Rev Port Pneumol. 2016;**22(Esp Cong 2):**1-9

revista portuguesa de
PNEUMOLOGIA
portuguese journal of pulmonology

ORAL PRESENTATIONS

5th CIMAGO Congress

University of Coimbra, Coimbra, Portugal, 27-28 January 2016

The abstracts are the author's responsibility.

Session "New therapeutics and approaches in clinical oncology"

OP1. THE SYNERGIC EFFECT OF PHOTODYNAMIC THERAPY AND ACETYLSALICYLIC ACID IN COLORECTAL AND ESOPHAGUS CANCER CELLS

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Introduction: The colorectal and esophageal cancers are situated in the top 10 of the most common cancers in terms of incidence and mortality, having both of them a high cyclooxygenase expression. Photodynamic therapy is a low invasive therapy that is approved for the treatment of various disorders, particularly cancer.
Objective: Evaluate the use of cyclooxygenase inhibitors such as acetylsalicylic acid (aspirin) in combination with photodynamic therapy for the promotion of anti-proliferative effects in WiDr and OE19 cancer cells in order to increase the effectiveness of the treatment.
Methods: WiDr cell line was cultured in DMEM medium supplemented with 5% fetal bovine serum (FBS), 1% antibiotics and sodium piruvate, while OE19 cell line was propagated in culture with RPMI also with 5% FBS, 1% antibiotics and sodium piruvate. For the studies of proliferation evaluation 80,000 cells/ml (MTT assay) or 250,000 cells/ml (SRB assay) were plated in 48 multiwell plates, with the addition of a photosensitizer, previously synthetized by us, at the concentrations of 5 nM, 50 nM, 200 nM and 500 nM past 24h. Subsequently the plates were irradiated with a flow 7.5 mW/cm^2 to achieve 10J, followed by the addition of aspirin at the concentrations of 2.5 mM and 10 mM. After 24 hours, cell proliferation was assessed by the MTT assay or SRB assay. For the flow cytometry studies, the cell cultures were stained with annexin V/propidium iodide-FITC probe for evaluating cell death type, JC-1 probe for evaluating mitochondrial membrane potential, propidium iodide for evaluating cell cycle, DHE probe for evaluating the presence of superoxide ion, DCF probe for evaluating the presence for peroxides and finally mercury orange probe for evaluating the glutathione activity.
Results: The results following the MTT Assay and SRB Assay suggest that the combination of photodynamic therapy with acetylsalicylic acid diminishes both cancer cell lines metabolic activity and protein mass in a manner dependent of the concentrations of the photosensitizer. For the treatment with 50 nMPS and 2,5 mM acetylsalicylic acid the metabolic activity decreases 82 ± 10% in WiDr cell line and 79 ± 10% in OE19 cell line. Considering flow cytometry, necrosis is the major cell death type, in a manner dependent of photosensitizer concentration, although cell death by apoptosis also occurs. In terms of cell cycle, there is a measurable retention in G0 in some conditions. The presence of reactive oxygen species increases in a manner dependent of photosensitizer concentrations and the activity of GSH seems not to be highly affected by it. Finally, the membrane mitochondrial potential seems to decrease which indicates death by apoptosis.
Conclusion: The combination of photodynamic therapy and acetylsalicylic acid is potentially synergistic being dependent on the concentration of the photosensitizer in both cell lines, playing also role in the type of cell death activated in both cell lines studied.
Funding: FCT, Portugal (UID/NEU/04539/2013), COMPETE-FEDER.

OP2. EVALUATION OF THE CYTOTOXIC RESPONSE OF PHOTODYNAMIC THERAPY IN COMBINATION WITH DOXORUBICIN AND METHOTREXATE INOSTEOSARCOMA

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Introduction: Osteogenic Sarcoma (OS) is a malignant tumor that arises from primitive mesenchymal cells and is characterized pathologically by spindle cells and formation of osteoids. Doxorubicin and methotrexate are chemotherapeutics currently used. Combined treatments may target different key signal transduction pathways, may be more efficient in destroying cancer cells and in eluding cellular resistance mechanisms. Photodynamic therapy (PDT) is a non-mutagenic therapeutic modality for treating cancer. Several studies reported that combination treatment of chemotherapy and PDT may overcome tumor drug resistance, increase anticancer activity and became a therapeutic approach in cases that surgery is not possible.
Objective: To evaluate the effect of chemotherapy alone and in combination with photodynamic therapy in osteosarcoma cells.
Methods: MNNG-HOS (OS cell line) was cultured in DMEM supplemented with 10% fetal bovine serum. For each experiment, 50,000 cells/mL were plated in 48 multiwells. After 24, 48 and 72 hours, cells were incubated with several concentrations of doxorubicin and methotrexate. In order to determine the IC_{50}, cell proliferation was evaluated through MTT assay. In order to evaluate the effect of PDT in combination with chemotherapy at 72 hours, we incubated the cells with selected concentrations of doxorubicin and methotrexate. After 24 hours we administered the photosensitizer (5,15-bis(2-bromo-3-hydroxyfenil)porphyrin), previously synthetized by us and irradiated the cells with a flux of 7.5 mW/cm^2. Forty eight hours later, we proceeded to MTT and SRB assay. In order to evaluate the types of cell death, the mitochondrial membrane potential, cell cycle and oxidative stress we proceeded to flow cytometry technique.
Results: The results showed that the combination therapy induces a decrease in metabolic activity and cell viability. For the treatment with 86 nM PS in combination with doxorubicin it was obtained a value of 16.48 ± 5.35 ($p < 0.001$) and 46.60 ± 20.35 ($p = 0.001$), metabolic activity and cell viability values, respectively. The combination therapy with doxorubicin, showed that there was a sharper loss of mitochondrial membrane potential in cell cultures subjected to combined treatment and the type of predominant cell death induced by therapy was later apoptosis/necrosis (59.80 ± 6.24 ($p < 0.001$)). The cells subjected to this treatment also showed a higher intracellular production of reactive oxygen species.
Conclusion: The combined therapy presented a more evident cytotoxic effect than that shown by individual therapies. It was now possible to test this approach in an animal model. These results reveal the potential of this combination as a future therapeutic approach in osteosarcoma.
Funding: FCT, Portugal (UID/NEU/04539/2013), COMPETE-FEDER.

