1ST ASPIC INTERNATIONAL CONGRESS

PROCEEDINGS BOOK

FUNDAÇÃO CALOUSTE GULBENKIAN

25-26 NOVEMBER 2014

ASPIC
ASSOCIAÇÃO PORTUGUESA DE INVESTIGAÇÃO EM CANCRO

LETTER OF WELCOME

The Portuguese Association for Cancer Research – ASPIC – was officially created in February 2013 and, after a kick-off meeting on "Public Benefit of Cancer Research", held in 2013 (www.aspic.pt/conference2013), is now organizing its 1st Congress.

The program has an outstanding group of invited speakers from Portugal and abroad and ample time for discussion in Plenary Symposia as well as Oral and Poster sessions. A joint Symposium ASEICA-ASPIC addresses new research approaches and will establish a link with cancer researchers from Spain, with lectures by Manuel Serrano, Paula Soares and Goreti Sales. Other symposia will cover early cancer detection (lecture by Rebecca Fitzgerald), cancer genes and what we have learned from them (lectures by Moshe Oren, Manuel Teixeira and Branca Cavaco), and mechanisms and implications of tumour heterogeneity (lectures by Samuel Aparicio and Luís Costa).

The scope of the congress is necessarily limited – it is a two day event – but the poster sessions will be open to presentations in all cancer research areas (we know we eventually learn more listening about subjects that are outside our "small world"). Everybody is most welcome to listen, to discuss and to participate. Even for those outside the "biobox", such as chemists and physicists, there will be space for discussions and even for "burning news" at the final session of the congress.

The topics of the congress and the high quality of the speakers are attractive to a wide range of professionals and researchers. We expect to bring together most cancer researchers from Portugal and also from Spain and other countries. The scientific program and the congress venue – Fundação Calouste Gulbenkian – will synergize to make this congress a great moment for the cancer research community.

I am confident this is the first of many ASPIC Congresses to come!

Kind regards,

Leonor David
Congress Coordinator and President of ASPIC

CONTENTS

ASPIC

ASPIC, the Portuguese Association for Cancer Research, was created in 2013 and promotes cancer research in all its aspects and in public benefit. The association encourages excellence, disseminates results, analyzes and proposes solutions for relevant questions for cancer research and cancer investigators in Portugal and at the international level taking advantage of its affiliation with the european partner – EACR.

Membership benefits include:

Automatic affiliation to the European Association for Cancer Research

Reduced registration rates at meetings organized by ASPIC, ASEICA, EACR and ECCO

Access to scholarships and awards

Contacts and opportunities for collaborative research

Reduced subscription rates to the European Journal of Cancer

Membership fees:

Active Member   40€/year, 120€/4 years

Post-doc students   25€/year

Master or PhD students   20€/year

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ASPIC

CONGRESS COMMITEES

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Leonor David, President of ASPIC

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Chairs: Sérgio Dias, Carmen Jerónimo

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João Nuno Moreira

Maria Gomes da Silva

CONGRESS PROGRAM

Tuesday, 25 November

11.00 **Pre-Congress Meeting**
REUNIÃO DA ASPIC COM ASSOC. DE DOENTES
Chairs: Manuel Sobrinho Simões, Jorge Soares, Alfredo Carrato, Leonor David

12.30 Registration and Poster mounting
Lunch

13.30 **Opening Session**

Symposium I
EARLY CANCER DETECTION
Chairs: António Dias Pereira, Carla Oliveira

14.00 Rebecca Fitzgerald . United Kingdom
Changing the face of cancer through early detection: examples of new technologies for oesophageal cancer.

15.00 6 selected oral presentations

16.30 Coffee-break

Symposium II
NEW RESEARCH APPROACHES FROM ASPIC –ASEICA
Chairs: Alfredo Carrato, Leonor David

17.00 Manuel Serrano . Spain
Pre-clinical and clinical data on the efficacy of anti-Notch therapies for lung adenocarcinomas.

