IS INHIBITED BY RAPAMYCIN

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Introduction: The mammalian target of rapamycin (mTOR) is hyper-activated in diabetes, cardiovascular diseases and cancer. It has been reported that mTOR protein levels (PL) are maintained in stress conditions in which protein synthesis is globally inhibited by regulators of the canonical translation mechanism. A recent study resolved this conundrum by demonstrating that mTOR translation occurs by an alternative cap-independent mechanism that allows maintenance of mTOR PL in adverse conditions. Maintenance of mTOR signals is beneficial in several stress conditions, as this kinase contributes to a proper cellular response. Nevertheless its over-activation induces exacerbated cell proliferation and growth, promoting cancer development. Accordingly, mTOR is an attractive therapeutic target and several efforts have been made to develop mTOR inhibitors (mTORis). FDA approved the mTORis everolimus and temsirolimus for treatment of several tumor types and although both possess antiproliferative properties, their efficacy is lower than expected.

Objective: Accordingly, the goal of this study is to inhibit mTOR, by impairing its cap-independent translation (CIT).

Materials and methods: For that, we transfected HEK293T cells with dicistronic plasmids and treated, subsequently, with 4EGI-1 or thapsigargin (translation inhibitors) or rapamycin (mTORC1 inhibitor).

Results: Results demonstrated that treatment with 4EGI-1 or thapsigargin is not effective at reducing mTOR PL. On the other hand, rapamycin diminishes mTOR PL through inhibition of CIT. As the observed decrease at both PL and CIT was very modest, we performed the same test but using higher doses of this compound. By using rapamycin at higher doses, a pronounced decrease of mTOR CIT was observed, which was accompanied by a marked reduction of PL.

Conclusions: From these results we conclude that mTOR is refractory to some known translation inhibitor compounds, and that rapamycin not only inhibits mTORC1 but also the mTOR protein itself, by acting on mTOR CIT. The inhibitory extent to which rapamycin decreases mTOR translation and, concomitantly, mTOR PL is dose-dependent. Our work suggests that mTORis targeting mTOR CIT might be a good therapeutic strategy.

P18. NOVEL 4,5,6,7-TETRAHYDROPYRAZOLO[1,5-A]PYRIDINE FUSED CHLORINS AS VERY CYTOTOXIC COMPOUNDS AGAINST MELANOMA CANCER CELLS

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Introduction: Photodynamic therapy (PDT) is a clinically approved, minimally invasive therapeutic procedure, which is entering the mainstream of cancer treatments. Nowadays PDT has been successfully used in the treatment of skin cancers, but the use of photodynamic therapy against melanoma can be compromised due to the natural resistance mechanism of some melanoma cancer cells. Thus, the search for new photosensitizers able to overcome the resistance of melanoma to photodynamic therapy is a relevant research goal.

Materials and methods: A375 (Human melanoma cells) were propagated according to standard procedures. 24 hours later a series of new the photosensitizers were administered and 24 hours later irradiated with a proper device. For each experiment, cells were plated and kept in the incubator overnight. The formulation of the sensitizers consisted in a 1 mg/mL solution in DMSO and the desired concentrations being achieved by successive dilutions. The sensitizers were administered in several concentrations (from 1 nM to 10 mM) and cells were incubated for 24 h. Controls were performed on every test. Cells were washed with PBS and new drug-free medium was added. Each plate was irradiated with a fluence rate of 7.5 mW/cm², to reach 10 J. Evaluation by MTT assay was performed 24 h after the photodynamic treatment in order to evaluate the cytotoxic effect.

Results: Our previous in vitro PDT studies demonstrated that increasing the hydrophilicity of the chlorins leads to a higher activity against A375 melanoma cells. Therefore, a series of novel 4.5.6.7-tetrahydropyrazolo[1.5-a]pyridine-fused chlorins bearing dicarboxylic acid and monocarboxylic moieties were developed showing an interesting biological activity in the A375 human melanoma cells. Inhibition of the metabolic activity seems to be dependent on the concentration of the sensitizers used. With the experimental metabolic activity values, it was possible to calculate the concentration of the sensitizers that inhibits the proliferation of cultures in 50% (IC50). For this series of compounds, IC50 values ranged from mM to nM concentrations. Nevertheless, a new molecule with an IC50 value of 67.93 nM stood out.

Conclusions: The compounds tested were active against human melanocytic melanoma A375 cells. MTT assay showed that the metabolic activity was inversely proportional to the concentration of the photosensitizer. Interestingly low IC50 values in the nanomolar range encourage further studies.

P19. MICROGLIA PROMOTE ENDOTHELIAL CELLS DYSFUNCTION IN GLIOBLASTOMA

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Introduction: Glioblastoma multiforme (GBM) is the most common and aggressive brain tumor, with an average life expectancy of 12-15 months. GBM is highly infiltrated by microglia (MG) that under the tumor microenvironment acquire an activation phenotype with tumor-supportive features that promote the tumor growth and invasiveness. Additionally, it appears to induce alterations in the permeability of the blood-brain barrier (BBB), although the mechanism is not clarified. Herein, we evaluated the effects of reciprocal interactions between MG and GBM cells in the integrity of endothelial cells (ECs) monolayer.

Materials and methods: Microglial BV-2 cells were co-cultured with U87 GBM cells in a transwell system during 48h. A monolayer of the human brain endothelial cell line hCMEC/D3 was exposed to condi-

tioned medium of the co-culture (CM-CC). The transendothelial electrical resistance (TEER) and the macromolecular flux of 4 kDa-FITC and 70 kDa-RITC across the ECs monolayer were measured. The intercellular junction proteins β -catenin and zonula occludens (ZO)-1 in ECs was analyzed by immunocytochemistry.

Results: The exposure of ECs monolayer to the conditioned medium harvested from the MG/GBM co-culture induced a decrease in the TEER and an increase in permeability of both fluorescent dyes across the confluent ECs in relation to control cells. These effects were accompanied by a decrease in the expression of the intercellular junction proteins, namely in β -catenin and ZO-1 that are important elements of the intercellular junction structure.

Conclusions: The present work shows that MG under the influence of GBM cells creates an inflammatory environment that destabilizes the intercellular junction proteins with subsequent disruption of the endothelial cells monolayer integrity, highlighting the role of microglial activation in BBB dysfunction in brain tumors.

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P20. IMPACT OF SPLENIC ARTERY LIGATION AFTER ALPPS ON LIVER VIABILITY, REGENERATION AND FUNCTION - OUTCOMES OF AN EXPERIMENTAL STUDY IN ANIMAL MODEL

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Introduction: The ALPPS (“Associating Liver Partition and Portal vein ligation for Staged hepatectomy”) has been recently described as a revolutionary strategy in hepatobiliary surgery, becoming widely used. However, the complex mechanisms for rapid hepatocellular regeneration associated with ALPPS are not well known. On the other hand, the technique bounds to a high rate of morbidity and mortality due, mainly, to postoperative liver failure.

Materials and methods: Thirty-one rats were submitted to portal ligation and in situ splitting (ALPPS) with (n = 15) and without splenic artery ligation (n = 16). The control group (laparotomy and/or pedicle transection) included 14 animals. After animal occlusion (12, 24, 48 and 120h after surgery), blood and hepatic tissue samples were collected to evaluate hepatic function, regeneration and viability.

Results: The animals submitted to splenic artery ligation at 12h revealed better hepatic function and less reactive species production. When evaluated at 48h, the group submitted to splenic artery ligation had a higher percentage of cell death by apoptosis and a lower reactive species production. 120h after surgery there is higher cell viability and lower reactive species production in the group submitted to splenic artery ligation.

Conclusions: This experimental study suggests that splenic artery ligation in ALPPS, by modulating the portal flow, promotes an increase in hepatocellular viability and regeneration, with no func-

tion impairment, probably related to a decrease in oxidative stress.

P21. VALIDATION OF A SIRNA TARGETING PI3KCA GENE TOWARDS COLON CANCER THERAPY

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Introduction: PI3K is an enzyme comprised by two subunits, a regulatory and a catalytic one. Although there are several isoforms, the catalytic subunit p110 α , encoded by the gene PI3KCA, is often mutated in several cancers including colon cancer. The mutant p110 α shows a gain of function in enzymatic and signalling activity that can lead to an increased cell growth, survival, proliferation and motility. Therefore, the silencing of this gene could be an interesting and promising therapy. For this purpose, small interfering RNA molecules designed to target a specific mRNA for degradation, thus used for silencing protein coding genes, are a possible solution. The aim of this work is to validate a siRNA against PI3KCA as a therapy for colon cancer and to understand the extent of its effects.

Materials and methods: In this study, we used HCT 116 colon cancer cell line, which contains a mutation in the PI3KCA gene. This cell line was transfected with a siRNA against PI3KCA using Lipofectamine RNAiMAX Reagent (Invitrogen) in a concentration of 30 nM (previously optimized with a fluorescently labelled siRNA). Its effects were evaluated 72 h after the transfection through western blotting, Sulforhodamide B (SRB) assay and cell cycle analysis.

Results and conclusions: Western blotting results revealed a decrease of 24% in the levels of p110 α in transfected cells in comparison with the controls. However, the SRB assay demonstrated no relevant differences between transfected cells and the controls. Therefore, this siRNA does not affect the proliferation of this cell line, unlike what happens in other colon cancer cell lines such as RKO. In the cell cycle, it was possible to observe an accumulation of cells in the G0/G1 phases, which could mean a cell cycle arrest at this point. Additional experiments are needed to better understand the effects of this siRNA and its potential as a therapy for colon cancer.

P22. RADIATION EFFECTS ON HUMAN CELL LINES OF OSTEOSARCOMA AND RETINOBLASTOMA

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Introduction: In paediatric ages, osteosarcoma (OS) is the most common bone sarcoma and retinoblastoma (RB) is the most common

intraocular cancer. Additionally, it is known that patients with RB during early childhood are at risk of developing OS during adolescence. Aim: study radiation effects on viability and proliferation on human cell lines of osteosarcoma (MNNG/HOS) and retinoblastoma (Y79) as well as cell cycle and type of cell death after irradiation and calculate the half lethal dose (LD50).

Materials and methods: MNNG/HOS and Y79 cells were cultured and exposed to ionising radiation (IR) doses ranging from 0 Gy (control) to 12 Gy. Cell survival was evaluated by clonogenic assay. Cell viability and proliferation were assessed by trypan blue exclusion assay and Ki-67 expression. Flow cytometry was used to evaluate the cell cycle and cell death. The cell morphology was assessed by optical microscopy with May-Grünwald Giemsa staining.

Results and conclusions: After irradiation, we observed a cytotoxic and an antiproliferative effect in both cell lines in a dose-dependent way for MNNG/HOS cells and in dose and a time-dependent way for Y79 cells. For both cell lines, the LD50 was calculated using the linear quadratic model. The Y79 cells present a lower LD50 (1.21 Gy) than the MNNG/HOS cells. The last ones showed a higher radioresistance with an LD50 twice higher (2.42 Gy) than the Y79 cells. The higher doses of IR cause a cell cycle arrest in G2/M phase and cell death mainly by apoptosis in the MNNG/HOS cell and also in the Y79 cells. Radiotherapy has an anti-tumour effect in different types of malignancies by inhibiting tumour cell growth, promoting cell death and inducing cell cycle arrest. As is showed in our results, IR aggression induced a cytotoxic and antiproliferative effect, a cell cycle arrest and cell death in both cell lines. The cell cycle arrest in G2/M phase that can be related to the significant increase of reactive oxygen species (ROS) or to the attempt of the cell to repair damages, like DNA double-strand breaks, caused by the radiation. When repair mechanisms fail the cell death happens mainly by apoptosis.

P23. CELLULAR RESPONSE TO IONIZING RADIATION: IN VITRO STUDIES ON HUMAN PROSTATE CANCER CELL LINES

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Introduction: Prostate cancer (PCa) is one of the most commonly diagnosed malignancies among men in the world, being the second most common cancer in men and the fourth most common cancer among all cancers. Radiotherapy (RT), usually hypofractionated, is one of the therapeutic options to treat PCa, however, the cellular and molecular mechanisms involved in RT still need to be deepened. **Objective:** To study the cellular and molecular effects of ionizing radiation (IR) in two human prostate cancer cell lines (PC3 and LNCaP).

Materials and methods: PC3 and LNCaP were cultured and exposed from 0 Gy (control) to 12 Gy of IR. Cell survival was evaluated by

clonogenic assay. Cell viability and proliferation were assessed by trypan blue exclusion assay and Ki-67 expression through immunocytochemistry. Levels of oxidative stress (OS) (peroxides, superoxide anion) and antioxidant defenses (GSH) were evaluated 48 hours after irradiation, as well, cell cycle and cell death assessed by flow cytometry, besides cytomorphology using optical microscopy.

Results: IR induced cytotoxic and anti-proliferative effects for both PCa cell lines in a dose and time-dependent way. The main type of cell death observed for PC3 cell line was by apoptosis, while for LNCaP cell line necrosis was the most common type of cell. For both cell lines, there was an alteration in OS levels, with an increase of the peroxides and superoxide anion levels and a decrease of GSH expression. The cell cycle was blocked in the G2/M phase for both cell lines.

Conclusions: Our results showed a decrease in viability and proliferation, supported by a decrease in Ki-67 expression. The linear aggression model was the best fit for both cell lines, with LD50 of 1.75 ± 0.07 Gy for PC3 cells and of 1.68 ± 0.03 Gy for LNCaP cells. We verified an increase of cells with apoptotic characteristics by May-Grünwald Giemsa with the increased radiation doses for PC3 cell line and an upsurge of necrotic cells for LNCaP cell line. The IR induced a blockage in G2/M phase in both cell lines. For PC3 cell line, we presume that this block could be explained by the fact of this cell line be characterized as P53null and also by the increase of ROS production. In another hand, on LNCaP cell line the blockade observed could be clarified by the effect of IR on cells that lead to an activation P53 independent pathways. These results highlight the importance of ionizing radiation as a valuable therapeutic target in prostate cancer.

P24. RADIOBIOLOGICAL EFFECTS UNDERLYING THE CLINICAL EFFICACY OF RADIUM-223 IN METASTATIC PROSTATE CANCER: IN VITRO STUDIES

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Introduction: Regarding to Prostate Cancer (PCa), metastatic Castration-Resistant Prostate Cancer (mCRPC) presents the greatest challenge in terms of therapy. Recently, the radiopharmaceutical Radium-223 (²²³Ra) has contributed to the hope of these patients, however, little is known about the mechanisms of action involved in the therapeutic response. In view of this, the main objective of this study is to clarify and deepen the knowledge about the mechanisms underlying the clinical efficacy of ²²³Ra.

Materials and methods: Two PCa cell lines were irradiated, PC3 (hormone-resistant, representative of mCRPC) and LNCaP (hormone-sensitive, representative of a less advanced stage of PCa). These cells were irradiated with ²²³Ra at different doses (0.25-10 mGy), to determine the cell survival factor (SF), by clonogenic assay. Kinetic assays allowed to determine the ability of ²²³Ra to reach the nucleus of cells. Also, studies of viability, DNA damage, oxidative stress and the study of active signaling pathways in radiation response were carried out.

Results: We found that LNCaP cells (LD 50-1.41 mGy) had a radiosensitivity significantly higher than PC3 cells (LD 50-4.22 mGy). The 223Ra was shown to be uptake, internalized in the nucleus and retained by PC3 cells in percentages of 1.43, 2.29 and 3.99, respectively. For LNCaP cells, the percentages assume values of 1.39, 3.62 and 5.45, respectively. Concerning viability there is a significant decrease between the control and the doses of 1, 4 and 10 mGy. In terms of oxidative stress, results demonstrate a tendency to increase ROS associated with a decrease in AD. These results are further associated with a distinct activation of CHK2, a nuclear protein involved in cell cycle entrainment and mediator of various pathways of cell death and/or senescence.