OP3. DRUG DESENSITIZATION IN ONCOLOGY: THE EXPERIENCE OF AN ALLERGY AND CLINICAL IMMUNOLOGY DEPARTMENT – A 5 YEARS PERIOD

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Introduction: Any cancer drug can potentially trigger a hypersensitivity reaction (HSR), particularly chemotherapy agents and monoclonal antibodies. The more frequent drugs inducing HSR are the platins (IgE-mediated reactions) and taxanes (non-immunological reactions). The clinical approach following an HSR has been discontinuation of treatment and avoidance of the trigger drug. Desensitisation protocols are based in the reintroduction of chemotherapy agents and must be considered when there is no valid and effective alternative treatment. This is especially relevant for cancer patients who are thus able to continue their first line treatment. Desensitisation induces only temporary tolerance (lasting days) and, in cases of chemotherapy drugs, patients have to undergo drug desensitization using this method in each of the following treatment cycles.
Objective: To describe the experience of an Immunoallergology Department with desensitization to chemotherapy agents.
Methods: Retrospective review of charts of oncology patients desensitized in the Immunoallergology Department of CHUC in the last 5 years.
Results: There were a total of 72 desensitisation procedures corresponding to 15 patients treated (11 female) during the period in question, with a mean age of 56 years (range: 28-73 years). The patients had ovarian cancer (8 patients), lung cancer (3 patients), colon cancer (2), rectal cancer (1) and breast cancer (1). The HSR were all with moderate to severe immediate reactions: rash, urticaria, laryngeal stridor, bronchospasm, syncope and anaphylaxis (6 patients). Eleven patients were desensitized to platins (carboplatin n = 6, cisplatin = 3, oxaliplatin n = 2), three to taxanes (docetaxel n = 2, nabpaclitaxel n = 1) and one to monoclonal antibodies (panitumumab). In the total of 72 desensitisation procedures, the range of desensitisation procedures for patient was 17 (maximum) to 2 (minimum) treatments with carboplatin. There were 5 desensitisation procedures in 2011, 12 in 2012, 19 in 2013, 18 in 2014 and also in 2015. In the 72 desensitisation did not have any reaction in 59 procedures (82%) and in the others, the reactions were milder than the initial HSR (only 1 patient had anaphylaxis in the third desensitization with cisplatin). All patients received their daily programmed dose. Five patients discontinued the desensitization programme (3 patients due to progression of the oncological disease, 1 patient due to neurological toxicity and another for anaphylaxis during de desensitization).
Conclusion: In the majority of patients, desensitization procedures allowed safe reintroduction of chemotherapy agents in Immunoallergology centers with experience. This approach must be considered by Oncologic doctors in the treatment of specific oncologic patients with previous history of HSR to these drugs.

OP4. IS NF-KB EXPRESSION RELEVANT IN MULTIPLE MYELOMA PATIENTS TREATED WITH BORTEZOMIB AS FRONTLINE REGIMEN?

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Introduction: Nuclear factor kappa B (NF-κB) is a heterodimeric transcription factor that promotes transcription of several anti-

apoptotic growth factors, proteins, and cytokines, after its nuclear translocation. NF-κB is normally present in the cytoplasm in association with its inhibitor, IκB. Bortezomib inhibits degradation of IκB and therefore blocks NF-κB activity. As NF-κB is highly expressed in myeloma cells, its inhibition by bortezomib promotes their apoptosis. Bortezomib is frequently included in the frontline regimens to treat multiple myeloma (MM) patients. However, the prognostic impact of NF-κB expression levels in plasma cells from newly diagnosed MM patients, treated with bortezomib, is largely unknown.

Objective: To analyze the expression levels of NF-κB in CD138+/CD19- and CD138+/CD19+ plasma cells from MM patients, at diagnosis, and to determine its impact in response and in overall survival of patients treated with bortezomib as frontline regimen.

Materials and methods: We evaluated 24 newly diagnosed MM patients, between April 2010 and April 2013, treated with bortezomib (+ dexamethasone, ± cyclophosphamide) as first-line regimen. NF-κB expression levels were analyzed with monoclonal antibodies by flow cytometry in CD138+/CD19+ and CD138+/CD19- plasma cells from bone marrow samples collected at diagnosis. NF-κB expression levels are mentioned in mean intensity of fluorescence (MIF). Response evaluation was determined according to International Myeloma Working Group response criteria. For statistical analysis, software IBM SPSS Statistics v22 was used.

Results: Twenty-four patients (42% male) were studied, with a median age of 61 (41-75) years; 21/24 (88%) patients presented response to bortezomib. We analyzed NF-kB expression levels in CD138+/CD19+ and CD138+/CD19- plasma cells in patients with and without response to bortezomib (1 CR, 4 VGPR, 16 PR). According to our results, patients with response to bortezomib presented higher NF-κB expression levels in CD138+/CD19- plasma cells (26.2 ± 1.7 MIF vs 19.7 ± 1.8 MIF; p = 0.032). In CD138+/CD19+ plasma cells, we didn't find differences in NF-κB expression levels (38 ± 2.3 MIF e 33.6 ± 5.6 MIF, respectively). Based on these results, we searched for a prognostic impact of NF-κB expression levels in MM patients with response to bortezomib. Among these patients, median NF-κB expression levels in CD138+/CD19- and CD138+/CD19+ plasma cells were 26.2 ± 1.7 MIF and 38 ± 2.3 MIF, respectively (p = 0.0001). We also found that NF-κB expression levels higher than or equal to 25 MIF in CD138+/CD19- plasma cells are associated to a longer survival: median survival not reached compared with 26.1 months (95%CI; 22.4-29.8); p = 0.022, for NF-κB expression levels lower than 25.

Conclusion: Plasma cells from MM patients with different phenotypes present distinct NF-κB expression levels. Higher NF-κB expression levels in CD138+/CD19- plasma cells are associated with increased response rates to bortezomib and with a benefit in overall survival. These results suggest that NF-κB expression levels in CD138+/CD19- plasma cells from MM patients might be considered a potential biomarker for response to bortezomib and prognosis.