18.00 Paula Soares . Portugal
Telomerase promoter mutations in cancer: an emerging molecular biomarker?

18.30 Goreti Sales . Portugal
New biosensors to monitor cancer biomarkers.

19.00
19:30 **ASPIC General Assembly**

20.30 Congress Dinner

Wednesday, 26 November

Symposium III
CANCER GENES: WHAT DID WE LEARN FROM THEM?
Chairs: Sergio Dias, João Nuno Moreira

09.00 Moshe Oren . Israel
The many faces of P53.

10.00 4 selected oral presentations

11.00 Coffee-break

11.30 Manuel Teixeira . Portugal
Li-Fraumeni syndrome: the prime example of pleiotropism in cancer predisposition.

12.00 Branca Cavaco . Portugal
Identification of familial cancer susceptibility genes – novel genomic approaches.

12.30 Lunch

13.30 **Poster Session**

Symposium IV
MECHANISMS AND IMPLICATIONS OF TUMOUR HETEROGENEITY
Chairs: João Barata, José Dinis Silva

14.30 Samuel Aparicio . Canada
EACR sponsored lecture:
Clonal evolution and cancer medicine.

15.00 4 selected oral presentations

16.00 Luís Costa . Portugal
Cancer Metastases: is bone a useful paradigm?

16.30 Coffee-break

17.00 **Closing Session**
EACR Awards

ACCREDITATION INFORMATION

The 1st ASPIC International Congress is accredited by the **European Accreditation for Continuing Medical Education** (EACCME) to provide the following CME activity for medical specialists. The EACCME is an institution of the European Union of Medical Specialists (UEMS), www.uems.net.

The 1st ASPIC International Congress is designated for a maximum of 9 hours of European external CME credits. Each medical specialist should claim only those hours of credit that he/she actually spent in the educational activity. The EACCME credit system is based on 1 ECMEC per hour with a maximum of 3 ECMECs for half a day and 6 ECMECs for a full–day event.

European Accreditation is granted by the EACCME in order to allow participants who attended the above-mentioned activity to validate their credits in their own country.

Through an agreement between the European Union of Medical Specialists and the American Medical Association, physicians may convert EACCME credits to an equivalent number of AMA PRA Category 1 Credits™. Information on the process to convert EACCME credits to AMA credits can be found at: www.ama-assn.org/go/internationalcme.

Live educational activities, occurring outside of Canada, recognized by the UEMS-EACCME for ECMEC credits are deemed to be Accredited Group Learning Activities (Section 1) as defined by the Maintenance of Certification Program of The Royal College of Physicians and Surgeons of Canada.

ABSTRACTS

INVITED SPEAKER

SI 1. Changing the face of cancer through early detection: examples of new technologies for oesophageal cancer.

Rebecca Fitzgerald

(*no abstract available*)

Rebecca Fitzgerald is a Professor of Cancer Prevention and a tenured Programme Leader at the MRC Cancer Unit, Hutchison/MRC Research Centre, University of Cambridge. She is a practicing clinician at Addenbrooke's Hospital Cambridge.

The focus of her research is to improve methods for early detection of oesophageal cancer through better understanding of the molecular pathogenesis. Rebecca has seen her work recognized with several international distinctions, including the prestigious Westminster medal and prize, an NHS Innovation prize, a Lister Prize Fellowship, an NIHR Research Professorship, the Goulstonian Lecture at the Royal College of Physicians, the Sir Francis Avery Jones Medal from the British Society of Gastroenterology and was elected a Fellow of the Academy of Medical Sciences.