Conclusions: Studies have shown that 223Ra decreased survival and cell viability and that it can effectively reach the nucleus of PCa cells. The similar results in both cell lines pointing to a possible therapeutic application in earlier stages of this pathology. These results also suggest that 223Ra has a direct effect on CaP cells that are in the bone metastatic niche, with potential to reduce the aggressiveness of these cells. Due to the direct and indirect damage occurs the activation of important signaling pathways and systems that lead to cell death, contributing to the clinical efficacy of the use of this radiopharmaceutical.

P25. PRODUCTION OF REACTIVE OXYGEN SPECIES IN BREAST CANCER WITH COLD ATMOSPHERIC PLASMA TREATMENT

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Introduction: Breast cancer is among the most diagnosed cancers with about 1.67 million new cases. The need for new, effective and free of side effects therapies is growing as aging is modifying the epidemiology of cancer. Cold atmospheric plasma (CAP), a gas with enough energy to ionize a significant fraction of particles, has come into attention as a potential anti-tumour therapy. Our previous studies showed that CAP determined the decrease of breast cancer cells viability after an exposure of only 60 seconds. Aim: the goal of this study was to evaluate the effect of cold atmospheric plasma on breast cancer cell lines concerning the production of reactive oxygen species (ROS).

Materials and methods: In this study, we used two different representative breast cancer cell lines: hormonal receptor positive breast cancer (MCF7) and triple negative breast cancer (HCC1806). Cells were cultured, plated and exposed to CAP, using a homemade CAP ejector, for different periods of time: 60 and 120 seconds. To investigate the ROS, cell cultures were evaluated through specific probes, namely 2',7'-dichlorodihydrofluorescein diacetate (DCFH2-DA) and dihydroetide (DHE). The levels of glutathione antioxidant defense (GSH) were also evaluated. Studies were performed 2 and 24 hours after CAP exposure.

Results: After 2 hours of CAP therapy, ROS levels do not show any variation compared to the control on both cell lines. For example, intracellular content of peroxides was (97.84 ± 21.74)% on MCF7 and (91.61 ± 9.53)% on HCC1806 at 120 seconds. Evaluation after 24 hours of exposure to treatment showed that intracellular content of peroxides increased with an exposure of 60 seconds. It was (143.62 ± 15.59)% on MCF7 cell line. Levels of glutathione remain similar to the control in both cell lines.

Conclusions: Levels of oxidative stress and antioxidant defenses suggest that other events besides ROS formation might be involved

in the plasma effect on breast cancer cells. The results obtained encourage further studies.

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P26. EFFECT OF COLD ATMOSPHERIC PLASMA IN HUMAN CANCER CELLS LINES

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Introduction: The need for effective and free of side effects new therapies is growing as aging is modifying the epidemiology of cancer. Cold atmospheric plasma (CAP), a gas with enough energy to ionize a significant fraction of particles, has come into attention as a potential anti-tumour therapy. Aim: the goal of this study was to investigate the effect of cold atmospheric plasma on different tumour cells.

Materials and methods: To evaluate the cytotoxicity of plasma in different human tumours: osteosarcoma (MNNG-HOS), melanoma (A375), lung cancer (H1299), colon carcinoma (WiDr), prostate cancer (PC3 and LnCap), extrahepatic bile duct carcinoma (TFK-1), urinary bladder grade three carcinoma (HT-1376), esophageal adenocarcinoma (OE19), hormonal receptor positive breast cancer (MCF7), triple negative breast cancer (HCC1806) and endometrial cancer (ECC-1) cell lines were cultured, plated and exposed to CAP, using a homemade CAP ejector, for different periods of time ranging from 15 to 120 seconds. Cell cultures were evaluated through colorimetric MTT and SRB assays 24 hours later.

Results: Most cell lines showed a decrease in metabolic activity and protein content after 60 seconds, being more evident after 120 seconds CAP exposure. H1299, LnCap, ECC-1 and cells were the most sensitive to CAP within this study. The observed metabolic activities were 3.39 ± 0.48% (p < 0.001); 4.58 ± 0.89% (p < 0.001) and 8.43 ± 1.90% (p < 0.001), respectively, while protein content was of 12.40 ± 1.69%, (p < 0.001); 6.21 ± 0.56%, (p < 0.001) and 20.18 ± 2.93%, (p < 0.001). Although, decrease of protein content on PC3 and OE19 cells was not as pronounced as in the remaining tumour cell lines tested, the metabolic activity was lower.

Conclusions: The effects of CAP treatment suggest that this new form of therapy is cytotoxic in several types of human cancer cells. The results obtained encourage further studies.

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P27. NOVEL PHOTOSENSITIZERS AS PROMISING THERANOSTIC AGENTS FOR CANCER TREATMENT

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Introduction: We recently developed of a new type of photochemically stable platinum (II) 4,5,6,7-tetrahydropyrazolo[1,5-a]pyridine-fused chlorins, which are remarkable photosensitizers that can be

used in photodynamic therapy (PDT), due to its therapeutic capacity. Simultaneously, due to its highly luminescence properties, in the biological relevant 700-850 nm red and NIR spectral region, they may be used for biological imaging. In addition, photophysical studies indicate that they may be used as ratiometric oxygen sensors.

Materials and methods: Photocytotoxicity studies were carried out against two human tumour cell lines, the OE19 line of oesophageal carcinoma and the A375 line of melanocytic melanoma, in order to test their potential therapeutic effects in PDT. Four compounds were tested (MADCA 7, 8, 12 and 14). The new compounds are characterized by different degree of hydrophilicity, with compounds MADCA 12 and 14 being more hydrophilic than MADCA 7 and 8.

Results: IC50 values were calculated. Preliminary studies indicate that MADCA 7 and 8 show IC50 values greater than 10 μM against both cell lines. MADCA 14 presents values of 2149 nM and 4813 nM for A375 and OE19 cell lines, respectively. Interestingly, MADCA 12 proved to be the more active PDT agent exhibiting IC50 of 571 nM and 1562 nM against A375 and OE19 cell lines, respectively.

Conclusions: The study demonstrated that platinum (II) 4.5.6.7-tetrahydropyrazolo[1.5-a]pyridine-fused chlorins with more hydrophilic characteristics require lower doses of photosensitizers to induce a significant photocytotoxic effect on tumour cells.

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P28. CAN WE EAT TO PREVENT CANCER? THE INFLUENCE OF DIET ON COLORECTAL CANCER

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Introduction: Industrialization, western lifestyle and changes in environmental and dietary factors are possible causes of the increasing prevalence of colorectal cancer (CRC). The Mediterranean diet (MDiet) is considered one of the healthiest diet models because it has positive functional effects on the health and well-being of the individual, being its main benefit the synergy between combinations of nutrients and not isolated nutrients. The type of diet accounts for about 30% to 50% of the worldwide incidence of CRC. Several studies suggest that the adherence to a dietary pattern based on the MDiet prevents the development of certain types of cancer, due to the role of dietary fiber, as well as, the diversity of vitamins and substances with antioxidant properties. To date, there are few studies conducted in the Portuguese population. This work constitutes an exploratory study in the “Região Centro” of Portugal and aims to understand the impact of diet on CRC.

Materials and methods: 247 subjects (M-148, H-99, 52 \pm 20 years) were asked to answer a Food Frequency Questionnaire (FFQ) to

evaluate their eating habits. The questionnaire was done in accordance to the instructions provided by the survey itself and available at the site <http://higiene.med.up.pt/instrucoes.htm>. Food surveys were included in a database Access and the conversion of food into nutrients was done using Food Processor Plus software. Nutritional information allowed the determination of the adherence to the MDiet through the MediScore scale and the influence of exercise by metabolic equivalent values (METs) calculation. The information obtained allowed to compare several parameters between the control group (n = 125) and the group with CRC (n = 122), using SPSS.

Results: CRC group showed a statistically significant increase in calories, proteins, carbohydrates, fat, sugar and cholesterol intake compared to the control group (p < 0.05). The ingestion of these nutrients conditioned the onset of CRC, with predominance in the descending colon and rectum (p < 0.05). In contrast, the control group had METs significantly higher than the CRC group (p < 0.001). No differences were observed on MediScore values.

Conclusions: Diet of CRC patients was characterized by the generalized high consumption of nutrients. This high consumption is correlated with the appearance of CRC in the terminal region of the colon. Physical exercise was associated with a lower risk of CRC.

P29. TARGETING RETINOBLASTOMA WITH PLASMA-ACTIVATED MEDIUM

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Introduction: Retinoblastoma (RB) is the most common childhood malignant tumor affecting primarily the retina being highly dependent of vascular supply. In the last years, the possibility to use plasma as a therapeutic approach against cancer has been studied. Plasma is a half-ionizing gas, normally used in its non-thermal form. Our previous results with direct cold atmospheric plasma (CAP) treatment have showed that the metabolic activity, the protein content and the mitochondrial membrane potential were decreased in treated RB cells. Nevertheless, these effects were less evident in normal fibroblasts. Considering the vascularization of RB, the interaction of CAP with surrounding fluids is a very relevant topic. In this way, administration of plasma-activated liquids could be an interesting approach. Emerging evidences supports that plasma-activated media (PAM) can be useful in killing various cancer cell lines. In this sense, the aim of this work was to evaluate the effect of PAM in human retinoblastoma cells.

Materials and methods: Cell culture media was conditioned by CAP up to 120 seconds. Y79 cell cultures were incubated with this medium for 24 hours. Trypan blue was performed to assess PAM cytotoxicity in the RB cells while Alamar blue was used to evaluate the metabolic activity. Furthermore, SRB assay was carried out to quantify the protein content of the treated cells.

Results: Preliminary results showed that CAP treatment affects cell viability. The presence of CAP led to a cell dead of (33.24 \pm 2.53)% of RB cells. Likewise, treated cells with PAM had a lower metabolic activity (77.98 \pm 8.90)% and a lower protein content (38.67 \pm 5.37)% when compared to control.

Conclusions: The selective properties of CAP, already described, together with these results point to a new cancer therapy based on PAM. These positive results suggest that PAM should be investigated as a potential adjuvant treatment for the present therapies.

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P30. MICROVASCULAR INVASION: DETERMINANT FACTOR FOR PATIENTS OUTSIDE MILAN CRITERIA IN LIVER TRANSPLANTATION

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Introduction: Hepatocellular carcinoma (HCC) is an aggressive tumor with rising incidence worldwide and represents a major global health problem. Microvascular invasion (MVI) is an important prognostic factor after liver transplantation (LT) in patients with cirrhosis and HCC, but cannot be evaluated pre-LT by imaging studies or hepatic biopsy.

Materials and methods: From January of 2010 to December of 2015, 333 LT were performed, 81 for HCC (58.7 ± 6.7 years), predominantly male patients (85%). Median of MELD was 13 and 52% Child-Pugh B/C. Fifty-six percent of patients were inside Milan criteria (MC). Study of prognostic factors (PF) of overall survival (OS) and disease free-survival (DFS). Univariate and multivariate analysis (p < 0.05).

Results: At 90 days mortality rate was 4.9% and major morbidity 37%. MVI was the most important PF in OS (p = 0.018) and in DFS (p = 0.006). At 5 years, OS was 91.1% vs. 76.4% (p = 0.131) and DFS was 91.3% vs. 71.5% (p = 0.064) for patients that were inside or outside MC. For patients outside MC, OS (p = 0.026) and DFS (p = 0.009) were significantly worse when MVI was present. The same was not observed for patients inside MC, OS (p = 0.816) and DFS (p = 0.816).

Conclusions: Morphologic parameters are insufficient to predict recurrence of HCC or its biology. Using MC can lead to the exclusion of patients that could benefit from LT. The diagnosis of MVI in pre-LT by imaging exams or the knowledge of genetic and molecular biology of HCC is of greater relevance for its effective treatment.

P31. HEPATOCELLULAR CARCINOMA - THE IMPORTANCE OF AN INDIVIDUALIZED APPROACH IN BCLC D PATIENTS: BEST SUPPORTIVE CARE?

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Introduction: Hepatocellular carcinoma (HCC) is an aggressive tumor with rising incidence worldwide and represents more than 90% of primary hepatic malignancies. Tumor extension, liver function and performance status are the most important prognostic factors.

Liver transplant (LT) is the only therapeutic option with potential to simultaneously cure HCC and subadjacent chronic liver disease. For patients with end-stage disease BCLC D (bad performance and/or Child-Pugh C) palliative treatment is the only indicated, with survival less than 3 months.

Materials and methods: From January of 2010 to December of 2015, 333 LT were performed, 81 for HCC (58.7 ± 6.7 years), predominantly male patients (85%). Median of MELD was 13 and 52% Child-Pugh B/C. The BCLC classification was 8.8% in very early stage (0), 45% in early stage (A), 31.2% in intermediate stage (B), 0% in advanced stage (C) and 15% in end-stage disease (D). Study of prognostic factors of overall survival (OS) and disease free-survival (DFS). Univariate and multivariate analysis (p < 0.05).

Results: At 90 days mortality rate was 4.9% and major morbidity 37%. After the median follow-up of 23 ± 22 (1-80) months, OS was 68 ± 3 (62-74) months (5-yr OS 83.2%), and DFS was 60 ± 4 (52-67) months (5yr DFS 76.8%). Differences in survivals between BCLC stages were not observed. At 5 years, OS was 83.3% vs. 85.6% (p = 0.717) and DFS was 83.3% vs. 83.7% (p = 0.794) for BCLC D patients vs. other stages (0, A, B, C) respectively.

Conclusions: LT is a good therapeutic option for HCC with acceptable morbidity and mortality. A good selection of patients with an initial indication for palliative treatment (BCLC D) can allow similar outcomes to those with formal indication for LT, increasing significantly survival and quality of life.

P32. ELACRIDAR AS A MODULATOR OF IMATINIB RESISTANCE

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Introduction: In chronic myeloid leukemia (CML), the most relevant mechanisms associated with the acquisition of resistance to tyrosine kinase inhibitors (TKIs) are those dependent on the therapeutic target, the BCR-ABL oncoprotein. However, intracellular drug concentration has proved to be very important in therapy response and acquisition of drug resistance. Thus, modulation of efflux transporters, such as P-gP and BCRP, may contribute to a greater efficacy of TKIs. The objective of this study was to evaluate the therapeutic potential of Elacridar (P-gP and BCRP inhibitor) in monotherapy and in combination with Imatinib in *in vitro* models of CML.

Materials and methods: To achieve this goal, 3 CML cell lines were used: K562 cells (sensitive to Imatinib), K562-RC (8x resistant) and K562 RD (18x resistant). P-gP and BCRP activity was evaluated by flow cytometry (FC). The therapeutic potential of Elacridar was evaluated in cells incubated in the absence and presence of Elacridar in monotherapy and in combination with increasing doses of Imatinib by the resazurin method. Cell death was evaluated by optical microscopy (May-Grünwald-Giemsa staining) and by FC (Annexin V/7-AAD and Apoptosis, DNA Damage, and Cell Proliferation Kit). The cell cycle was evaluated by FC (PI/RNase). The data were analyzed statistically and the differences were considered significant when p < 0.05.

Results: Resistant cell lines show higher expression and activity of P-gP and BCRP compared to the sensitive one. Elacridar in monotherapy, in the used concentrations, did not reach the IC50 in any cell line. However, the association of 250 nM of Elacridar with Imatinib modulated the resistance and re-sensitized resistant cells to Imatinib. In mechanistic terms, Elacridar in monotherapy induced

cell death by apoptosis/necrosis, showing no effect at in cell cycle progression. In combination with Imatinib was observed cell death by apoptosis, accompanied by increased caspase-3 activation, cleaved PARP and DNA damage (phosphorylated H2AX). This effect was accompanied by a cell cycle arrest in S phase.