This work was supported by CIMAGO (project no 23/09)

OP5. NEW THERAPEUTIC MODALITIES IN RETINOBLASTOMA TREATMENT

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Introduction: Retinoblastoma is the most common malignant eye tumour in paediatric population, representing 3% of all tumours in children less than 15 years of age. In Portugal the estimated incidence is approximately 10 cases a year. Diagnosis and early treatment are essential, since treatment of retinoblastoma while intraocular carries a high probability of cure and the survival rate is usually higher than 95%. Retinoblastoma treatment is complex and implies a strategic choice of the different methods of treatment. Since the 90's that chemotherapy is the main therapeutic weapon in retinoblastoma allowing retention of the eye in a higher percentage of children. Recently new forms of chemotherapy administration have been used in particular super-selective ophthalmic artery infusion chemotherapy (SOAIC) and intravitreal chemotherapy, increasingly considered as first line treatments for this type of tumour. SOAIC replaces intravenous chemotherapy achieving higher intraocular concentrations, lower systemic concentrations and therefore minor side effects. Also it is associated with a higher rate of globe retention and preservation of useful vision in some eyes. Intravitreal chemotherapy is indicated, as adjunctive treatment when vitreous seeding is present, which is an important limiting factor in the success of any eye conservative treatment. SOAIC treatments began in the CHUC, in July 2015, becoming the only center in the country to ensure retinoblastoma treatment. In October 2015 the CHUC Ophthalmology department was recognized by the Ministry of Health as the sole national reference center in the treatment of ocular tumours.

Objective: To present the new treatment modalities of retinoblastoma, their indications, advantages and disadvantages.

Methods: Description of the new therapeutic modalities for retinoblastoma treatment and a retrospective analysis of the first cases treated in the Ophthalmology department of CHUC, through consultation of the clinical records and complementary examinations conducted during the patients diagnostic and therapeutic approach. Seven SOAIC sessions were held in two patients in the CHUC since July 2015.

Results and conclusion: SOAIC and intravitreal chemotherapy seem to be promising treatments in retinoblastoma treatment. The intra-arterial chemotherapy causes tumour regression and cure without the systemic side effects associated with systemic chemotherapy. Intravitreal chemotherapy is an adjunctive treatment effective in treating vitreous seeding. It is necessary to perform a careful election of patients for the application of these new forms of treatment in order to minimize the risk of extraocular metastasis. Despite the great advantages brought by these new therapeutic modalities, definitive positions cannot be made based on the evidence currently available. Prospective randomized studies are needed to enable conclusions and develop treatment protocols. Retinoblastoma treatment is constantly evolving and probably will change in the coming years.

OP6. LEUKEMIC STEM CELLS DEFINED BY DIPEPTIDIL-PEPTIDASE IV (CD26) EXPRESSION IN CD34+CD38- STEM CELLS AND TYROSINE KINASE INHIBITOR THERAPY IN CHRONIC MYELOID LEUKEMIA

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Introduction: Recently, dipeptidil-peptidase IV (DPP IV; CD26) has been described as a specific marker of leukemic stem cells (LSC) within the $CD34^+CD38^-Lin^-$ compartment present in chronic myeloid leukemia (CML) patients. These cells seem to be difficult to eradicate by tyrosine kinase inhibitors (TKI), although the efficacy of different generations of TKI remains unknown. In addition, the negative influence of these LSC on optimal therapy responses (time

to achieve major molecular response) is under investigation in CML. In this study, we aimed at the evaluation of the presence of LSC in chronic phase CML patients to establish the additional parameters to better define the efficacy of different therapeutic strategies.
Methods: Peripheral blood samples from chronic phase CML patients (n = 45) under Interferon-alpha 2b (IFN-α 2b), imatinib, dasatinb, nilotinib, bosutinib and ponatinib therapy were analyzed by multi-parametric flow cytometry for the characterization of LSC. Buffy coats (n = 3) from healthy blood donors were used as control. Cytokines and chemokines were evaluated in a 34-plex panel by xMAP technology (Luminex®).
Results: Circulating CD34 positive cells were increased in chronic phase CML patients when compared to controls (0.15 ± 0.05% vs 0.06 ± 0.05%). CD26 was found expressed in CML $CD34^+CD38^-Lin^-$ stem cells (12.07 ± 4.14%) and absent in controls. CD26 expression was higher in LSC compared to progenitor cells (MFI 704.6 ± 200.8 *vs*. 270.4 ± 34.8). We also found SDF-1 significantly overexpressed in CML patients undergoing TKI treatment when compared to IFN-α 2b. An impairment of LSC to respond to SDF-1, associated with less migration to stem cells niche, has been observed, although they express high levels of CXCR4. All of our chronic phase CML patients with detectable LSC in peripheral blood samples needed to change therapy and/or increase TKI dose and the majority of these patients present high or intermediate clinical prognostic risk score (Sokal) on initial evaluation.
Conclusion: Detection of LSC defined by CD26 in CD34+CD38-Lin- stem cells might be an important tool to improve CML treatment strategy in the near future.
Financial Support: FEDER (Programa Operacional Factores de Competitividade – COMPETE) and FCT (Fundação para a Ciência e a Tecnologia) through project PEst-C/SAU/LA0001/2013-2014.

OP7. VISMODEGIB FOR ADVANCED BASAL CELL CARCINOMA – EXPERIENCE OF A DERMATOLOGY DEPARTMENT

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Introduction: Advanced basal cell carcinomas (aBCC) are a small subset of basal cell carcinomas (BCC) that are difficult to manage due to their locally aggressive behavior or distant metastases. Wide surgical excision, the standard treatment for most sporadic BCC, radiotherapy or classic chemotherapy are not feasible in most cases. A targeted therapy acting on *hedgehog* pathway, vismodegib, became available in Europe for aBCC since 2013. We aim to describe the experience of our Dermatology department with vismodegib.
Methods: Retrospective analysis of clinical files of all patients with aBCC treated with vismodegib at our Dermatology department.
Results: We treated 4 patients with aBCC (3 males/1 female), ranging from 17 to 78 years. Case 1: A 17 years-old male with Gorlin-Goltz syndrome was submitted to surgical excision of a medulloblastoma, by the age of 2, and received adjuvant radiotherapy. During the childhood he developed multiple BCC over the irradiated skin. Despite several surgical excisions, the BCC progressively increased in number. Due to the extension of the affected skin he started vismodegib 150 mg *id*, and by week 16 a complete clinical and histologic regression was observed. As side effects he suffered moderate alopecia and muscle spasms, which resolved with drug interruption at week 20. He further developed a few BCC in the same region, effectively treated with surgery or cryosurgery. Case 2: A 62 year-old male underwent, by the age of 57, wide left orbital exenteration of a recurrent frontal BCC. After 5 years of follow-up a relapse was detected on the left orbital apex, extending to left posterior sphenoid and adjacent cavernous sinus, as well as to left and right ethmoidal labyrinth. After 15 weeks of vismodegib 150 mg *id* the sphenoidal and ethmoidal involvement markedly decreased and continued to improve until week 28, the time of our last evaluation. During treatment he complained of low grade dysgeusia and muscle spasms. Case 3: A 78 years-old male presented with a large and poorly demarcated recurrent BCC extending from frontoparietal to occipital area. The lesion was too large for surgical resection and radiotherapy was not feasible due to the risk of scalp radionecrosis. An 80% reduction in tumour size was observed after 30 weeks of vismodegib 150 mg *id*. However, the patient died during the treatment due to ischemic stroke. Case 4: A 68 years-old female, who presented with new-onset dyspnoea, was diagnosed with lung and bone metastases of BCC. During childhood she had been treated with scalp radiotherapy for *tinea capitis* and by the age of 60 she had already developed multiple scalp BCC, including a histologically aggressive one (micronodular type), ultimately treated with surgery and radiotherapy, after several recurrences. The treatment with vismodegib 150 mg *id* led to disease stabilization and symptomatic improvement, despite some side effects, as muscle spasms and dysgeusia. Nevertheless, the disease progressed after 8 months with development of peritoneal carcinomatosis. The drug was discontinued and the patient died 4 months later.
Conclusion: The aBCC cases are difficult to manage, but vismodegib seems to be largely advantageous facing the classic therapeutic options. In the case of Gorlin-Goltz syndrome, vismodegib induced a complete response. Constitutive activation of *hedgehog* pathway that characterizes this genodermatosis, due to loss of heterozygosity of *PTCH1* tumour suppressor gene, may explain the subsequent development of BCC, mainly in a previously radiodamaged skin. In the cases with locally extensive disease vismodegib induced at least partial responses, which could, for instance in case 3, enable a posterior surgical approach with curative intention. This patient suffered an ischemic stroke, but the relationship between vismodegib and this event is unknown. In the case of metastatic disease, the metastases progressed after a transient stabilization. The possible occurrence of mutations conferring resistance to vismodegib might have played a role in the disease progression. Our data are consistent with those of literature, where objective response rates range from 30% in metastatic disease to 43% in locally aBCC.

Session "Epidemiology and Oncology"

OP1. EXPOSURE OF HUMAN EPITHELIAL BRONCHIAL CELLS TO HEXAVALENT CHROMIUM, A LUNG CARCINOGEN, DOWNREGULATES HSP90α AND CONFERS RESISTANCE TO STRESS

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Introduction: The carcinogenicity of hexavalent chromium [Cr(VI)] compounds as encountered in certain industries has long been established by the International Agency for Research on Cancer (IARC) and other regulatory agencies. Still, despite numerous studies, the molecular basis of Cr(VI)-induced neoplastic transformation remains elusive. It is generally accepted that cells exposed to Cr(VI) experience several types of cellular stresses, namely oxidative and metabolic stresses. These stresses are expected to activate the stress response of the exposed cells and this activation might protect the cells from further stresses, such as those encountered during neoplastic transformation. In this

study, we determined the effects of Cr(VI) on cellular stress, using acute thermal shock as a model. To this end, we monitored the expression of three components of the stress response implicated in carcinogenesis: heat shock proteins 90 alpha (Hsp90α) and 70 (Hsp70) and heat shock factor 1 (HSF1). Additionally, we determined the doubling times of cultures that survived Cr(VI) insults that were not overly cytotoxic (0.1-2 μM), to verify previous published findings by our group suggesting that their proliferation rates were higher than those of their non-exposed counterparts.
Methods: The human airway epithelial cell line BEAS-2B was used throughout this study. Acute thermal shocks were induced by replacing spent medium with cold medium (4 ºC), in the case of cold shock, or heated medium (43 ºC), in the case of heat shock. Effects of a 48h exposure to Cr(VI) on the expression of Hsp90α, Hsp70 and HSF1 were evaluated at the mRNA level by quantitative PCR (qPCR), and at the protein level by ELISA. Commercially available kits were used for total RNA extraction, cDNA synthesis and ELISA. Total protein was determined by the Bradford method, with BSA as the standard. Doubling times were calculated by monitoring total cell numbers in cultures in the exponential phase of growth using the Trypan Blue exclusion method. Differences with a $p < 0.05$ (paired Student's *t* test or One-Way ANOVA followed by Dunnett's post test) were considered statistically significant.
Results: Our results show that BEAS-2B cells exposed for 48 h to 1 μM Cr(VI) were less affected by acute thermal shocks than their non-exposed counterparts (as assessed by the magnitude of the inhibitory effect that these shocks produced on cell proliferation). This suggests that Cr(VI) confers a certain resistance to this type of shock. Contrary to what might have been expected, resistance was not acquired through elevated protein levels of the components of the heat shock response evaluated in this study. In fact, Hsp90α levels were found to be significantly decreased, whereas those of Hsp70 and HSF1 remained essentially unaltered (despite a not statistically significant trend towards reduced HSF1 protein levels). Of note, the observed decrease in Hsp90α levels upon exposure to a heavy metal was not totally unexpected, as it was in line with earlier reports for this and other cell lines. In contrast with our findings at the protein level, Hsp90α and HSF1 mRNA levels remained unaltered, whereas those of Hsp70 were significantly decreased. Finally, determination of doubling times confirmed higher proliferation rates for cells that survived Cr(VI) insults not overly cytotoxic (0.1-2 μM Cr(VI)).
Conclusion: Our results indicate that, in BEAS-2B cells, Cr(VI) downregulates Hsp90α, a critical component of the stress response. This is accompanied by increased resistance to acute thermal shocks, as well as augmented proliferation rates.
Acknowledgments: AMU acknowledges funding from CIMAGO, Portugal (grant 16/12). AMU and TCO acknowledge funding from FCT, Portugal (grant UID/MULTI/00070/2013, to Química Física Molecular Research Unit; grant SFRH/BPD/101169/2014, to TCO).