Conclusions: In conclusion, our results suggest that Elacridar in therapeutic combination with Imatinib re-sensitize resistant cell lines to Imatinib, with involvement of the efflux transporters. These results, if translated into clinical practice, may contribute to therapy response improvement in patients resistant to Imatinib. This work was supported by CIMAGO and FCT (SFRH/BD/51994/2012).

P33. MICRORNAS PROFILE IN CML - A POTENTIAL BIOMARKER TO IMATINIB RESPONSE

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Introduction: The therapeutic efficacy of Imatinib and other Tyrosine Kinase inhibitors (TKIs) in Chronic Myeloid Leukemia (CML) change dramatically the course of this disease. The actual challenge is to predict which patients will develop resistance to TKIs, to improve therapeutic selection. The microRNAs (miRs) are important regulators of gene expression and could play an important role in Imatinib resistance. This work was investigate the role of miR-21, miR-519c, miR-451 and miR-26 expression levels in CML patients at diagnosis and correlated the expression levels of this miRs with therapy response. **Materials and methods:** For that, we assessed the expression levels of miR-203, miR-21, miR-519c, miR-451, miR-26 and miR-16 (endogenous control) by TaqMan MicroRNA Assays, in 29 CML patient samples at diagnosis. The population in the study presented a median of 53 years old, with 52% of males and 86% of the patients were diagnosis at chronic phase. The proper statistical analysis was performed and was considered a significance level of 95% ($p < 0.05$).

Results: The miR-451 was the miR with higher expression levels (median: 7.3), a median of expression of 0.086 for miR-26, and the miR-21 presented the lowest levels (median: 0.0003). The expression of miR-519c was not detected in any sample. The phase of disease at diagnosis did not influence the expression levels of this miRs. We evaluated the potential use of miRs expression as a biomarker of response to Imatinib at 6 and 12 months of treatment follow-up. The ROC analysis show that patients with miR-451 expression levels higher than 5.69 and lower expression levels of miR-21 than 1.16×10^{-4} have an optimal response to Imatinib at 12 months [AUC 0.77 (CI95% 0.58-0.95); Sensibility = 92%; Specificity = 64%; $p = 0,017$; AUC 0.77 (CI95% 0.57-0.97); Sensibility = 55%; Specificity = 100%; $p = 0,021$, respectively]. The patients with this profile combining miR-451 and miR-21 according to the cut-off levels have 44.8x higher probability of achieving optimal response at 12 months.

Conclusions: The preliminary results suggest that miRs profile in CML at diagnosis could constitute a new biomarker of Imatinib response, particularly the levels of miR-451/miR-21. However, more studies are necessary with a higher number of patients.

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P34. THE EVALUATION OF ALDH INHIBITORS IN ENDOMETRIAL CANCER STEM CELLS

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Introduction: Endometrial cancer is the most common malignant disease of the female genital tract. About 20% of the diagnosed endometrial carcinomas are aggressive and invasive neoplasms, presenting risk of recurrence. Some of these patients have poor outcomes with conventional therapies. Cancer stem cells (CSC) are a cell subpopulation of undifferentiated cancer cells responsible for tumor initiation, resistance to therapy and metastatic phenotype. Resistance to therapy results from several mechanisms, such as pathways of aldehyde dehydrogenase (ALDH), an enzyme also related with tumor volume, lymphatic nodal invasion, recurrent disease, and poor prognosis. ALDH increases activity was found in CSC of several tumors, as endometrial cancer, where the ALDH1 expression was associated with tumorigenesis and chemotherapy resistance. In fact, our previous work pointed ALDH increased expression as a putative marker of endometrial CSC. **Materials and methods:** Therefore, we aimed to evaluate the ALDH inhibition through Western Blot, in two human endometrial cancer cell lines, ECC-1 and RL95-2, submitted to three ALDH inhibitors, all-trans retinoic acid (ATRA), diethylaminobenzaldehyde (DEAB) and JQ1. ECC-1 and RL95-2, were submitted to 5-10 μM of ATRA, 50-250 and 50-100 μM of DEAB, and 100-500 and 100-250 nM of JQ1, respectively, for 48 hours. Beforehand, we evaluated the inhibitors cytotoxicity in both cell lines, through MTT assay.

Results: The cytotoxicity assay results showed that the inhibitors presented no toxicity in both cell lines, at concentrations of 1-10 μM of ATRA, 10-250 and 10-500 μM of DEAB and 5-250 and 5-500 nM of JQ1, to RL95-2 and ECC-1, respectively. The preliminary results obtained from the evaluation of ALDH expression showed that there is a tendency for a decrease in ALDH expression in cells submitted to DEAB, in both cell lines.

Conclusions: In conclusion, the concentrations of ALDH inhibitors studied do not present cytotoxicity in endometrial cancer cell lines and DEAB seems to be the most promising ALDH inhibitor. In the continuation of these studies, it is intended to study the influence of these inhibitors in the response to therapeutics.

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P35. THE POTENTIAL THERAPEUTIC EFFECT OF HEAT SHOCK PROTEIN 90 INHIBITION IN CHRONIC MYELOID LEUKEMIA

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Introduction: HSP90 facilitates the maturation, stability, activity and intracellular folding of more than 200 proteins, called 'client proteins'. In cancer cells, HSP90 helps to overcome multiple environmental stresses, including genomic instability/aneuploidy, proteotoxic stress, increased nutrient demands, reduced oxygen levels, and to prevent destruction by the immune system. One of these client proteins of HSP90 is BCR-ABL, the oncoprotein responsible for Chronic Myeloid Leukemia (CML). Alvespimycin (17-DMAG) is an HSP90 inhibitor that has better pharmacokinetic properties and fewer side-effects compared to others benzoquinone ansamycins. This work aims to study the effect of alvespimycin in chronic myeloid leukemia cell lines (sensitive and resistant to imatinib) and to explore the role of HSP family in the sensitivity to imatinib.

Materials and methods: In this context, we used 3 CML cell lines: the K562 cells, sensitive to Imatinib, and the K562-RC and K562-RD cells resistant to Imatinib. Cells were incubated in the absence and presence of increasing concentrations of 17-DMAG (from 1 to 1,000 nM), in single dose. The dose-response curves were determined by resazurin 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. The Apoptat Probe was used to evaluate caspase expression levels and JC-1 probe to determine the mitochondrial membrane potential, by FC. Cell cycle was evaluated by FC, using PI/RNase assay. The protein expression levels of HSP family were analyzed by western blot.

Results: Our results showed that 17-DMAG induce a reduction in cell lines viability, with an IC50 of 50 nM for K562 and K562-RD cells and lower than 50 nM for the K562-RC cell line, after 48 hours of treatment. This compound induces cell death predominantly by apoptosis, confirmed by morphological analysis, FC and by the increase of JC-1 Monomers/Aggregates ratio. The cell cycle analysis showed that 17-DMAG induces cell cycle arrests in all the tested cell lines. The HSP protein analysis showed that K562-RC have slightly more expression of HSP90 than K562.

Conclusions: In conclusion, our results suggest that inhibition of HSP90 by alvespimycin (17-DMAG) could be used as a new potential approach in the treatment of CML, even in case of Imatinib resistance.

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P36. PARTHENOLIDE AS A NEW THERAPEUTIC APPROACH IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: Acute Lymphoblastic Leukemias (ALLs) are hematologic malignancies characterized by dysregulated cell proliferation and differentiation arrest, resulting in the accumulation of immature lymphoid progenitors (B or T). Although the progress in the therapeutic approaches of patients with ALL, the survival rates have not increased substantially in recent years and relapses are frequent. One of the altered signaling pathways in ALL is the kappa-B nuclear factor (NF- κ B) pathway. In this sense, this study evaluated the therapeutic potential of parthenolide (PRT), an inhibitor of the NF- κ B pathway, in in vitro models of T-ALL and B-ALL.

Materials and methods: To this end, T-ALL (CEM, JURKAT, and MOLT-4) and B-ALL (697 and KOPN-8) cell lines were incubated in the absence and presence of PRT in single and fractionated administration schemes. Metabolic activity was assessed by resazurin assay. Cell death was evaluated by optical microscopy (May-Grünwald-Giemsa staining) and by Flow Cytometry (FC; Annexin V/7-AAD staining). The expression of FAS, FAS-L, activated-caspase-3, phosphorylated NF- κ B, mitochondrial membrane potential ($\Delta\psi$ mit; JC-1) and oxidative stress [superoxide (O₂⁻; DHE), hydrogen peroxide (H₂O₂, DCFH₂DA), and reduced glutathione (GSH)] were also analyzed by FC. The results were statistically analyzed considering a level of significance of 95% ($p < 0.05$).

Results: The results indicate that PRT reduced metabolic activity in a time-, dose- and cell line-dependent manner. KOPN-8 and CEM (IC₅₀ 50-75 μ M) were the most sensitive cells and MOLT-4 and JURKAT (IC₅₀ 100 μ M) the less sensitive. Single and fractional administration regimens showed similar results. PRT induced cell death by apoptosis associated with a decrease in $\Delta\psi$ mit and an increase in oxidative stress levels (increased ROS and decreased GSH) in all cell lines. In addition, PRT also induced an increase of FAS and FAS-L levels in cell lines, except CEM and JURKAT cells. A cytostatic effect was also observed in JURKAT (G₀/G₁ phase arrest) and MOLT-4 (G₂/M phase arrest) cells. Decreased levels of phosphorylated p65 (a subunit of NF- κ B) confirm the inhibition of the NF- κ B pathway.

Conclusions: In conclusion, these results suggest that PRT may represent a new potential therapeutic approach in ALL. However, the therapeutic efficacy may depend on ALL subtype.

P37. EPIGENETIC MODULATORS IN B CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: B cell acute lymphoblastic leukemia (B-ALL) is the most frequent hematologic neoplasia in children and is characterized by deregulated B cell proliferation and differentiation arrest. Epigenetic alterations, namely DNA methylation and histone modifications, are involved in B-ALL development and progression and may constitute new therapeutic targets for in these neoplasms. Thus, the aim of this study was to evaluate the therapeutic potential of hypomethylating agents ([DNMTi: Azacitidine (5-AC) and Decitabine (DAC)]) and histone deacetylase inhibitors [HDACi: Panobinostat (PAN) and Vorinostat (SAHA)], in monotherapy and in combination therapy, in in vitro models of B-ALL.

Materials and methods: Two B-ALL cell lines, the KOPN-8 and 697 cells, were incubated in the absence and presence of DNMTis and/or HDACis, in monotherapy and in therapeutic combination. Cell viability was determined by the FMCA assay (Fluorometric Microculture Cytotoxicity Assay). Cell death was evaluated by optical microscopy (May-Grünwald-Giemsa stain) and Flow Cytometry (FC; Annexin V/7-AAD double stain). The 5-methylcytosine (5-mC) levels were analyzed by FC and the methylation status of 24 tumor suppressor genes (TSGs) was carried out by MS-MLPA. The results were analyzed statistically considering a significance level of 95% ($p < 0.05$).

Results: The results showed that the studied drugs reduced cell viability in a time-, dose- and cell line-dependent manner, being the 697 cells more sensitive than KOPN-8 cells. B-ALL cells were found to be more sensitive to DAC (697 10 μM ; KOPN-8 > 15 μM) than to 5-AC (697 15 μM ; KOPN-8 > 20 μM), after 72h of treatment. They were also more sensitive to PAN (697 7.5nM; KOPN-8 > 20 nM) than to SAHA (697 750 nM; KOPN-8 1,000 nM). In addition, therapeutic associations of DAC with PAN or SAHA reduced more effectively the cell viability when compared to monotherapy treatments. These drugs induced predominantly apoptosis in 697 cells and apoptosis and necrosis in KOPN-8 cells. Moreover, SAHA induced cell cycle arrest in phase S and G0/G1, respectively in 697 and KOPN-8 cells. Finally, both DNMTi and their association with PAN induced a decrease in 5-mC levels and the demethylation of at least two TSGs (697: TP73, KLLN, MGMT, and CD44; KOPN8: MGMT and STK11).

Conclusions: Our results suggest the therapeutic potential of epigenetic modulators in the treatment of B-ALL in monotherapy and in association with panobinostat.

P38. DETECTION OF *HELICOBACTER PYLORI* IN EXTRAGASTRIC TISSUES: ASSOCIATION OF INFECTION WITH CARCINOGENESIS

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Introduction: *Helicobacter pylori* (HP) is a gram-negative micro-aerophilic bacterium that has been described as the main pathogen of several benign and malignant diseases of the digestive tract, such as gastric and duodenal ulcers, chronic gastritis, hepatobiliary diseases, gastric lymphoma and carcinoma. HP was classified as class I human carcinogen in 1994. The relationship between HP infection and extragastric diseases has been investigated over the years with contradictory conclusions. The objective of this work is to perform a bibliographic review on the association between HP infection and the development of extragastric pathologies, particularly of pancreatic carcinoma. It is also intended to determine the best method for the detection of the bacterium.

Materials and methods: work was based on the literature from the following databases: b-on, PubMed, SciELO, Wiley Online Library and Scientific Repository of the Polytechnic Institute of Lisbon, under the terms “*Helicobacter* spp.”, “*Helicobacter pylori*”, “pancreatic cancer”, “extragastric diseases”, “risk of pancreatic cancer” and “CagA”. From a total of 32 analyzed articles, 18 were selected, excluding those with dubious methods and results, that were not performed in human samples and meta-analysis.

Results: In the selected studies the presence of HP DNA was detected by Polymerase Chain Reaction (PCR) in more than 50% of the pancreatic and hepatocellular cancer samples. There was also an association between the presence of this bacterium and colonic cancer, primary sclerosing cholangitis, primary biliary cirrhosis, cholecystitis and biliary tract carcinoma. However, detection by enzyme-linked immunosorbent assay provided ambiguous results, with 2 of the studies showing no association between infection and pancreatic cancer, while others showed positive results below 50%. There are also 3 studies that related the seroconversion of cytotoxin-associated gene A negative to pancreatic carcinoma. Other methods like histochemical and immunocytochemistry (ICQ) techniques were compared to each other and the results demonstrated that ICQ had the greatest consistency.

Conclusions: The majority of the studies demonstrated an association between HP infection and the development of extragastric diseases. Therefore, HP is a potential risk factor for the etiology of these pathologies. Comparing previous methods, it was possible to observe that PCR was the most sensitive and specific technique for HP detection.

P39. CONSERVED EMBRYONIC SIGNALING PATHWAYS INHIBITORS AS NEW THERAPEUTIC STRATEGIES IN B-CELL NEOPLASMS

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Introduction: B-cell neoplasms are a heterogeneous group of diseases that include B-cell lymphomas and plasma cell disorders. Multiple myeloma (MM) is a malignant neoplasm originated by the proliferation of monoclonal plasma cells that remains incurable. Diffuse large B-cell lymphoma (DLBCL) is an aggressive lymphoma, with a fast growing that is rapidly fatal if untreated. Inappropriate activation of conserved embryonic signaling pathways critical for stem cell self-renewal and differentiation in hematopoiesis, such as WNT/ β -catenin and Hedgehog, has been implicated in these B-cell neoplasms. The main goal of this study was to evaluate the therapeutic potential of WNT/ β -catenin and Hedgehog inhibitors, respectively IWR-1 and vismodegib, alone and in combination in MM and DLBCL cell lines.

Materials and methods: The therapeutic potential of IWR-1 and vismodegib in monotherapy and in therapeutic combination was evaluated in two in vitro models of MM and DLBC, the H929 and FARAGE cell lines, respectively. The metabolic activity was evaluated by resazurin assay and cell death by optical microscopy (May-Grunwald staining) and flow cytometry (FC; Annexin V/7-AAD staining). Cell cycle analysis was evaluated by FC, using a PI/RNase solution. Proteins related to apoptosis (BAX, BCL2, caspases) and some molecules related to WNT and HH signaling pathways (β -catenin and p-ERK) were tested by FC. The results were statistically analyzed considering a level of significance of 95% ($p < 0.05$).