OP2. ASSOCIATION BETWEEN IL-1A, IL-1B, IL-1RA AND IL-1R POLYMORPHIC VARIANTS AND COLORECTAL CANCER

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Introduction: Multiple studies have reported strong associations between colonic inflammation and colorectal cancer (CRC) development. Altered expressions of pro/anti-inflammatory cytokines have a crucial role in CRC proliferation. Although there are many studies related to cytokines polymorphisms involvement in CRC risk, the role of IL-1 in intestinal inflammation process is not yet clarified. This study aimed to investigate the impact of common IL-1A, IL-1B, IL-1RA and IL-1R polymorphisms in CRC development risk.
Methods: A total of 50 CRC biopsies and 50 blood samples from healthy subjects, used as control group, were included in this case-control study. IL-1A -889T > C; IL-1B -511C > T and 3954C > T, IL-1RA 11100T > C, IL-1R 1970C > T polymorphisms were genotyped using commercially available kits.
Results: IL-1A and IL-1B, low producer genotypes, IL-1A -889CC (OR = 0.2; 95%CI 0.1 to 0.5); IL-1B -511CC (OR = 0.2; 95%CI 0.1 to 0.6) and IL-1B 3954CC (OR = 0.2; 95%CI 0.1 to 0.5) were found associated with CRC absence ($p < 0.005$). While IL-1B high producer genotypes, IL-1B -511TC (OR = 4.0; 95%CI 1.7 to 9.5) and IL-1B 3954TT (OR = 5.5; 95%CI 1.5 to 20.8) were found more prevalent among CRC subjects ($p < 0.01$). We also verified that IL-1RA 11100TT and IL-1R 1970TT, IL-1 low binding genotypes, were both linked with CRC lower risk level ($p < 0.05$), being more prevalent among controls.
Conclusion: IL-1 genes appear to have an important role in inflammatory status and CRC risk mechanisms. It seems that IL-1A, IL-1B, IL-1RA, IL-1R common polymorphisms control IL-1 A and IL-1B production and receptor binding, leading to uncontrolled pro-inflammatory responses and genetic epithelial remodelling and/or carcinogenesis. Inflammation controlled by these genes may be understood as pre-neoplasic conditions in this multifactorial disease.

OP3. AGE-DEPENDENT SENSITIVITY TO ANTI-MITOTICS CHEMOTHERAPY

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Oncogenic transcription factor FoxM1 is overexpressed in the majority of human cancers and it was recently described as a major predictor of adverse outcomes. Emerging data suggest that targeting FoxM1 in mono- or combination therapy may have promising therapeutic benefits for the treatment of cancer. FoxM1 expression is associated with the proliferative capacity of the cell, consistently with its role in primarily driving the expression of G2/M specific genes, with associated phenotypic expression of mitotic defects and chromosome aberrations when defective. Previously, we found that expression of FoxM1 and its downstream targets decreases progressively during chronological ageing. Therefore, we asked i) whether spindle poisons (or anti-mitotic drugs) commonly used in chemotherapy, could be more efficient in killing old cells that under express FoxM1, and ii) whether a combination therapy using both FoxM1 inhibition and anti-mitotic drugs could act effectively to kill tumor cells overexpressing FoxM1. We used phase-contrast time-lapse live cell imaging to observe individual cell behavior of primary human dermal fibroblasts (young and old age) and fibrosarcoma HT-1080 cancer cells in response to anti-mitotics, i.e., whether they die in mitosis or slippage out of mitosis without dying. This methodology circumvents the confusing interpretations of population-based assays. Independently of the anti-mitotic drug used, we observed that old fibroblasts display an increased sensitivity to these drugs. FoxM1 accounts for this increased sensitivity to anti-mitotics as i) FoxM1 downregulation in young cells increases the percentage of cells dying in mitosis and ii) FoxM1 overexpression in old cells decreases the percentage of cells dying in mitosis. Consequently, we tested combination treatment using both FoxM1 downregulation and anti-mitotics in a fibrosarcoma cell line overexpressing FoxM1. FoxM1 repression

significantly induced mitotic cell death in this cell line in response to anti-mitotics. Overall, this validates the role of FoxM1 in the mitotic cell fate decision in response to anti-mitotics, as low FoxM1 levels correlate with a higher sensitivity to these drugs. We found evidence for an increased pro-apoptotic efficacy of anti-mitotics in combination with FoxM1 downregulation that might be explored in the future as a chemotherapeutic strategy.

OP4. POLYMORPHISMS IN OXIDATIVE STRESS RELATED GENES AS PROGNOSTIC AND THERAPEUTIC BIOMARKERS IN CHRONIC MYELOID LEUKEMIA

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Introduction: Oxidative stress (OS), resulting from an imbalance between Reactive Oxygen Species (ROS) production and antioxidant defenses, contributes to cell damage, apoptosis and ineffective hematopoiesis. Chronic myeloid leukemia (CML) is a clonal neoplastic disease associated with the reciprocal translocation t(9;22), encoding the BCR-ABL1 oncogene. BCR-ABL protein induces, among other mechanisms, production of reactive oxygen species (ROS) by activation of the PI3K pathway, increase glucose metabolism and mitochondrial dysfunction. The antioxidant enzymes superoxide dismutases (SOD) and catalase (CAT), as well as DNA repair enzymes, such as OGG1, are important cell defense components against OS. Polymorphisms in genes that codify these enzymes may contribute to differences in susceptibility of individuals to oxidative damage, since it can lead to reduced protection against OS, influencing CML development and therapeutic response.
Objective: In the present study we investigate the influence of polymorphisms in genes related with oxidative stress (CAT, GPX1, MPO, SOD1, and SOD2) and DNA repair (OGG1, NEIL1, and XRCC1), and the transcription factor NFE2L2, as a prognostic risk marker in CML patients [namely in overall survival and tyrosine kinase inhibitors (TKIs) response]. Moreover, we also analyzed it participation in the development of mutations in BCR-ABL1 gene.
Methods: This study enrolled 75 patients diagnosed with CML. The genetic polymorphisms of CAT (rs1001179), GPX1 (rs1050450), MPO (rs2333227), SOD1 (rs2070424), SOD2 (rs4880), OGG1 (rs1052133), NEIL1 (rs5745920), XRCC1 (rs1799782), and NFE2L2 (rs13001694), were assessed by RFLP-PCR and Tetra-primer-ARMS-PCR. The statistical analysis was carried out by variance analysis, χ^2 test and Fisher exact test ($p < 0.05$).
Results: Our results show that SOD2 genotype influence mutation status of BCR-ABL1 (CC genotype: odds ratio 9.25x, 95%CI 1.24-18.82; p = 0,007). On the other hand, patients with MPO GG and AG genotypes have a high rate of sub-optimal response to TKIs (odds ratio 4.92x, 95%CI 1.24-9.10; p = 0,043). Moreover, the overall survival of CML patients can be influenced by NEIL1 [CML patients with CT genotype had lower survival (166 ± 5 months) than patients with CC and TT genotypes (204 ± 6 months; p = 0.041)] and NFE2L2 [CML patients with TT genotype had lower survival (88 ± 7 months) than patients with CT and TT genotypes (216 ± 8 months; p = 0.003)].
Conclusion: These results suggest that genetic polymorphisms in oxidative stress and DNA repair related genes influence the prognosis of CML patients, the TKI response and the development of BCR-ABL1 mutations, which could constitute new prognostic and therapeutic biomarkers.
This work was supported by CIMAGO (Project 22/09) and R. Alves is supported by the FCT fellowship SFRH/BD/51994/2012.

OP5. NOVEL REGIONS OF GAIN AND LOSS ON CHROMOSOME 11 DETECTED IN THE ORAL SQUAMOUS CELL CARCINOMA

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Introduction: Oral squamous cell carcinoma (OSCC) is the most common neoplasm of head and neck with high incidence and mortality worldwide. Gain of the chromosomal region 11q13 is one of the most prominent genetic alterations in these tumors and is associated with poor prognosis and metastasis (Noorlag et al. Virchows Arch. 2015;466:363-73), being the *CCND1* the candidate target gene in this region. With this study we aimed to identify separate recurrent chromosomal bands with genomic alterations mapped in the chromosome 11 in order to clearly delineate the most frequent regions of amplification and deletion in OSCC.
Methods: Biopsies of oral squamous cell carcinoma were acquired from 100 patients and array-CGH was performed using an Agilent oligonucleotide microarray 4x180K. Healthy donors were used as controls.
Results: We identified several minimal regions of gain in chromosome 11, including 11p11.2-p11.12, 11q12.2-q13.1, 11q13.1-q13.2, 11q13.2-q13.4, 11q13.5-q14.1 and of loss, including 11p15.5-p15.4, 11p15.4, 11p11.1-q12.1 and 11q22.3. These regions may harbor candidate OSCC – associated oncogenes and tumor suppressor genes. In this study, gain of 11q13.2-q13.4 was the most frequently detected chromosomal aberration, which is in agreement with the literature. Within this amplicon, concordant amplification of several oncogenes was found, namely: *CCND1, FGF19, FGF4, FGF3, ANO1, FADD, CTTN* genes. The most frequent loss is located at the 11p15.5-p15.4, where are mapped several genes that could be an important role in the OSCC initiation and progression. Loss of distal 11q and amplification of chromosomal band 11q13 are associated with poor prognosis in OSCC. We observed several patients with loss at 11q22.3, where is mapped the *ATM* gene. This gene has been associated with reduced sensitivity (resistance) to ionizing radiation (IR) in OSCC cell lines (Sankunny et al. Genes Chromosomes Cancer. 2014;53:129-43).
Conclusion: We have demonstrated that there are multiple genomic alterations occurring on chromosome 11, where are mapped several genes that could be important for oral carcinogenesis process. In few of those regions there are already some genes indentified as important for OSCC development and for patients' outcome. Thus, the correlation between these novel molecular findings and the clinic-pathological data is pivotal to identify and validate precise and accurate biomarkers of prognosis and therapeutic response in OSCC.

OP6. CLINICAL AND PATHOLOGIC PROGNOSTIC FACTORS AFTER HEPATECTOMY FOR HEPATOCELLULAR CARCINOMA: VASCULAR INVASION, BLOOD TRANSFUSION AND HEPATIC PEDICLE CLAMPING ARE ASSOCIATED WITH DECREASED SURVIVAL