Results: The results showed that IWR-1 and vismodegib reduced metabolic activity in a time- and dose-dependent manner. H929 cells were more sensitive to IWR-1 (IC50 40 μM) than FARAGE cells (IC50 75 μM), while FARAGE cells were more sensitive to vismodegib (IC50 57 μM) compared to H929 (IC50 70 μM). The reduction of metabolic activity was more pronounced in the combination regimens as well as in the fractional administration of drugs compared to single-dose regimens. IWR-1 and vismodegib induced cell death by apoptosis, associated with increased activated-caspases, and G0/G1 cell cycle arrest. In addition, both drugs induced a decrease in β -catenin and p-ERK expression levels, more pronounced in MM cells (H929).

Conclusions: The results suggest that MM cells are more sensitive to WNT/ β -catenin pathway inhibitors and LDGCB cells to Hedgehog pathway inhibitors. However, both drugs may represent potential therapeutic approaches in these lymphoid neoplasms, especially in therapeutic combination.

P40. FADU RESPONSE TO CISPLATIN, DOCETAXEL AND 5-FLUOROURACIL - IN VITRO PRELIMINARY RESULTS

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Introduction: Tumours of the oral cavity and pharynx are the most common forms of head and neck squamous cell carcinoma (HNSCC). The drugs commonly used for chemotherapy are cisplatin (CDDP), docetaxel (DTX) and 5-fluorouracil (5-FU). However, patients with late diagnoses, in advanced stage, show poor prognosis, multidrug resistance and lower progression free survival requesting therapy improvement. The aim of this study was to evaluate the cytotoxic effects of CDDP, DTX and 5-FU [half maximal inhibitory concentration (IC50)] in the FaDu cell line. Besides this proliferation, cell cycle and death and reactive oxygen species (ROS) induced by IC20, IC50 and IC80 of CDDP alone, 48h after exposure, will be also analyzed.

Materials and methods: For IC50 determination, cells were incubated with different concentrations of CDDP (0.5-33 μ M), DTX (0.01-180 nM) and 5-FU (0.5-3,844 mM) for 24, 48 and 72h, and the sulphorhodamine B assay was performed. To evaluate cell cycle (propidium iodide) and death (Annexin V and propidium iodide), ROS [reduced glutathione (GSH), dihydroethidium (DHE) and 2,7-dichlorodihydrofluorescein (DCFH2)], flow cytometry was used, 48h after treatment with CDDP IC20; 50; 80.

Results: Related to proliferation 48h after drug incubation the IC20, IC50, IC80 values were for CDDP (6.2, 12.3, 24.4 μ M), for DTX (1.35, 5.57, 23.01 nM) and for 5-FU (11.30, 158.25, 2,216.80 nM). After 72h of incubation the values were for CDDP (5.92, 10.20, 17.54 μ M), for DTX (1.21, 2.658, 5.83 nM) and for 5-FU (9.89, 77.26, 603.48 nM). Preliminary results about viability and oxidative stress reveal that the decrease on cell proliferation is followed by significant reduction on viability by necrosis ($p = 0.001$) and apoptosis ($p = 0.0001$). After 48h of CDDP incubation a significant cell cycle block in G2/M ($p = 0.001$) occurred with lower drug concentrations while higher doses significantly block cells in G0/G1 ($p = 0.0001$) and S phases ($p = 0.001$). DHE ($p = 0.03$) and DCFH2 ($p = 0.011$) levels significantly increased with CDDP exposure as well as GSH ($p = 0.05$) levels.

Conclusions: All the chemotherapeutic agents used demonstrated time and dose-dependent cytotoxic and anti-proliferative effect. CDDP exposure caused a decrease in viability and an increase in oxidative stress. Cells try to reduce ROS levels through increasing GSH expression. The FaDu cell line demonstrated to be more sensitive to DTX.

P41. IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE FADU CELL LINE BEFORE AND AFTER IONIZING RADIATION - PRELIMINARY RESULTS

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is the sixth leading cancer worldwide. Hypopharyngeal cancer (HPC) despite its low incidence, often presents a poor prognosis with an advanced stage and distance metastasization. Treatment failure may be due to tumour heterogeneity, particularly the presence of cancer stem cells (CSC). Identification and pattern of CSC markers could help to improve therapy, with new therapeutics targets providing personalized therapy to HPC patients. The aim of this study was to evaluate and characterize the expression of proliferation, adhesion and CSC markers in a hypopharyngeal cell line (FaDu) before and after ionizing radiation exposure.

Materials and methods: Cells were processed and paraffin cell blocks were performed. Through immunohistochemistry (IHC) epithelial characterization (cytokeratin CAM 5.2, leukocyte common antigen (LCA), vimentin), tumoral aggressiveness (P16, P53, OCT4 and SALL4), proliferation (Ki-67) and CSC markers (EpCAM, CD10, CD44, CD117, CD133, beta-catenin) were evaluated. Additionally, 14 subtypes of high-risk HPV (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 16, and 18), EBV, Akt and Wnt expressions were analyzed. For radiotherapy, 0.5×10^6 cells/mL were exposed to increased doses of X-ray (from 0.5 to 10 Gy), except the control cells, to perform the clonogenic assay and IHC. The treated cells plated to IHC were fixed with alcohol 96%, 48h after treatment and posteriorly analyzed by IHC (Ki-67, EpCAM, CD133, CD44, beta-catenin, CD117, CD10, Akt, Wnt, OCT4 and P53).

Results: The FaDu cell line expressed cytokeratin+, vimentin+ and LCA-. Relatively to tumoral aggressiveness and proliferation, it was observed P16+, P53-, OCT4-, SALL4- and Ki-67+. Stemness was characterized by EpCAM-, CD10-, CD44+, CD117-, CD133+ and beta-catenin+. From the 14 high-risk HPV subtypes analyzed only HPV18 was stained positive and EBV-. Moreover, control cells expressed Akt+ and Wnt+ in normal conditions. As preliminary results, a decrease on cellular survival with the increase of ionizing radiation dose was observed. In addition, this X-ray radiation induced alterations on Wnt expression.

Conclusions: According to the obtained results we can conclude that FaDu survival is affected by ionizing radiation exposure. These results could be related with Wnt expression which is altered after irradiation. This molecule is involved in cell fate determination, cell polarity, migration and cell proliferation.

P42. CAN γ -SECRETASE INHIBITORS BE A GOOD THERAPEUTIC APPROACH IN ACUTE LYMPHOBLASTIC LEUKAEMIA?

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Introduction: Acute Lymphoblastic Leukaemia (ALL) is a malignant disease characterized by an accumulation of early lymphoid precursors in bone marrow, and can affect both linages, B and T cells (B-ALL and T-ALL). NOTCH signalling plays a significant role in cell fate decision during development, stem cell self-renewal and differentiation in haematopoiesis, and activating NOTCH1 mutations are present in more than half patients with T-ALL. Therefore, modulation of NOTCH signalling pathway, for example with gamma-secretase inhibitors, might provide a new therapeutic approach in ALL. In this context, the aim of this study was to evaluate the therapeutic potential of a γ -secretase inhibitor, GSI-XXI, in two in vitro models of ALL.

Materials and methods: For this purpose, we used two different cell lines, a T-ALL (CEM) and a B-ALL (KOPN-8). These cells were incubated in the absence and presence of GSI-XXI. Cell viability and proliferation were assessed by trypan blue exclusion assay. Cell death was evaluated by optical microscopy and flow cytometry (FC) using annexin V/propidium iodide double staining and JC-1 probe. Apoptotic protein levels (BAX and BCL-2) and cell cycle distribution were also evaluated by FC. The expression levels of *CCND1*, *CCNB1*, *CCNE1* and *NF- κ B* genes were determined by RT-PCR. Results were considered statistically significant when $p < 0,05$.

Results: Our results suggest that GSI-XXI reduced cell proliferation and viability in a dose- and cell type dependent manner with an IC50 at 24h of approximately 40 μ M for CEM and 30 μ M for KOPN-8 cells. This compound induced cell death mainly by apoptosis in both cell lines, mediated by an increase in BAX/BCL-2 ratio and a decrease in mitochondrial membrane potential. The analysis of cell cycle progression also revealed a significant arrest in G0/G1 phase in CEM cells. This analysis also showed a sub-G1 peak in KOPN-8 treated cells which correspond to DNA fragmentation a typical feature of apoptosis. Finally, GSI-XXI did not induce significant changes in the expression levels of *CCND1*, *CCNB1*, *CCNE1* and *NF- κ B* genes.

Conclusions: In conclusion, if these results can be translated to clinical practice, they suggest that γ -secretase inhibitors, like GSI-XXI, might be a good therapeutic approach in acute lymphoblastic leukaemia patients.

P43. THE INFLUENCE OF FIXATION TEMPERATURE IN IN VITRO DNA ANALYSIS

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Introduction: Formalin-fixed and paraffin-embedded (FFPE) are important sources for molecular studies, namely to identify cancer-related biomarkers. Nevertheless, the quality of FFPE-obtained DNA is lower than that of fresh/frozen tissues, since fixation induces several chemical modifications. The impact of tissue fixation duration, fixative type and pH on the integrity of FFPE-extracted DNA has been the subject of several studies. It's well established that fixation using formalin for less than 72 hours allows extraction of high quality DNA. Although it is known that DNA stability is highly dependent on temperature, the influence of the fixation temperature on the quality of FFPE-extracted DNA is not understood.

Objective: Evaluate the influence of the fixation temperature in the quality of FFPE-extracted DNA through a systematic literature review.

Materials and methods: The search was performed in PubMed for studies published in English, up to December 2017. The included studies compared two or more fixation temperatures using formalin, to perform DNA analysis.

Results: 7 studies met the defined criteria. All compared room temperature (RT) with 4 °C (5 studies), 0-4 °C (1 study) or 37 °C (1 study). DNA integrity was evaluated by agarose gel electropho-

resis, PCR, multiplex ligation-dependent probe amplification (MLPA) and whole gene amplification (WGA). In 5 studies the best results were obtained for fixation at 4 °C (electrophoresis, PCR and WGA); whereas RT allowed the best results in 2 cases (PCR and MLPA), one of which demonstrated that fixation at 37 °C preserved DNA similarly to RT.

Conclusions: DNA stability is highly temperature-dependent as DNases are inhibited at low temperatures. Accordingly, it is not surprising that fixation at 4 °C allows the extraction of less degraded DNA in most studies. Nevertheless, RT seems to be also an acceptable temperature, as it allowed successful MLPA and PCR when using small DNA fragments. From these observations one may conclude that tissue fixation must be performed at 4 °C if the goal is to analyse high-molecular-weight DNA. As RT is the standard fixation temperature in diagnostic lab units, these conclusions may imply the adequate adjustments in order to prevent false conclusions from molecular analysis. Nevertheless, additional studies that analyse not only DNA integrity, but also DNA purity, yield and concentration should be done to determine the optimal fixation temperature to perform molecular analysis.

P44. THE ROLE OF RADIATION THERAPY IN PEDIATRIC POPULATION: A 5 YEAR EXPERIENCE IN AN ONCOLOGY CENTER

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Introduction: The incidence of childhood cancer has been increasing over the last years. It is estimated that approximately 43 children are diagnosed every day, and 1 out of 8 patients will not survive the disease. Depending on the diagnosis and the staging degree, treatment may include surgery, chemotherapy and radiotherapy, or an association of these. The treatment approach by radiotherapy is responsible for the improvement of loco-regional tumoral control and overall survival. Childhood cancer represents a very heterogeneous group of diseases, which suffered major treatment advances in the last years. The impact of those advances in the quality of life and survival in this population is still not completely understood. The aim of this study is to evaluate the impact of the treatment in the tumoral response, toxicity profile and overall survival of the patients.

Materials and methods: Retrospective study that included all the children treated with radiotherapy in the IPOC, between August 2012 and March 2017. Statistical assessment was calculated by IBM SPSS.

Results: This study included a total of 64 children with a median age of 9 years (range 0-17). The most common diagnosis were lymphoblastic leukemia (16%), rhabdomyosarcoma (14.1%) and Hodgkin lymphoma (10.9%). At the time of the diagnosis, only 14% of the patients presented metastatic disease, more frequently observed in the lung. The majority were treated with curative intent (88%), including adjuvant radiotherapy and chemo/radiotherapy, with 3D-conformal therapy (77%) and intensity modulated radiation therapy (14%). The early toxicities most commonly seen were radiodermatitis (34%), headaches (14%) and alopecia (12%). In the clinical and imaging assessment following the conclusion of the treatment, a partial tumoral response was the most common outcome. The median follow-up was 30 months and the overall survival was 54.3 months.

Conclusions: Overall, pediatric cancer is relatively rare; however it has shown a progressive increasing incidence in the last few decades. With the improvements in cancer treatment there has been a major growth in the population of survivors. This

study has verified that regardless of the treatment intent, the majority of patients presented partial or even complete tumoral response after radiotherapy. The treatment was relatively well tolerated, with mild side effects, and plays an important role in the improvement of tumoral control and overall survival of the population.

P45. HNSCC CELL LINES WITH DIFFERENT RADIOSENSITIVITIES HAVE DISTINCT RESPONSES TO RADIATION TREATMENT

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer type worldwide. Even with treatment advances, the 5-year survival rate remains around 50%. One treatment option for these patients is radiotherapy, which is based on a “one-size fits all” model, with the administration of standard doses (2 Gy) of radiation to all patients, regardless of tumor heterogeneity between patients. Resistance to radiotherapy is common in HNSCC, however the mechanisms behind remain to be elucidated. Here, we evaluate two different cellular responses to radiotherapy in a radioresistant and a radiosensitive cell line for further understanding of radiation response in HNSCC.

Materials and methods: HSC-3 and BICR10 cell lines were cultured in DMEM supplemented with 10% fetal bovine serum and 1% of penicillin and streptomycin. For BICR-10, 1% of hydrocortisone was also added. Cells were exposed to different radiation doses (0.5 Gy to 10 Gy) for clonogenic assay and median lethal dose (LD50) evaluation. Intracellular levels of reactive oxygen species (ROS) - dihydroethidium (DHE) and dichlorofluorescein (DCF) -, and antioxidant activity - reduced glutathione (GSH) expression - were measured using a fluorescent microplate reader at 2, 6 and 24 hours after irradiation. Cell cycle analysis was performed by flow cytometry after 2 and 8 days of radiation. For ROS and cell cycle analysis, cells were irradiated with standard treatment dose (2 Gy).

Results and conclusions: The results obtained from the clonogenic assay allowed the assessment of the different LD50 between both cell lines allowing the classification of one cell line as radio-sensitive (HSC-3) and the other as radioresistant (BICR10). HSC-3 cell line shows a slight blockage in S and G2/M phases. Radiation in BICR10 cell line seems to arrest cells in G0/G1 phase, blocking cell division. ROS measurements after radiation differ between both cell lines. BICR10 has an enhancement in DCF and DHE production after 6 hours of irradiation. HSC-3 cell line has an increase in both DHE and DCF production immediately after radiation (2h) that decreases at the 6h time-point, where an increase in GSH production is observed. These results show that the radiation effect is dependent on the cell line and suggest that the distinctive outcomes regarding radiotherapy response could be associated to different molecular responses after irradiation.

P46. IS SIRT1 AND MTOR INTERPLAY AN ARISING THERAPEUTIC TARGET FOR BLADDER CANCER?

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Introduction: Bladder cancer (BC) has a high incidence, a high recurrence rate and patients have a poor survival expectancy. BC heterogeneity is mediated by several signalling pathways. The mammalian target of rapamycin (mTOR) pathway is altered in 72% of BC cases but there is no suitable therapy directed to this pathway. Interestingly, the role of SIRT1 in tumorigenesis is a matter of controversy since it acts as a tumour promoter or suppressor suggesting that SIRT1 has different functions according with the type and grade of the tumour. In addition, it may interact with factors that regulate tumour aggressiveness and growth. Herein, we hypothesize that SIRT1 has a role in BC progression and interacts with mTOR pathway. To test our hypothesis, we used two BC cells lines representative of different BC grades.

Materials and methods: We cultured two different cell lines HT-1376 and TCCSUP, representative of BC stage III and IV, respectively. Cells were cultured during 24 hours with increasing doses of EX527 (0.1 μ M, 1 μ M and 10 μ M), a SIRT1 inhibitor; YK-3-237, a SIRT1 activator (0.1 μ M, 1 μ M and 10 μ M) and Rapamycin (0.01 μ M, 0.1 μ M and 1 μ M), a mTOR inhibitor. Cell and mitochondrial toxicity were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Sulforhodamine B (SRB) and Mitochondrial potential (JC-1) assays.

Results and conclusions: Our results show that SIRT1 inhibition and activation have differential effects on BC cells from lower and higher grade illustrating a possible therapeutic target for BC progression. SIRT1 inhibitor decreased BC cell viability after exposure to all concentrations tested in the lower grade, while exposure to SIRT1 activator increased cell viability of higher grade BC cells. mTOR inhibition decreased cell proliferation though in BC cells from higher grade only the highest concentration promoted that effect. Interestingly, SIRT1 activation at the highest concentration decreased cell proliferation of BC cells of higher grade thus illustrating that SIRT1 pathway is also involved in BC progression. Finally, mitochondrial toxicity was also detected in BC cells of high grade tumour after inhibition of SIRT1 with the highest concentration illustrating that SIRT1 is at the interplay with mitochondrial functionality. Overall our results suggest that both, SIRT1 and mTOR are key players in BC physiology. Further studies are needed to unveil the interplay of both pathways in BC establishment and progression.

P47. ENZALUTAMIDE VERSUS CHEMOTHERAPY IN MCRPC: WHICH IS THE BEST OPTION FOR TREATING IN FIRST LINE?

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Introduction: Enzalutamide is an oral androgen-receptor inhibitor that could be used as first option or after docetaxel with improved survival in metastatic castration-resistant prostate cancer (mCRPC). The aim is to evaluate and compare the outcome of patients that started enzalutamide without prior chemotherapy (group 1) with those that underwent chemotherapy prior to starting enzalutamide (group 2) until July 2017.

Materials and methods: A retrospective observational single-center study was performed involving all patients treated with enzalutamide at our institution (40 patients): 25 (62.5%) in group 1 and 15 (37.5%) in group 2 and the outcome was compared. The software used was IBM SPSS Statistics 23, with $p < 0.05$.

Results: The age at prostate cancer diagnosis was 66.3 ± 9.9 years with an initial PSA of 20.05 ± 158.0 ng/dL and a predominantly Gleason score of 7 (40.5%). The initial disease was mainly localized (58.3%), the majority (37.8%) treated with surgery. All patients developed metastases after 69.7 ± 12 months. The hormone therapy was initiated 42.6 ± 11.4 months after the initial treatment. The castration resistance state was reached 88.5 ± 12.5 months after initial treatment with a PSA of 50.0 ± 27.8 ng/dL. Patients started enzalutamide after 108.8 ± 12.4 months after the initial treatment, with a PSA of 123.9 ± 143.8 ng/dL. The duration of time that patients were treated with enzalutamide was 385.1 ± 38.6 days. The side effects were rare. The discontinuation rate was 62.5% (25 patients), mainly due to death (37.5%). The PSA at discontinuation was different (group 1- 194.0 ± 88.8 versus group 2- 412.0 ± 516.2 ng/dL, $p: 0.04$). The hospital length of stay since the mCRPC diagnosis was 8.1 ± 2.7 days, not different between groups. The number of observations in the Emergency Department was different (group 1- 1.6 ± 2.1 vs group 2- 8.3 ± 11.4 times, $p: 0.01$). Overall mortality was 37.5% (17 patients), occurring mainly in group 2 (10 patients), $p: 0.02$. For a follow-up of 9.7 ± 6.4 years from the prostate cancer diagnosis, the survival after enzalutamide onset was 20.3 ± 1.6 months in group 1 and 17.1 ± 3.6 months in group 2, $p: 0.03$. There was a PSA reduction, not different between groups, mainly in the first month after enzalutamide onset (reduction of 66 ng/dL). **Conclusions:** Enzalutamide seems to have good results especially prior to chemotherapy: increased survival from prostate cancer diagnosis, fewer visits to Emergency Department, decrease of PSA and fewer side effects were some advantages.

P48. NON-SMALL-CELL LUNG CANCER - SURGICAL TREATMENT, RELAPSE AND FIVE-YEAR SURVIVAL - THE EXPERIENCE OF A TERTIARY HOSPITAL

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Introduction: Surgical resection, typically lobectomy, is the first therapeutic option in individuals with early-stage non-small cell lung cancer (NSCLC), and some with resectable stage IIIA disease. However, the evidence regarding surgical treatment for curative purposes in NSCLC is not conclusive, particularly in locally advanced cases. Observational studies are fundamental in the validation of definitive treatment recommendations. The objective of this study was to evaluate the relapse and survival rates of patients with NSCLC undergoing curative surgery in a tertiary hospital.

Materials and methods: A retrospective study of patients with NSCLC undergoing surgical therapy at a tertiary hospital in a 3-year period. Review of clinical data, histopathology, tumour location, pathological stage, surgical procedure, adjuvant therapy, relapse, disease-free survival and 5-year survival rate.

Results: We identified 53 patients, with a mean age of 62 ± 10.5 years. The majority were male (69.8%) and active (39.6%) or former (28.3%) smokers. Pathological stage was IA in 19 cases, IB in 10, IIA in 9, IIB in 7 and IIIA in 8. The most frequent surgical procedure was

lobectomy, in 68.4% of stage IA, 70% in IB, 77.8% in IIA, 85.7% in IIB and 50% in IIIA. Pneumectomy was performed in 37.5% of stage IIIA patients. In the earlier stages, IA and IB, the relapse rate was, respectively, 23% and 40%; in stage IIIA was 75%. Disease-free survival varied according to stage, between 27 months in stage IA and 11.3 months in stage IIIA. The five-year survival rate was 77% in the IA stage, 60% in the IB, 44% in the IIA, 57% in the IIB and 25% in the IIIA.

Conclusions: Our results regarding relapse and survival rates were similar to previous series. This evidence supports the efficacy of the surgery in localized non-small cell disease. Still the significant recurrence rates demonstrate the need for further investigation of prognostic factors, specifically in stage IIIA patients.

P49. LUNG CARCINOID TUMOURS - A POPULATION BASED REVIEW

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Introduction: Lung carcinoid tumours account for less than 2% of lung tumours. They are histologically classified as typical and atypical, according to the mitotic index and existence of necrosis. Surgery is the recommended approach in localized disease. In advanced or metastatic disease there is no effective treatment. Given its rarity, there is limited data regarding prognostic factors.

Materials and methods: A 5-year retrospective evaluation of the clinical data of patients diagnosed with lung carcinoid tumour and who underwent surgical treatment in a tertiary hospital.

Results: A total of 34 patients with lung carcinoid tumours were reviewed, 16 men and 16 women, with a mean age of 54.75 ± 12.88 years. Of these, 11 were asymptomatic at diagnosis, and 21 patients were symptomatic, mainly due to respiratory complaints. The histological analysis classified 31 patients as typical carcinoids and one case as atypical carcinoid; 2 cases presented a Ki67 index greater than 20%. The majority had central ($n = 19$) and right sided lung ($n = 18$) location. Staging defined 71.87% as stage IA, 18.75% as IB, 6.25% as IIA, and 3.13% as IIB. All patients underwent radical surgery and no adjuvant treatment was needed. The surgical procedure was lobectomy in 24 cases (75%), atypical sublobar resection in 8, bilobectomy in 1 and pneumectomy in 1. Disease recurrence occurred in 3 cases (9.38%), with a free-disease survival time ranging from 4 to 35 months. The mortality rate was 28.1% ($n = 9$), including the relapsed cases, and corresponding to an average survival time of 61.32 ± 58.34 months. The remaining 25 patients continue in follow-up.

Conclusions: In agreement with previous data, typical carcinoid tumours were the most frequent histological type, the majority with central and right-sided location. Although considered indolent and non-aggressive tumours, when recurrence occurs the response to available treatment is generally poor. Larger series and identification of prognostic factors are far-reaching for future management.

P50. ADJUVANT RADIOTHERAPY IN PN2 NON-SMALL CELL LUNG CANCER - OUR RESULTS

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Introduction: Lung cancer is the 4th most frequent malignant disease in Portugal, with almost 80% non-small cell lung cancer (NSCLC).

Several studies demonstrate a benefic effect of adjuvant chemotherapy (ChT) after completely resected NSCLC with pN2. However, most studies fail to proof survival benefit of adjuvant radiotherapy (PORT), although it results in significant lower risk for local recurrence. Lung ART protocol (2011) helped to enhance homogeneity in volume delineation.

Materials and methods: Retrospective analysis of patients with NSCLC pN2, treated with adjuvant ChT followed by PORT to the mediastinal stations according to LungART protocol at our department between January 2011 and December 2017. Dose of 50-54 Gy/25-27 fr (2 Gy/fr). Toxicity scored according CTCAEv4.0 scale. Survival analysis by Kaplan-Meier method. Type I error of 0.05.

Results: 17 patients were included, 70.6% male, with a median age of 62 years (48-73). The clinical stage was predominantly cT1b-2 (70.6%) and cN0 (58.8%). Lobectomy with mediastinal lymphadenectomy was performed in 94.1%. Histologically 100% were adenocarcinomas and pathological staged as pT1a in 11.8%; pT1b in 29.4%; pT2a in 23.5% and pT3 in 35.3%. The mediastinal lymph node station most prevalently involved was station 7 (41.2%) and 5 (35.3%). Cisplatin + vinorelbine was delivered in 52.9%. Toxicity during ChT was predominantly hematological with anemia grade 1-2 in 58.8%, leucopenia G1-2 41.2% and neutropenia G3-4 in 17.6%. RT was delivered using 3D-conformal technique in 94.1% and volumetric arch therapy in 5.9%. Prescription dose was 54Gy/27fr in 82.4%. All patients completed the treatment. RT-related acute toxicity was anemia G1 in 52.9%, radiation dermatitis G1-2 in 58.8% and dysphagia G1 in 52.9%. There was no grade 3-4 toxicity. Radiation pneumonitis was found in 41.2% of which 57.1% were G2. With a median follow-up of 44 months, 2-year locoregional control (LRC) was 100%, disease-free survival (DFS) 69.5% and overall survival (OS) 88.2%. Although there are no significant differences, patients with more than 1 involved nodal station had poorer OS (66.7% vs 92.9%, $p = 0.062$) and patients with more than 2 involved nodes had poorer DFS (50.0% vs 83.3%, $p = 0.101$).

Conclusions: PORT using LungART protocol in pN2 patients was well tolerated, with a high LRC. No differences in survival were observed regarding number of nodal stations or nodes involved, or hilar involvement. Yet, an inclusion of more patients will increase statistical power of this study.

P51. CHEMORADIOTHERAPY OR INTENSIVE RADIOTHERAPY IN LOCALLY ADVANCED HEAD AND NECK CANCER

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Introduction: Locally advanced head and neck (H&N) cancer requires a multidisciplinary approach in order to offer patients definitive treatment regarding function preservation and minimal toxicity. The standard of care to inoperable locally advanced H&N cancer is definitive chemotherapy and radiotherapy (CRT). In our center, patients were treated with an infusion of cisplatin 100 mg/m² at 1st, 22nd, 43rd days associated to intensity modulated radiotherapy (IMRT), with a total dose of 69,96 Gy/33 Fr/6,5 Weeks on the tumor volume. In certain cases, due to performance status or associated comorbidities, the patient received intensive radiotherapy (RT) instead of CRT. Toxicity associated with CRT in patients with H&N cancer may have a significant impact on therapeutic tolerance and can sometimes justify interruption of treatment. Breaks or pro-

longations of the treatment time with curative intent are associated with lower survival rates and loco-regional control.

Materials and methods: Retrospective review of patients with locally advanced H&N cancer in treatment between November 2013 and January 2016 in our center - Portuguese Institute of Oncology of Coimbra. Primary endpoint: objective response rate (ORR) with CRT or RT. Descriptive analysis and survival evaluation by Kaplan-Meier method.

Results: From the 70 patients included, 94% were male ($n = 66$) and the mean age was 60 years (± 12). The most frequent locations were oropharynx ($n = 23$), hypopharynx ($n = 10$), larynx ($n = 10$) and oral cavity ($n = 8$). 79% ($n = 55$) were treated with concomitant CRT, of which 40% ($n = 22$) completed three cycles, 44% ($n = 24$) completed two cycles and 16% ($n = 9$) completed one cycle. 21% ($n = 15$) patients were treated with RT. Treated patients with RT obtained an ORR of 80% and with CRT obtained an ORR of 96,4%. Only two patients died within 30 days after definitive therapy. 71,4% ($n = 50$) developed mucositis, with 46% ($n = 23$) grade II mucositis and 24% ($n = 12$) grade III mucositis. None of the patients developed grade IV mucositis. The severity of mucositis was greater in patients with oropharyngeal and oral cavity tumors ($p < 0.05$), 92% ($n = 11$) of the patients who developed grade III mucositis were from this group. The 12-month overall survival rate (OS) was 75% and 24-month OS was 61%.

Conclusions: In our study, patients treated with CRT had a superior ORR compared to RT alone. 84% completed two to three cycles of chemotherapy, in none of the cases there was suspension of radiotherapy.

P52. PROSTATE CANCER MANAGEMENT IN RENAL TRANSPLANT RECIPIENTS

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Introduction: Renal transplantation has evolved greatly in recent years, with graft and patient survival increasing. Part of this success is due to the development and application of immunosuppressive drugs. Immunosuppressed organ transplant patients have an elevated risk of malignancies. In this study we aim to determine the incidence of prostate cancer (PCa) in renal transplant recipients, as well as to evaluate their monitoring, treatment and oncological outcomes.

Materials and methods: We conducted a retrospective review of data from 1835 male patients who underwent renal transplantation between January 1987 and December 2016. Recipients presenting PCa were evaluated regarding the type of histology, age, posttransplant duration, immunosuppressive regimens, allograft functional status and PSA value. We retrospectively assessed the stage of the disease, treatment performed and consequent oncologic outcome. **Results:** We found 29 PCa in allograft recipients. The incidence of PCa in men with renal grafts was 1.6%. The mean age at transplantation was 53.4 ± 10.7 (range, 29-69 years) and the mean age at the time of diagnosis of PCa was 62.6 ± 6.1 years (range, 50-73 years). The mean time between renal transplantation and the diagnosis of carcinoma was 108 ± 85 months. Twelve recipients (41.4%) had graft failure and returned to dialysis. The median prostate-specific antigen (PSA) level at diagnosis was 7.4 ng/ml (range, 1-780 ng/ml). Twenty-four patients (82.8%) were diagnosed with prostate biopsy and five were detected incidentally by transurethral resection of prostate hyperplasia. The length of follow-up after PCa treatment ranged from 3 to 96 months. The overall survival rates at 1, 5 and 10 years after PCa diagnosis were 86.2%, 86.2% and 79.3%, respectively. Only one patient died of PCa. The remaining patients died of PCa-independent causes (cardiac failure and infections). One patient presented with osseous metastases and was managed with androgen deprivation with luteinizing hormone releasing hormone

agonists. All others had localized disease when the diagnosis was made. Radical prostatectomy was performed in 19 patients (65.5%) and radiation therapy in 5 (17.2%).