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Introduction: Hepatectomy (HP) is, along with liver transplantation, the only potentially curative treatment for hepatocellular carcinoma (HCC). Survival after HCC treatment is associated with several prognostic factors, such as vascular invasion (VI). Hepatic Pedicle Clamping (HPC), used to reduce perioperative bleeding during HP, has been theorized to increase the risk of recurrence.
Methods: Clinical and blinded pathological review of 39 patients undergoing HP for HCC between 2005 and 2013. Chronic liver disease in 33 patients (84-6%), with cirrhosis in 56.4% [most frequent etiologies: alcoholism (59%), HCV (17.9%) and HBV (10.3%)]. Major HP was performed in 15 patients (38.5%), Blood Transfusion (BT) in 11 patients (28.2%) and HPC in 29 patients (74.4%), with a mean time of 18.2 ± 17 minutes (5-60 minutes). Statistical analysis with SPSS™ 21.0. Survival tests (Kaplan-Meier, log rank and Cox regression). Statistical significance with $p < 0.05$.
Results: Major morbidity in 30.8% of patients. Mortality in three cases (7.7%), caused by liver failure. Overall survival (OS) of 36 months and Disease-Free Survival (DFS) of 17 months. After multivariate analysis, VI (HR = 6.176; $p = 0.001$) and HCV infection (HR = 3.335; $p = 0.036$) were associated with worse OS. BT (HR = 7.05; $p = 0.011$), HPC (HR = 12.596; $p = 0.005$) and VI (HR = 29.448; $p < 0.001$) were associated with worse DFS.
Conclusion: VI and HCV infection are associated with worse OS. BT and HPC were independent factors of worse DFS. The ischemia-reperfusion injury (IRI) produced by HPC could promote a more angiogenic and angioinvasive phenotype of tumor cells, resulting in higher recurrence. Strategies aiming at reducing IRI are critically important in liver surgery.

Session "Emergent research of biomedicine in oncology"

OP1. CYTOTOXIC AND REGULATORY FUNCTIONS OF NATURAL KILLER CELLS IN CHRONIC MYELOID LEUKEMIA DEPEND ON TYROSINE KINASE INHIBITOR THERAPY DEFINING A SPLIT ANERGY STATUS

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Introduction: Previous studies indicate that Natural Killer (NK) cells are deficient in chronic myeloid leukemia (CML) patients, although the mechanisms behind the dysfunction are not completely understood. Current therapeutic strategies influence these innate lymphoid cells and successful results may be partially explained by the advantageous effects on their cytotoxicity against cancer cells. Due to recent advances in the knowledge of NK cell´s biology, there is an increasing interest in mapping NK-cell responses in cancer.
Objective: To analyze NK cells in CML patients and the effect of therapy and dose dependent mechanisms on essential features of NK cells.
Methods: In this study, we analyzed blood samples from 67 CML patients treated with IFN-α and/or different generations of tyrosine kinase inhibitors (TKI). Extended analysis of NK-cell receptor repertoire and functional properties was performed by multiparametric flow cytometry, cell sorting, Luminex xMAP technology and real-time quantitative PCR.
Results: Relative frequency of NK cells was found reduced at CML diagnosis and recovered after treatment. CML therapy induces an increase of $CD62L^+$ $CD56^{bright}$ NK cells, associated to the capacity of migration to secondary lymphoid organs. Activation of NK cells and the increased expression of CD137 and CD137L were interpreted as a significant effect of therapy response. Activatory (KIR2DS1) and inhibitory (KIR2DL1, KIR2DL2) receptors were found altered in CML. The expression of KIR2DS1 by $CD56^{dim}CD16^+$ NK cells was highest in CML patients undergoing Dasatinib therapy. Treatment also increased the NKG2C/NKG2A (activatory/inhibitory) ratio. Lower expression of NKp30 and NKp44 was compensated by the increase of NKp46+ NK cells. Production of IFN-γ and suppression of TGF-β^+ and IL-10^+ NK cells was also a beneficial effect of treatment protocol. IFN-γ production decreased with an increased TKI dose.
Conclusion: Expansion and activation of NK cells was observed in TKI treated CML. Cytotoxic and regulatory functions of NK cells are TKI dependent defining a split anergy status. NK cell receptor repertoire is modulated by TKIs in CML. Information based on immune status of CML could help to define patients needing to change TKI and those that are "ready" to stop TKI therapy. NK cells are affected during CML and current therapeutic protocols ameliorate NK-cell performance. In the future, combination of NK cell-based immunotherapy with pharmacological interventions should be investigated in order to eradicate cancer cells and discontinuation of therapy.
Financial Support: FEDER (Programa Operacional Factores de Competitividade – COMPETE) and FCT (Fundação para a Ciência e a Tecnologia) through project PEst-C/SAU/LA0001/2013-2014.

OP2. CAN COLD ATMOSPHERIC PLASMA TREATMENT KILL CANCER CELLS?

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Introduction: Retinobastoma is a malignant tumor arising from the nuclear layer of the retina, being the most common primary intraocular malignancy in children. Worldwide, the incidence of retinoblastoma, in children younger than 5 years, is recorded nearly 11 cases per million. Currently available therapy methods include enucleation, radiotherapy, chemotherapy, thermotherapy, laser photocoagulation and cryotherapy. These therapies lack selectivity and are associated with serious side effects as loss of vision, loss of the eyeball and secondary tumours due to mutagenic effects. Plasma, the fourth state of matter, is a gas where a significant fraction of its atoms are dissociated to form an ionized state. Its effects on tumor cells have recently came into attention as it revealed a potential method of non-inflammatory selective anti-cancer therapy.

Objective: The aim of this work was to evaluate the cytotoxicity of cold atmospheric plasma (CAP) in a human retinoblastoma cell line.
Methods: We developed an electronic device capable of generating high output voltage (HV electrode; ~4kV). This equipment was designed to initiate an electrical discharge between the HV electrode and multiwell plates where cell cultures acted as the target. Human retinoblastoma Y79 cells were seeded in the multiwell plates, in volumes of 100 µl and 200 µl at a concentration of 5×10^5 cells/ml and $2,5 \times 10^5$ cells/ml, and left overnight. For treatment, the CAP was generated in open air, 2 mm above the surface of the cell cultures medium. Several short periods of time, ranging from 5 to 60 seconds, were tested. In order to evaluate the metabolic activity, colorimetric tests, Alamar Blue and MTT, were performed, 24 hours after treatment, for the higher concentration. In addition, cell proliferation was also assessed, using SRB assay, for the lower concentration.
Results: After CAP treatment, the metabolic activity and proliferation of retinoblastoma cells decreased accordingly to the exposure time and volume. After 60s of CAP exposure a reduction in metabolism, measured with Alamar Blue assay, of more than 60% ($p = 0.001$) and 50% ($p = 0.001$) was achieved both in the lower and higher volumes, respectively. MTT Assay showed similar trends, with a reduction of almost 50% ($p = 0.001$) and 30% ($p = 0.047$) in the lower and higher volumes, respectively. Moreover, a protein content of only (26.64 ± 9.20)% was obtained after the same time exposure, for the lower volume, as determined by SRB assay.
Conclusion: CAP treatment was able to induce significant decrease of the metabolic rate even at short periods of exposure. Furthermore, the observed reduction of protein content as a result of decreased number of cells, indicate that cellular death is the most likely cause of these effects. These results suggest great anti-cancer potential and characterization of cell death mechanisms is currently being held.
Funding: FCT, Portugal (UID/NEU/04539/2013), COMPETE-FEDER.