Conclusions: There appears to be an increased incidence of PCa in this population. These tumors can be approached in the same way as in the general population, and due to the potentially worse prognosis related to immunosuppression, a more regular follow-up is required in this specific population.

P53. SHORT COURSE RADIOTHERAPY IN LOCALLY ADVANCED RECTAL CANCER

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Introduction: Locally advanced rectal carcinoma (LARC) can be treated with short course radiotherapy (SC-RT: 25 Gy/5 F/1 wk) with surgery historically performed immediately. Studies show that delaying surgery may increase downstaging. An RT-surgery interval 1-4 weeks long is associated with higher postoperative complications rates. This study aim is to evaluate oncological outcomes and toxicity of patients with LARC treated with SC-RT.

Materials and methods: LARC patients treated with SC-RT between 2002-2017. Response assessed by pathological stage and modified Ryan's tumor regression grade (TRG), toxicity assessed by CTCAE 4.0 scale, survival estimated using Kaplan-Meier's method. $\alpha = 0.05$.

Results: 118 patients were evaluated, of which 24 had distant metastasis and were excluded. Of the remainder 94 patients, 64.9% were male, median age 81years, Karnofsky $\leq 80\%$ in 40.4%. Clinical staging: cT3 in 81.9% and cT4 in 12.8%; 58.5% had cN+. 85 patients were submitted to surgery, with a median RT-surgery interval of 8.5weeks (1-22 weeks). Conservative surgery was performed in 75.3%, with postoperative complications in 36.5%. 10.8% underwent adjuvant chemotherapy. Complete pathologic response (ypT0N0) was identified in 11.8%, with TRG 0-1 in 15.3%. Lymphovascular invasion present in 23.5% of surgical specimens, with R0 resections in 83.6%. Surgery was performed more than 4 weeks after RT in 61.2%, and was associated with higher ypT0N0 rates than those undergoing surgery earlier (15.3% vs 3.8%, $p = 0.166$) and better TRG (0-1) (20.3% vs 3.8%, $p = 0.058$), with no differences in postoperative complications. With a median follow-up of 26 months, survival rates at 5-years were as follows: locoregional disease-free survival (LRDFS) 90.6%, disease-free survival (DFS) 70.5%, cancer specific survival (CSS) 64.1% and overall survival (OS) 39.5%. Comparing ypT0N0; ypT1-2N0; ypT3-4 or ypN+, there were significant differences regarding DFS ($p = 0.002$) and CSS ($p = 0.006$), with better outcome for good responders.

Conclusions: SC-RT was a good option for neoadjuvant irradiation in LARC, considering age and comorbidities. Although not significant, delayed surgery provided a higher ypT0N0 rates and better TRG status with no increase of postoperative complications. Improved outcome was registered when good response was obtained.

P54. RADICAL CYSTECTOMY IN ELDERLY - IS IT A SAFE OPTION?

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Introduction: Muscle-invasive bladder cancer incidence increases with age and peaks in the octogenarians. Even though radical cyst-

tectomy (RC) is the gold standard for this disease, it is frequently not offered to elderly patients because of supposed increased risks of complications. The aim of our study was to determine if significant differences exist in the perioperative outcomes of patients aged > 75 years compared to younger patients treated with radical cystectomy (RC).

Materials and methods: We conducted a retrospective analysis of all patients with bladder cancer (BC) that underwent RC in our urology department from January 2014 to June 2017. Eighty-eight patients were identified and included in the study. Patients were divided into two groups: Group 1, aged < 75 years (58 patients) and Group 2, aged ≥ 75 years (30 patients). Co-morbidities and perioperative outcomes were compared between the groups. Fisher's exact test was used for statistical analysis.

Results: The mean age was 69.1 (35-84) years. There were 65 patients with muscle-invasive disease and 23 with non-muscle-invasive disease. There was no significant difference in median hospital stay between the two groups (12 vs 14 days) ($p > 0.05$). The 30-day mortality rate was 3.4% for those aged ≥ 75 years (30.05). Most complications were minor (Clavien-Dindo Grade I-II) and there was no statistically significant difference between the two groups ($p > 0.05$). Ileal conduit diversion was the most common form of urinary diversion in group 1 ($n = 35$, 60.3%), while cutaneous ureterostomy was the most common derivation in group 2 ($n = 13$, 43.3%).

Conclusions: RC in elderly patients has similar perioperative morbidity when compared with younger patients and can be offered to selected patients. Thus, age should not be an absolute contraindication for RC. Despite RC is not contraindicated in the elderly, we observed a greater tendency to choose simple urinary derivations, contributing to the reduction of complications in these patients.

P55. ADRENAL CORTICAL CARCINOMA: CLINICOPATHOLOGICAL FEATURES FROM A REFERENCE CENTRE

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Introduction: Adrenal cortical carcinomas (ACC) are rare tumors of the adrenal gland with variable mortality rate with unpredictable biologic behaviour. The use of special scores and immunohistochemistry (IHC) may be helpful in the stratification of these patients. The goal of this research is to perform a retrospective clinicopathological review of all the ACC diagnosed at our institution between January 2004 and December 2017.

Materials and methods: In the period of interest a total of ten cases (4M:6F) were identified (of a total of 217 surgical specimens - 0,05%), with median age of 53 ± 6.52 years (46-63) at time of presentation. Mostly were incidental discovery (6); the remain were the result of investigation due to pain complains. Only two had hormone production. Adrenal glands had a median weight of 213 ± 642.99 g (34-2,200). Tumors had a median size of 9.1 ± 6.77 cm (5.5-20) and were equally distributed (5 for each side). All were diagnosed as ACC using Weiss/Modified Weiss criteria. Ancillary studies were performed using silver staining (reticulin) and IHC for Ki67 and P53.

Results: After a median follow up of 34.5 ± 34.7 months (3-110), 4 patients were dead - overall survival (OS) of 65.5 ± 16.5 months with 70% alive at one and three years and of 46.7% at five years. Disease free survival (DFS) had a mean of 13.5 ± 37.3 months. Three

patients experienced local recurrence and three had distant metastasis. At time of diagnosis 2 patients did not have vascular invasion and two did not have necrosis. Mitotic index had a mean of 10.5 mitosis/50 HPF (1-40). Ancillary studies showed loss of reticulin fibres in all cases (8 in a diffuse pattern), P53 staining had variable intensity with more than 50% of cells in 4 cases. Ki67 had a mean index of 17.2% (2-43%). Regarding staging (ENSAT and TNM/AJCC 8th edition): 8 patients were Stage II/T2, 1 was Stage III/T3 and the remaining was on Stage IV/T4. All patients were subjected to adjuvant therapeutic with mitotane. On univariate analysis staging was predictor of worse OS. No factors were identified as predictors of worse DFS.

Conclusions: ACC are tumors of difficult prediction regarding biological behaviour. Loss of reticulin fibres was the most consistent pathological finding and staging is the most powerful predictor of worse OS. More studies are needed in order to provide biological markers of worse behaviour.

P56. "SHORT COURSE" RADIATION THERAPY IN OLDER GLIOBLASTOMA PATIENTS MULTIVARIABLE PROGNOSTIC ANALYSIS

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Introduction: Glioblastoma (GBM) is the most common malignant primary brain tumor in adults. Currently, conventional fractionated radiotherapy (RT) of approximately 60 Gy with concomitant/adjuvant use of temozolomide is recognized as the standard post-operative treatment for GBM, however most of the trials exclude patients older than 65 years. Roa et al demonstrated the non-inferiority of a hypofractionated "Short-Course" of RT in the elderly. Multivariate analysis of prognostic factors established that anemia, age and tumor volume may negatively influence outcome in patients with malignant gliomas. The aim of this study was to evaluate the influence of prognostic factors related to patient selection on survival outcomes, namely age, gross tumor volume (GTV), hemoglobin (HB), leukocytes (LEUC) levels at the beginning of treatment.

Materials and methods: Data analysis of patients submitted to "short-course" RT between 2009 and 2016. Statistical analysis by the Kaplan-Meier/Cox model for OS estimation and its own correlation with the age, HB value (12 g/dl), LEUC value (10.0 g/L), and GTV value (50 cm³).

Results: 38 patients submitted to RT (39.9 Gy/15 Fractions/3 weeks). The mean age for diagnosis was 71 years. 92.1% underwent partial surgical resection. The global overall survivor was 141.9 days. The mean GTV was 188 cm³. The mean values of HB and LEUC were respectively 13.0 g/dl and 11.0 g/L. HB values greater than 12 g/dl (HR 1.13/p = 0.74), LEUC values below 10.0 g/L (HR 1.22/p = 0.52), and a GTV below 50 cm³ (HR 1.65/p = 0.18), showed a trend towards a more favorable survival prognosis.

Conclusions: GBM is aggressive in the elderly with an average survival of 4.7 months. Higher HB is a variable with a statistical tendency for a favorable prognosis that may be associated to the degree of oxygenation of the tumor micro-environment/oxidative stress and the efficacy of the damage caused to the tumor DNA. The inflammatory/systemic immune status is associated with a less favorable prognosis and can translate interactions between the tumor, host, tumor environment, apoptotic regulation and associated comorbidities. The value of HB, LEUC and GTV can be an instrument of prognostic stratification with clinical relevance, although the authors did not find statistical significance. Further studies should be carried out in order to intensify the validation of the RT "short course" in the elderly with GBM and to explore the correlations mentioned above.

P57. PROGNOSTIC FACTORS OF GASTRO-INTESTINAL STROMAL TUMOURS

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Introduction: This work pretend to analyse the results of surgical treatment and prognostic factors of gastro-intestinal stromal tumours in a group of patients.

Materials and methods: A retrospective analysis of fifty-eight patients was carried out (30 males; 28 females). Mean age was 66 years (range: 28 to 89 years). The patients were submitted to surgical treatment between 2000 and 2014 (mean follow-up: 45 months). Clinical presentation was abdominal pain (25 cases) and digestive bleeding (21 cases). The tumours were located in: stomach (36 cases), jejunum/ileum (15 cases), duodenum (3 cases), oesophagus (2 cases) and colon (2 cases). **Results:** Radical resection was performed in 30 cases and atypical excision was performed in 28 cases. Complete resection was achieved in 86,2% of cases. The mean dimension of the tumours was 7,1cm. 57% were defined as Stage I and 51,7% presented spindle cell histology. The markers CD117, CD34 e AML were identified in 91%, 61% e 39% of cases respectively. At time of diagnosis, the tumour was confined to the organ in 51 cases, in 2 cases invades adjacent structures and in 5 cases presented metastasis. Seventeen patients received adjuvant therapy. During follow-up, 3 patients died due to the disease and 5 presented recurrence of the disease. The median 5-year overall survival was 94,7%. The survival was influenced by the following variables: age, symptoms at diagnosis, locations of the tumour, stage, invasion of adjacent structures, mitotic index > 5/50 HPF, tumour necrosis.

Conclusions: Prognosis was influenced by age, symptoms at diagnosis, locations of the tumour, stage, invasion of adjacent structures, mitotic index > 5/50 HPF, tumour necrosis. Complete resection was accomplished in the majority of cases.

P58. IMPORTANCE OF THE NUMBER OF RETRIEVED LYMPH NODES IN SURGERY FOR RECTAL CANCER TREATED WITH NEO-ADJUVANT CHEMORADIO THERAPY

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Introduction: Current guidelines recommend the excision of a minimum of 12 lymph nodes in rectal surgery for an adequate post-operative staging. After neo-adjuvant chemoradiotherapy the number of lymph nodes retrieved is smaller. This work pretend to evaluate the impact of the number of retrieved lymph nodes in the prognosis of rectal cancer patients treated with neo-adjuvant chemoradiotherapy (QRT).

Materials and methods: Between 2002 and 2012, 126 patients were submitted to QRT followed by surgery for rectal cancer. The variables that influenced global survival (GS) and disease-free survival (DFS) were determined. The number of lymph nodes retrieved during surgery associated with better survival was determined.

Results: The variables that were related with GS and DFS were: tumoral regression grade (p = 0.006); perivascular/perineural permeation (p < 0.001); positive circumferential margin (p < 0.001); type of surgery (p = 0.02); post operative stage (p < 0.001); number of lymph nodes retrieved and positive lymph nodes (p < 0.001). In the 126 cases, a mean of 13.3 lymph nodes were analyzed. The multivariable analysis determined that a minimum of 9 lymph nodes retrieved in surgery is correlated with GS and DFS (p = 0.03).

Conclusions: Histological analysis of a minimum of 9 lymph nodes in rectal cancer patients treated with QRT allows a correct post-operative staging.

P59. NATURAL KILLER CELL REPERTOIRE AND FUNCTIONAL DEFICIENCY IN RECURRENT OR REFRACTORY SOFT TISSUE SARCOMA PATIENTS

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Previous studies indicate that Natural Killer (NK) cells are deficient in Soft Tissue Sarcoma (STS) patients, although the mechanisms behind the dysfunction are not completely understood. Current therapeutic strategies influence these innate lymphoid cells and therapy failure may be partially explained by the incapacity ameliorate their cytotoxicity against cancer cells. Due to recent advances in the knowledge of NK cell biology, there is an increasing interest in mapping NK-cell responses in refractory or recurrent soft tissue sarcoma. The aim of the present study was to analyze Natural Killer cells in recurrent or refractory STS patients and the effect of therapy on receptor repertoire and functional capacity of these cells. We analyzed peripheral blood samples from STS patients (osteosarcoma, myxoid liposarcoma, liposarcoma, clear cell sarcoma, high-grade pleomorphic sarcoma, leiomyosarcoma, epithelioid leiomyosarcoma and giant cell tumor of the bone) and healthy blood donors as controls. Extended analysis of NK-cell receptor repertoire and functional properties was performed by target cell visualization assay (TCVA), multiparametric flow cytometry, cell sorting, Luminex xMAP technology (45-plex cytokine, chemokine and growth factor panel) and real-time quantitative PCR (gene expression analysis). Relative frequency of NK cells was found significantly reduced in refractory or recurrent STS patients with poor cytolytic capacity and normal IFN- γ production, defining a split anergy status. NK cells exhibited a mature status (increased CD57) and deficient early activation (decreased CD69), although CD62L NK cells were found similar to controls, meaning untouched capacity of migration to secondary lymphoid organs. NK receptor repertoire was found altered with significant upregulation of NKP44, NKG2A, NKG2D, PD-1 and CD137 (4-1BB) and down-regulation of CD226 (DNAM-1). Analysis of a large panel of 45 cytokines, chemokines and growth factors revealed an increase of IL-10, TGF- β , IL-4 and normal production of IFN- γ by STS NK cells. NK cells are significantly affected in recurrent or refractory STS with a split anergy phenotype - decreased cytotoxicity and normal production of IFN- γ . Combination of NK cell-based immunotherapy with pharmacological interventions should be investigated in order to reduce metastatic potential and to eradicate cancer cells. Financial Support: FEDER (COMPETE) and FCT through project PEstC/SAU/LA0001/2013-2014.

P60. SOLUBLE AND MEMBRANE-BOUND RECEPTOR-LIGAND IMMUNE CHECKPOINTS IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Blockade of immune checkpoints (ICP) seems to unleash the potential of the antitumor immune response in a fashion that is transforming human cancer therapeutics. Although its implications in immune response during chronic myeloid leukemia (CML) are obvious, literature regarding CML and ICP is scarce. This study aimed at the analysis of ICP expression by lymphocyte subsets and plasmatic levels during tyrosine kinase inhibitor (TKI) therapy and its correlation with molecular response. Chronic phase CML patients (n = 55), divided according to molecular response to TKIs and Interferon-alpha 2b therapy, and healthy donors (n = 25) were included in this study. Multi-parametric flow cytometry was used for the analysis of the expression of ICPs by different T, B, NK, monocyte and dendritic cell subsets. A 59-plex panel including ICPs, cytokines, chemokines and growth factors was analyzed by xMAP technology (Luminex®). Gene expression profiles of cell sorted populations and miRNA-mediated immune regulation is also presented. Expression of CD137 by several lymphocyte subsets and PD-1 by regulatory T cells and NK cells were found significantly altered in CML patients under TKI therapy. These associations were observed for the cell population frequency expressing the receptor, and also for density of these molecules at cell membrane. Increased plasmatic levels of BTLA, HVEM, PD-1, PDL1, and CD137 were associated with good molecular response to therapy. PD-1, PD-L1, TIM-3 and CD137 were found increased in patients that achieved deep molecular response (MR4.5). ICP gene regulation by miRNAs was correlated with CML for PD-1 (miR-16, miR-195), PD-L1 (miR-520d-5p), TIM3 (miR-326), GITR (miR-939), HVEM (miR-128) and CD80 (miR-22). Some immune checkpoints seem to be affected by TKI therapy in CML and their cell expression and plasmatic levels correlate to molecular response. Soluble and membrane-bound receptor-ligand immune checkpoints could represent interesting targets for future therapeutic monitoring and for pharmacologic interventions in CML. Funded by the FEDER Funds through COMPETE 2020 and by FCT within the framework of the Strategic Project with reference assigned by COMPETE: POCI-01-0145-FEDER-007440.

P61. ASSESSMENT OF MALNUTRITION RISK IN HOSPITALIZED PATIENTS

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Introduction: Malnutrition affects about 10% of the elderly population, and a large part of this population is at risk of malnutrition. In hospital admission between 20 and 60% of admitted patients are malnourished or at risk of malnutrition. Individualized nutritional assessment is important for the identification of cases at nutritional risk, and there is evidence of the benefits of patient follow-up, with a reduction in hospitalization time, patient nutritional status improvement, and a better prognosis. In this context, the aim of this work was to evaluate the nutritional status of patients admitted to an Internal Medicine Service (IMS) and its evolution during hospitalization.

Materials and methods: This was a prospective study of patients admitted to the IMS through Nutritional risk screening (NRS 2002), and other variables such as Body Mass Index (BMI), brachial perimeter, leg perimeter, analytical exams and weekly reassessment during hospitalization. Results were analyzed statistically using SPSS and a was considered a significance level of 95% (p < 0.05).

Results: Thirty-five patients were hospitalized in the IMS during May 2017, 24 (69%) women and 11 (31%) men, with a mean age of 83 years (± 8.37), ranging from 63 to 104 years. According to the NRS-2002 classification, 30 (85.7%) of the patients studied were at nutritional risk, with a score distributed between 3 (60%) and 4 (40%). There were significant differences between the nutritional risk groups and the various elderly groups ($p = 0.018$). Patients that were not at nutritional risk were younger (69 years) than those at nutritional risk (83 years). Moreover, the BMI of patients without nutritional risk was higher (31 kg/m²) than those at risk (26 kg/m²). The brachial and leg perimeter were normal in all patients that were not at nutritional risk, being low in more than 80% of those who were at nutritional risk. Finally, patients at nutritional risk had lower albumin and hemoglobin levels.

Conclusions: These preliminary results showed that the majority of patients admitted to the IMS were at risk of malnutrition and that the risk of malnutrition was positively correlated with age and negatively with BMI, brachial perimeter, leg perimeter, albumin and hemoglobin levels.

P62. STUDY OF GENETIC RISK VARIANTS IN RETINAL ANGIOMATOUS PROLIFERATION USING MLPA - AN UPDATE

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Introduction: Age-related Macular Degeneration (AMD) is a late-onset multifactorial disease characterized by a progressive destruction of the macula. Although the early stages are usually asymptomatic, the late forms of the disease - geographic atrophy and neovascular/exudative AMD (eAMD) - can lead to irreversible vision loss. Retinal angiomatous proliferation (RAP) is a particularly aggressive neovascular phenotype, estimated to represent up to 20% of all eAMD cases. The phenotypic differences between RAP and typical eAMD are thought to have a genetic basis. In fact, AMD is a multifactorial disease characterized by complex interactions between environmental (age, smoking, ethnicity, etc) and genetic factors, with the latter accounting for up to 70% of the disease burden. Most genetic alterations associated with the incidence and progression of AMD are single nucleotide polymorphisms (SNPs) in *CFH* and *ARMS2/HTRA1* genes. Moreover, copy number variations (CNVs) involving *CFHR3* and *CFHR1* genes have also been extensively reported.

Materials and methods: This is a cross-sectional study, aiming to evaluate SNPs and CNVs within the genes most commonly associated with AMD in patients with RAP. Taking advantage of multiplex ligation probe amplification (MLPA) technique, a SALSA probemix (MRC-Holland) was used to evaluate 198 samples: 42 RAP and 42 eAMD patients and 114 controls.

Results: Patients (eAMD+RAP) and controls have statistically significant differences concerning the presence of *CFH* intronic SNP (rs1410996; $p=0.022$, OR=2.86, 95% CI [1.17-7.02]) and *ARMS2* SNP (rs10490924; $p<0.001$, OR=3.06, 95% CI [1.90-4.93]). The combination of these two genetic variants increased the odds ratio of neovascularization ($p<0.001$, OR=3.80, 95% CI [1.90-13.24]). Furthermore, the presence of at least one rare allele in *ARMS2* SNP was able

to also distinguish RAP from controls, whereas only the presence of 2 risk alleles allowed a distinction between eAMD and controls. We also verified a tendency of eAMD to present no CNVs concerning *CFHR3* and *CFHR1* with comparing with controls ($p=0.045$).

Conclusions: The cohort's increase, as well as the addition of a new group of patients allowed a better understanding of RAP's genetic background, with promising results for the genetic distinction between this neovascular phenotype and typical eAMD. This represents one further step towards a new diagnostic approach of AMD and ultimately, the prediction of its development.

P63. TRANSCRIBED ULTRA CONSERVED NONCODING RNA (T-UCR) EXPRESSION PROFILES ASSOCIATED WITH TYROSINE KINASE INHIBITOR THERAPY IN CHRONIC MYELOID LEUKEMIA

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Transcribed ultraconserved regions (T-UCR) are a novel class of long noncoding RNAs. Many classes of noncoding RNAs have been implicated in human tumorigenesis. In addition to the different expression profiles of T-UCRs that could be used to distinguish human leukemias and carcinomas, they have also been reported to have direct interactions with miRNA with an important regulatory effect in disease development such as chronic myeloid leukemia (CML). In this study, we aimed at the correlation of T-UCR and miRNA:T-UCR pairs in CML, according to tyrosine kinase inhibitor (TKI) therapy, clinical risk scores and molecular response. We analysed peripheral blood samples from 45 CML patients and 15 healthy controls. Two panels of 481 T-UCR and 752 miRNA probes were used for RT-qPCR analysis. Differential expression was evaluated using the Mann-Whitney test followed by Benjamini-Hochberg multiple testing correction. CML samples presented significantly different expression of uc.164 ($p < 0.01$), uc.118 ($p < 0.01$), uc.125 ($p < 0.01$), uc.391 ($p < 0.01$), uc.153 ($p < 0.01$), uc.141 ($p < 0.01$), uc.143 ($p < 0.05$) and uc.145 ($p < 0.05$), when compared to healthy controls. This latter T-UCR (uc.145) was associated with development and immune regulation pathways. We analysed Sokal, EURO, EUTOS and ELTS risk scores and found uc.236 ($p < 0.0001$), uc.39 ($p < 0.05$) and uc.7 ($p < 0.05$) to be associated with EUTOS low risk. Concerning therapy, dasatinib was correlated with uc.187 ($p < 0.005$). For imatinib doses, uc.4 ($p < 0.05$) and uc.3 ($p < 0.05$) inversely correlated with 400 and 800 mg daily, respectively. Molecular response in CML samples presented a signature including uc.187 ($p < 0.001$), uc.107 ($p < 0.05$), uc.409 ($p < 0.05$), uc.198 ($p < 0.05$), uc.309 ($p < 0.05$), uc.102 ($p < 0.05$), uc.294 ($p < 0.05$) and uc.361 ($p < 0.05$). Major molecular response was identified by the altered expression of uc.198 ($p < 0.05$), uc.215 ($p < 0.05$) and uc.210 ($p < 0.05$). The negative regulation of T-UCRs by miRNAs, involving T-UCR:miRNA interaction, was associated with upregulated (miR720, miR8863p, miR1274a, miR101 and miR129) and downregulated (miR489 and miR1973) microRNAs. In the present study, we identified T-UCRs signatures and T-UCR:miRNA pairs associated with CML, risk scores, TKI therapy and molecular response. The expanded knowledge of RNA biology in general, together with the recent interest in the multitude of newly discovered elements such as T-UCRs, could help to improve CML therapy.

P64. CHARACTERIZATION OF GENOMIC PROFILE OF BLADDER CANCER: ARRAY-COMPARATIVE GENOMIC HYBRIDIZATION AS A HIGH-THROUGHPUT APPROACH

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Introduction: Bladder cancer (BC) is a solid tumor with high recurrence rates. It is the sixth tumor with the highest incidence and the eighth one with the highest mortality in the world. Since the prognostic tools currently available have limitations in identifying subgroups of patients at increased risk for recurrence or progression after treatment, we needed to increase knowledge of the genetic changes associated with the BC. The aim of this study was to characterize the genomic profile of bladder tumors using a whole genome technique, array-Comparative Genomic Hybridization (aCGH). **Materials and methods:** Bladder tumor samples were obtained from 28 patients when they performed a transurethral resection of bladder tumor (TURBT), and aCGH was done using an Agilent oligonucleotide microarray 4 × 180K. The controls used were bladder tissue samples from non-cancer donors. Clinical data from the patients were registered and histopathological information analyzed.

Results: With this whole genome approach, we verified that our samples presented few genomic imbalances. In these preliminary results, we did not verify a pattern of chromosomal alterations, as we did not find imbalances in more than 20% of patients. Besides that, the chromosomes with more frequent copy number gains were 1, 11, 13, 18 and 21 and the chromosomes with more frequent copy number losses were 1, 6, 10, 13, 20, 21, 22 and X. In addition, the sizes of aberrations detected for the same chromosome were often variable between patients.

Conclusions: In conclusion, we found that with this approach we identified some chromosomal regions altered in bladder cancer comparing to normal tissues. Thus, could be mapped fundamental genes related to disease initiation and progression. The correlation between molecular and clinical-pathological data will be essential to recognize accepted biomarkers with possible diagnostic and prognostic interest.

P65. INFLUENCE OF HISTOCHEMICAL STAINS ON DNA OBTAINED FROM FFPE SAMPLES

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Introduction: Formalin fixed, paraffin embedded (FFPE) samples are used for diagnostic and prognostic purposes. Histopathological analysis frequently includes not only histomorphological evaluation but also histochemical and molecular studies. In some cases FFPE samples are scarce and it is necessary to use the same histological section for histochemical analysis and DNA extraction. In molecular pathology labs this is a common practice, allowing the analysis of DNA specifically from altered cells. However, histochemical techniques use reagents that may induce chemical modifications on DNA. To perform a literature review about the influence of histochemical stains on DNA integrity.

Materials and methods: PubMed and Research Gate were used to survey original articles published until December 2017.

Results: For this review articles about the analysis of DNA extracted from stained FFPE sections were considered. The studies demonstrated that: DNA extracted from sections stained with Azure B, toluidine blue and methyl green (MG) was successfully amplified by Polymerase Chain Reaction (PCR) whereas Mayer's hematoxylin stain inhibits the reaction. Another study demonstrated that DNA amplification by PCR had better results with eosin Y and MG stains comparatively to Mayer's hematoxylin and May-Grunwald. Banaschak et al. 2001, showed that DNA analysis by PCR and capillary electrophoresis was successful with Hematoxylin Eosin (HE), Periodic Acid Schiff (PAS), Azan and Perls stains. Phosphotungstic acid hematoxylin (PTHA) and Gomori stains had negative results. Two different studies concluded that DNA is refractory to HE stain as capillary electrophoresis demonstrated similar degradation to that of unstained samples and it was successfully amplified by PCR.

Conclusions: Histochemical analysis allows demonstration of cellular components whose alterations are typical from pathological conditions. These techniques encompass reagents that may alter biomolecules. Nevertheless, from the analyzed studies it is possible to conclude that DNA integrity is maintained in techniques such as Azure B, toluidine B, MG, eosin Y, HE, PAS, Azan and Perls. On the other hand, Mayer's hematoxylin, May-Grunwald, PTHA and Gomori resulted in inhibition of DNA amplification. Since this analysis was not performed in common routine techniques such as Masson's Trichrome and PAS-Alcian Blue, it is important to deepen the knowledge, performing new studies for future appliance.

P66. SEVERE INTELLECTUAL DISABILITY, ABSENT SPEECH, EPILEPSY AND CRANIOFACIAL DYSMORPHISMS IN A FEMALE PATIENT WITH A 3(p25.3) PROXIMAL INTERSTITIAL DE NOVO DELETION

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Introduction: Deletions at the distal portion of the short arm of chromosome 3 cause a recognizable syndrome with characteristic features, most frequently arising de novo and with breakpoints at band 3(p25). Interstitial deletions involving only sub-band 3(p25.3) are less frequently reported, and within this region two deletion areas can be defined: distal and proximal deletions.

Case report: We report a 24 year old female with global developmental delay (DD), severe intellectual disability (ID), absent speech, epilepsy and craniofacial dysmorphisms. Due to her severe ID, absent speech and dysmorphic features, she was initially considered an Angelman syndrome patient. However, array-CGH analysis revealed a *de novo* 1Mb interstitial deletion at band 3(p25.3) between positions 10,364,749 and 11,421,309 (hg19).

Discussion: The reported deletion overlaps with deletions previously reported in the most proximal area of region 3(p25.3), although there are only 5 patients reported in the literature with this imbalance. These patients present a common phenotype consisting of DD, ID, absent or poor speech and epilepsy or EEG anomalies. The commonly deleted region includes the 3 last coding exons of *SLC6A11* gene, *SLC6A1* gene and its antisense gene, *HRH1* gene and part of *ATG7* gene. Both *SLC6A* genes code for Gamma-aminobutyric acid (GABA) transporters, responsible from removing GABA from the synapse. *SLC6A1* gene is reported in OMIM Morbid Map as heterozygous mutations are responsible for myoclonic-atonic epilepsy

and is considered haploinsufficient. These data support the association of *SLC6A1* gene and the phenotype of epilepsy among patients with 3(p25.3) deletion.

P67. CONFINED PLACENTAL MOSAICISM OR A TRUE FETAL CHROMOSOMAL ANEUPLOIDY? INTERPRETING CHORIONIC VILLUS SAMPLING RESULTS IN PRENATAL DIAGNOSIS

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Introduction: Whole-chromosome aneuploidy is currently known as the leading cause of miscarriage and congenital birth defects in humans. Consequently, this chromosomal abnormality is the most common indication for invasive prenatal diagnosis by chorionic villus sampling (CVS) or amniocentesis, allowing the study of fetal chromosome constitution. CVS is performed earlier than amniocentesis; however it has the disadvantage that about 1-2% of CVS results may reflect confined placental mosaicism (CPM) instead of true fetal chromosomal abnormalities.