OP3. THE CENTRAL ROLE OF MTOR EXPRESSION IN TUMOR MICROENVIRONMENT AND AGGRESSIVENESS: A PRELIMINARY STUDY IN BALB/C NU/NU MICE

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Introduction: In recent years, the reciprocal interplay between malignant cells and stromal structure, here named extracellular matrix (ECM), has been receiving growing attention due to its involvement with neoplastic cell transformation, tumour growth, progression and resistance of tumour cells from host immune response. Relevant events in carcinogenesis in order to understand cancer development and novel targets for prevention and early eradication of lesions are being explored. In this context the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/ mammalian target of rapamycin (mTOR) which is a survival pathway that is constitutively activated in many types of tumours, is being studied.
Objective: In this work we studied the co-evolvement of neoplastic cells and tumour stroma, by establishing xenotransplant in a mice model after cell implantation in three different microenvironments (tongue, cheek and back), using a human oral epidermoid carcinoma cell line with metastatic potential and evaluating mTOR protein expression in the resulting tumour nodules as well as in adjacent tissue.
Methods: The human oral squamous cell carcinoma HSC-3 cell line was cultured in order to establish ortothopic and heterotopic xenotransplant mice models (approved by Faculty of Medicine, University of Coimbra, ethics committee, 3-CE-2011). Balb/c nu/nu mice were inoculated with approximately 3, 15 or 30 million cells on the tongue, cheek and back, respectively (3 mice *per* location). After inoculation, Balb/c nu/nu mice were monitored over 3 to 7 weeks, and sacrificed by anaesthetic overdose. Tumour nodules and involving tissue were then excised for *ex vivo* studies. Samples were formalin-fixed paraffin-embedded and morphology was characterized in Hematoxylin-Eosin double stained slides followed by immunohistochemical study of epithelial and mesenchymal phenotypes (cytokeratins AE1/AE3 and LP34, p63 and Vimentin). The expression of mTOR protein was also evaluated.
Results: Each neoplasia was composed by sheets of epithelial cells with two different populations: one with immunophenotype of myoepithelial cell (p63+) and the other with immunophenotype of epidermoid/squamous cell (LP34+/p63-). The applied epithelial and mesenchymal markers revealed lower expression in tongue raised tumours than in cheek and back tumours. Tongue raised tumours expressed positive mTOR protein in stroma and also in some malignant epithelial cells. Cheek tumours seem to tag for the expression of mTOR protein which was apparent in tumour stromal mesenchymal cells that involved tumour cells. In heterotopic tumours mTOR protein was mainly positive in the stroma cells surrounding the tumour cells.
Conclusion: Our preliminary *in vivo* results showed mTOR as a constitutive marker of aggressiveness in oral epidermoid carcinoma xenotransplants related with defined stroma existence.
This work was financed by CIMAGO and a scientific research grant is supported by Núcleo Regional do Centro da Liga Portuguesa Contra o Cancro (NRC-LPCC) in partnership with CIMAGO. Strategic Project CNC.IBILI (FCT: UID/NEU/04539/2013).

OP4. PROGRAM DEATH 1 (PD-1) RECEPTOR DOWNREGULATION ON REGULATORY T CELLS DURING TYROSINE KINASE INHIBITOR THERAPY IN CHRONIC MYELOID LEUKEMIA: IMPLICATIONS FOR TREATMENT DISCONTINUATION

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Introduction: Programmed death-1 (PD-1) receptor and its ligands (PD-L1 and PD-L2) are involved in attenuating tumor immunity and facilitating tumor progression. PD-1, PD-L1 and PD-L2 therapeutic blocking agents have been reported to have significant antitumor effects. In chronic myeloid leukemia (CML), the expression of this receptor and its ligands is not fully characterized for the different subsets of cells of the immune system in which their expression is found constitutively or post-induction. In this study, we analyzed

the expression of PD-1 and its ligands on regulatory T cells (Tregs) in chronic phase CML patients to understand the mechanisms underlying suppressor effects that inhibit the anti-leukemia immune response.

Methods: Peripheral blood samples from chronic phase CML patients (n = 50) under Interferon-alpha 2b (IFN-α 2b), imatinib, dasatinb, nilotinib, bosutinib and ponatinib therapy were analyzed by multi-parametric flow cytometry for the characterization of regulatory T cells and surface expression of PD-1. Buffy coats (n = 13) from healthy blood donors were used as control. Cytokines and chemokines were evaluated in a 34-plex panel by xMAP technology (Luminex®). Gene expression analysis and miRNA profiling were also performed for these samples.

Results: PD-1 Tregs were found significantly decreased ($p < 0.0001$) in CML patients and down-regulation of this receptor was also observed ($p < 0.01$). Naïve and memory Treg subsets were equally affected. No significant alterations were observed for PD-L1 and PD-L2 ligands. Although TGF-β and IL-10 production were not significantly altered by down-regulation of PD-1 in Tregs, the overall effect of tyrosine kinase inhibitor (TKI) therapy suggests a negative impact in these cells concerning the anti-leukemic immune response.

Conclusion: Regulatory T cells represent a major population of suppressors in the immune response against leukemia. Down-regulation of PD-1 receptor in Tregs during chronic phase CML reinforces the notion that discontinuation of treatment must be carefully evaluated beforehand, since these suppressor cells could permit the proliferation of existent residual leukemic cells.

Financial Support: FEDER (Programa Operacional Factores de Competitividade – COMPETE) and FCT (Fundação para a Ciência e a Tecnologia) through project PEst-C/SAU/LA0001/2013-2014.