Materials and methods: From a cohort of around 4,000 samples (20% CVS, 80% amniocentesis) that were received in our laboratory for prenatal diagnosis, 23% had indication for rapid aneuploidy detection test. Highly polymorphic short tandem repeats (STRs) on chromosomes 13, 18, 21, X, Y were used to detect the most common aneuploidies by quantitative fluorescent polymerase chain reaction (QF-PCR).

Results and conclusions: Of all CVS received, 40% were tested and the main indications were: increased nuchal translucency (NT), a positive biochemical screening, hygroma, omphalocele or other ultrasound anomalies. A negative result for aneuploidy test was verified in 73% CVS, while the remaining 27% presented a positive result: 4% trisomy 13, 24% trisomy 18, 52% trisomy 21, 13% monosomy X, 6% triploidy and 1% 48,XXXX. Among the CVS with a positive result for aneuploidy testing, a rare case was detected: a trisomy 21 where only 2 different alleles were evident in 2:1/1:2 ratios meaning that 2 of the 3 alleles were exactly identical. As this result was obtained in a CVS, this could correspond to a CPM; however, the fetus presented a phenotype including increased NT (7.8 mm) and hygroma. Later, by conventional cytogenetics a trisomy 21 was observed. After pregnancy termination, STRs analysis on DNA from fetal skin biopsy confirmed the CVS result. Comparing the STRs profile of fetus and mother, it was possible to conclude that the trisomy 21 was not of maternal origin. Thus, the trisomy 21 may be derived from a paternal meiotic II nondisjunction, an unusual condition observed in about 1% of the cases; or had origin in an early mitotic nondisjunction (=2% of the cases). This particular result of a 2:1 ratio illustrates that caution should be made on the interpretation of rapid aneuploidy testing by QF-PCR, especially in CVS, as we tend to associate this type of results to CPM and indeed they can be due to other biological mechanisms.

P68. GINGIVAL FIBROBLASTS CAN DEDIFFERENTIATE INTO STEM-LIKE CELLS

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Introduction: Partial/total loss of dental structures due to cavities, trauma or periodontal disease represent the main oral health problem. Artificial substitutes for dental tissues only partially replace biological functions, a situation that regenerative dentistry aims to overcome. Dental regeneration is based on stem cells; however, the collection, selection, and the number of available stem cells are important limitations, besides the associated ethical and rejection issues. Based on this problem, we propose to obtain stem-like cells to use in regenerative dentistry, using easy to collect gingival tissue, through a dedifferentiation protocol.

Materials and methods: Mouse gingival fibroblasts were obtained through explants. A differentiation protocol was performed, using a dedifferentiation agent (DA) in concentrations from 1 to 5 μ M, with or without medium renewal. The obtained cells were evaluated regarding their metabolic activity (MTT assay), cell death and cell cycle analysis (flow cytometry), DNA content (crystal violet staining) and protein content (SRB assay).

Results: DA appears to be well tolerated by cells, since a decrease in metabolic activity was observed only in higher concentrations (above 2,5 μ M, no medium renewal), therefore with a cumulative effect. Similar results were observed to cell death, being apoptosis the main type. Regarding protein content, only at higher values (5 μ M, without medium renewal), a significant decrease was observed. DNA content analysis by crystal violet staining showed that the DA promotes an increase in DNA content, with the higher results observed at 2,5 μ M with medium change. Cell cycle analysis showed the appearance of a 4N population, being the number of 4N cells directly proportional to the DA concentration. Also, an increase in the cell population in G2/M phase was observed.

Conclusions: The dedifferentiation protocol seems to successfully induce a population with stem-like characteristics, as seen by the DNA and cell cycle analysis, together with morphological changes also observed. These results allowed to optimize our protocol, namely the selection of the DA concentration and to maximize the emergence of a tetraploid population with a minimum cell death. The obtained cells will now be tested by international criteria, to validate its future use in regenerative dentistry.

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P69. ANALYSIS OF PROTEIN AGGREGATES IN PLASMA OF HEART FAILURE PATIENTS: A CASE SERIES

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Introduction: According to WHO, cardiovascular diseases remain the leading cause of death worldwide. The increase of misfolded protein aggregates has been associated with aging and with several age-related pathologies, including cardiovascular diseases. Thus, the elucidation of the pathophysiological mechanism of protein aggregates may represent a potential therapeutic target of these highly prevalent and life-threatening diseases.

Objective: To describe the detergent-resistant protein aggregates loaded in the plasma of a series of 3 cases of heart failure with reduced ejection fraction.

Materials and methods: Three patients referred between November and December 2017 for an exercise testing and two age-matched healthy controls were invited to participate. Blood was collected before the exercise test; clinical data was abstracted from the clinical files and confirmed with the patients. We isolated and quantified detergent-resistant protein aggregates in plasma precleared from albumin and immunoglobulin by diagonal two-dimensional (D2D) SDS-PAGE. This methodology consisted in the following steps: 1) resolve the unheated protein samples by SDS-PAGE; 2) excise and boil the resultant gel strip in a SDS containing buffer; 3) perform a second dimension of SDS-PAGE of the heated gel strip. The result is a 2D gel with a diagonal pattern of non SDS-resistant proteins plus non-diagonal SDS-resistant proteins and SDS-resistant soluble aggregates.

Results and conclusions: Patients showed a low cardiorespiratory fitness (VO₂peak 13.6, 18.9 and 19.8 mL O₂/kg/min) and reduced left ventricular ejection fraction (23%, 23%, 37%). One patient showed also a reduced right ventricular ejection fraction (16%); this patient (VO₂peak 18.9 mL O₂/kg/min) was the only one showing a high level (2-4 fold higher) of protein aggregates (0.8%) comparatively to the healthy controls. The remaining patients presented very similar levels of protein aggregates (0.2 and 0.4%) in relation to the healthy controls (0.2 and 0.3%). In future works, to elucidate the role of the protein aggregates in the pathophysiology of the cardiovascular disease, the isolated components of these protein aggregates should be characterised by high-resolution mass spectrometry.

P70. INFLAMMATION, CARDIORESPIRATORY FITNESS AND BLOOD PRESSURE IN PATIENTS WITH RESISTANT HYPERTENSION

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Introduction: Resistant Hypertension, i.e. uncontrolled blood pressure (BP) with 3 antihypertensive drugs or controlled BP with four or more antihypertensive agents, increases cardiovascular and end-organ damage risk. The importance of low grade systemic inflammation in BP control has been acknowledged, yet its role in resistant hypertension remains unclear.

Objective: To determine whether BP is associated with C-reactive protein (CRP), a marker of systemic inflammation, and cardiorespiratory fitness in patients with resistant hypertension.

Materials and methods: Seventeen patients (10 men, 7 women; age, 59.1 ± 7.3 years old; weigh, 78.5 ± 10.1 kg; height, 1.64 ± 0.10 m; body mass index, 29.2 ± 3.0 kg/m²) with resistant hypertension were recruited in the Hospital Infante D. Pedro, Aveiro. Outcome measures included clinical data, cardiorespiratory fitness (VO₂peak), casual and ambulatory BP, and plasma levels of high-sensitivity C-reactive protein (hsCRP). Correlation analysis was conducted to assess the association between variables; independent t-tests were conducted to compare variables between those with CRP levels below and above 3 mg/L.

Results: A significant inverse correlation was found between VO₂peak (33.8 ± 1.5 mL O₂/kg/min) and 24h (127.5 ± 3.4 mmHg; r = -0.599, p = 0.014), day (132.3 ± 3.2 mmHg; r = -0.556, p = 0.025) and night (117.1 ± 4.1 mmHg; r = -0.590, p = 0.016) systolic BP as well as with 24h (91.7 ± 2.5 mmHg; r = -0.615, p = 0.011), day (93.6 ± 3.6 mmHg; r = -0.565, p = 0.023) and night (84.7 ± 2.7 mmHg; r = -0.600, p = 0.014) mean BP. hsCRP (4.2 ± 1.1 mg/L) was correlated with systolic and diastolic 24h (r = 0.664, p = 0.004; r = 0.563, p = 0.019), day (r = 0.634, p = 0.006; r = 0.526, p = 0.030) and night (r = 0.713, p = 0.001; r = 0.635, p = 0.006) BP as well as with night pulse pressure (50.5 ± 2.9 mmHg; r = 0.513, p = 0.035) and night mean BP (r = 0.677, p = 0.003). Those with higher values of CRP (n = 8) showed higher night systolic (108.7 ± 3.0 vs. 126.5 ± 6.9 mmHg, p = 0.026) and mean BP (79.6 ± 1.8 vs. 90.4 ± 4.8 mmHg, p = 0.045). The correlations remain significant when controlling for confounding variables (e.g. age, body mass index). hsCRP and VO₂peak showed no correlation with casual systolic (144.0 ± 3.8 mmHg) and diastolic BP (82.0 ± 1.9 mmHg).

Conclusions: In patients with resistant hypertension, higher ambulatory BP is associated with higher levels of low grade inflammation and lower cardiorespiratory fitness, an independent predictor of all-cause mortality.

P71. IMMUNOHISTOCHEMISTRY EVALUATION OF THE EFFECTS OF ORTHODONTIC FORCE APPLICATION ON DENTAL PULP STEM CELLS

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Introduction: The effect of orthodontic force application on the dental pulp, which ranges from vascular stenosis to pulp necrosis, could compromise the long-term dental pulp vitality. The dental pulp stem cells (DPSC) are responsible for the pulp homeostasis by forming new components that are essential to maintain the pulp integrity. Since the effects of orthodontic forces on the DPSC are still unclear, this study aims to evaluate the effects of the application of orthodontic forces in this cell population.

Materials and methods: Pre-molars subjected to orthodontic forces were divided into 4 groups (control T0, 1 week of orthodontic movement T1, 2 weeks T2 and 3 weeks T3) for immunohistochemistry analysis with the specific stem cells antibodies Nanog and Oct3/4. All the procedures were approved by the Ethics Committee of the FMUC, Coimbra, Portugal.

Results: At T1 both markers were expressed. The immunofluorescence showed that the activated DPSC distributed themselves in form of a cluster, mainly in the subodontoblastic region, more specifically in the cell-rich zone. No expression of Nanog or Oct3/4 was observed at T0, T2 and T3.

Conclusions: These findings suggest that the DPSC are compelled to enter the cell cycle after one week of force application. The lack of expression at week two and three suggests that the DPSC may have been compromised in a differentiation and/or dedifferentiation process. Further studies with force reactivation are necessary.

P72. HISTOLOGICAL EVALUATION OF THE EFFECTS OF ORTHODONTIC FORCE APPLICATION ON THE DENTAL PULP TISSUE

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Introduction: The orthodontic force responsible for the orthodontic tooth movement has a crucial role in the regulation of periodontal and dental pulp physiology. Although the histomorphological principle of tooth movement has already been described, the effects of orthodontic force application on human dental pulp are still quite unclear. Therefore, this study aims to evaluate histologically the responses of the dental pulp tissue to the orthodontic force.

Materials and methods: Premolars extracted for orthodontic treatment were divided into 4 groups: a control group T0 (teeth not subjected to orthodontic forces), and 3 test groups, T1, T2 and T3 (teeth subjected to the application of orthodontic force during one, two and three weeks, respectively). The premolars were histologically processed and serial sectioned for light microscopy examination. All the procedures were approved by the Ethics Committee of the FMUC, Coimbra, Portugal.

Results: Relatively to T0, at T1 it was observed an increase in vascularization and formation of tertiary dentin. In some samples of T1, was visible not only the formation of mineralized tissue, with histological characteristics compatible with acellular cement, but also the increase in cell density of odontoblast-like cells. In some samples of T2, pulp calcifications and mineralized nodules were observed in the central region of the pulp and in the proximity of blood vessels, findings no longer visible at T3. At T3 the overall appearance of the pulp was similar to that of T0.

Conclusions: The observation of mineralized tissue inside the dental pulp at the end of 2 weeks confirms that, even in the presence of orthodontic force, this is the mechanism of protection by excellence. Aggressive stimuli like this force induced odontoblasts death by either apoptosis or necrosis. The presence of odontoblast-like cells, probably originated in the cell-rich zone of the pulp, endorsed the replacement of the odontoblasts population.

P73. UNUSUAL MANIFESTATIONS OF SJÖGREN-LUPUS OVERLAP SYNDROME

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Sjögren's syndrome (SjS) and systemic lupus erythematosus (SLE) are systemic autoimmune inflammatory disorders with chronic evolution to serious organ damages. SjS is characterized by the presence of hyperreactive B lymphocytes and lymphocytic infiltrates in the exocrine glands, resulting in their inflammation and the eventual loss of their physiological functions. This explains the sicca symptoms (xerostomia and xerophthalmia) - the clinical hallmarks of SjS. The SjS-SLE association has been reported instead of previous considerations like a same disease. The authors report a case of a 25-year-old woman with cryptogenic organizing pneumonia (COP), hemolytic anemia and thrombocytopenic purpura (Evans-Syndrome). She presented an

history of polyarthralgia, fever, dry cough and a petechial rash with spontaneous haematomas. There were count 4G/L platelets, mild anemia and leucopenia, and CT images with bilateral pulmonary consolidations. Autoimmune etiology was admitted due to strongly positivity for SSA, accordant salivary study and exclusion of others hypotheses. According recent criteria the authors assumed first manifestation of Sjögren-Lupus Overlap Syndrome. She begun treatment with 1 mg/kg prednisolone with clear improvement. These serious and uncommon signs show the variability of presentations, difficulties the diagnosis and may compromise the begin of therapeutic for this autoimmune overlap syndrome. 2016-ACR/EULAR classification criteria for SjS already allows diagnosis without sicca symptoms. In this case, it facilitated clinical interpretation and promoted a prompt therapeutic reaction.

P74. THE PARADIGM OF AUTONOMY IN PALLIATIVE CARE: IMPLEMENTATION OF ANTICIPATED CARE DIRECTIVE

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Introduction: In the approach to palliative care, it is not only the health professionals' team but also the patient's have the power to decide. In Portugal, the legal regime of Diretivas Antecipadas de Vontade (DAV) is already implemented by Law no. 25/2012 of July 16, reinforced by Administrative Rule no. 104/2014. This document allows health professionals to know the will of the patient with chronic pathology, evolutionary and incurable. The adult person can express their willingness to accept palliative care or refuse unnecessary or disproportionate health care using DAV by drawing up their Testamento Vital (TV) or delegating this function to a Health Care Provider (HCP). Health professionals cannot be excluded from the whole process of planning, designing and complying with the DAV. What is the knowledge of health professionals about DAV and its implication in Palliative Care?

Materials and methods: We conducted a search of the terms DAV, autonomy, health professionals, decision on end of life and legal ethical aspects, dated from 2010 to 2016 in online search, resulting in 26 studies, 3 of which empirical.

Results: The professionals still have many doubts and the applicability of this document in the therapeutic process of the patient still reduced. The small dissemination to the patients was motivated by gaps in subject revealed by professionals. Analyzing the research studies carried out in Portugal pointed out many doubts and uncertainties regarding the DAV application. It was also noticed that 53% of health professionals report having knowledge on the subject, being doctors the most knowledgeable, and 73% agrees with the ideology inherent to the application of DAV in Portugal. Besides, none of contacted professionals have experience with DAV in real context, all of them would respect DAV in case of Palliative Care where only 33% would respect in patients without palliative criteria. Testamento Vital was the most well-known DAV (73%) and only 40% knew the possibility of HCP. Of the sample, only 40% of the professionals admitted recommending their patients to perform a DAV and only 33% admit that they would put this possibility to themselves. From 2014 until 2017 only 6190 Portuguese registered their Vital Testament in RENT-EV, which falls short of the 20000 documents expected.

Conclusions: There is a need for reflection and debate on the ethical issues DAV end-points, with a promotion of patient autonomy and team decisions, as well as the effectiveness of RENT-EV.