SI 2. Tert Hypermethylated Oncological Region - THOR predicts biochemical relapse amongst prostate cancer patients

Pedro Castelo-Branco, Ricardo Leão[1,4,5], Celia Domingos[2,3], Stefan Buerno[6], Ana Gomes[5], Michal Schweiger[6], Alexandre R Zlotta[4], Arnaldo Figueiredo[5], Helmut Klocker[7], Holger Sueltmann[8], Uri Tabori[1]

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Introduction: Prostate cancer (PC) is the most frequently diagnosed cancer in men. Matter of major concern in PC is the overdiagnosis and overtreatment. Strategies to select the patients in order to reduce the overtreatment and consequent morbidities are important to preserve the benefits of treatment and reduce the downstream harms of these curative approaches. Limitless cellular self-renewal is essential for disease progression; this hallmark of cancer is governed by telomere maintenance and results from constitutive telomerase activation through TERT expression. We recently identified a specific region in the promoter of hTERT (TERT Hypermethylated Oncological Region - THOR) that is specifically hypermethylated in malignant tissues (Castelo-Branco, P *et al* The Lancet Onc. 2013). Here we evaluated THOR as marker for benign and malignant prostate disease and related it with prostate cancer aggressiveness and biochemical relapse. Material & Methods Tissue samples and patient data from 2 different cohorts, Discovery Cohort (DC) (n=164) and Validation Cohort (VC) (n=103) were obtained from three different countries. THOR status was analyzed by

Pyrosequencing. Univariate comparisons for baseline clinical and demographic features were done between THOR levels (hypermethyated and hypomethylated), in benign and malignant tissue. Chi-square tests were used for categorical variables and t-tests for continuous variables. *Results and Discussion:* THOR is significantly more methylated in prostate cancer tissues when compared to matched benign samples (p<0.0001) in a DC. THOR levels increase from benign tissue to Gleason 6 and progressively up to Gleason 8 (p<0.0001). There is no correlation between PSA values and THOR methylation (p=0.71), but there is a strong relation between THOR and Gleason score (p=0.0082). Patients with higher values of THOR have a significant short time for biochemical relapse (DC, p=0.0263; VC, p=0.0042). Interestingly, THOR was able to distinguish among Gleason 6 patients the ones which relapsed in a shorter period of time (p=0.0167). THOR has a higher Area Under the Curve (AUC) (0.67) when compared with PSA and a higher prediction for relapse (Cíndex of 0.81) - DC. In VC THOR also has an higher AUC (0.641) and a Cindex of 0.795. We identified THOR as a novel independent biomarker for prostate cancer. THOR was shown to have significant independent prognostic value for prediction of biochemical recurrence in two different cohorts of patients. THOR (as a specific mechanism of telomere maintenance, regulating self renewal in cancer) can act as a specific marker for disease progression and help distinguish between those patients with low pathologic scores the ones that should be in active surveillance or undergo immediate treatment.

No conflict of interest.

SI 3. Detection of somatic alterations in plasma from lung cancer patients

José Luis Costa[1,2], Ana Justino[1], Gabriela Fernandes[1,2,3], Miguel Silva[1], Ana Barroso[4], Bárbara Parente[4], Venceslau Hespanhol[1,2,3] and José Carlos Machado[1,2]

[1]*IPATIMUP - Institute of Pathology and Molecular Immunology of the University of Porto, Portugal;* [2]*Medical Faculty of the University of Porto, Portugal;* [3]*Department of Pulmonology, Hospital of São João, Portugal;* [4]*Unit of oncological pulmonology, Hospital Center of Vila Nova de Gaia-Espinho, Portugal.*

Introduction: Tumor-specific (somatic) mutations in plasma can serve as biomarkers for tumor detection, monitor tumor response to specific therapies, detect residual disease after surgery, and long-term follow-up. The intrinsic low abundance of circulating cell-free tumor DNA (cfDNA) makes the detection and quantification of such mutations in plasma a challenging task. This small scale study aims to establish a comprehensive strategy to be used in the detection of clinical relevant somatic alterations in plasma of lung cancer patients. *Material and Methods* Plasma samples obtained at different stages of disease progression and/or treatment were collected from a group of 11 lung cancer patients and used to isolate cfDNA. Genetic alterations in the EGFR gene (p.E746_A750del and p.L858R) identified at diagnosis in tumor biopsies were used as surrogate markers to optimize and validate the next generation sequencing strategy and data analysis workflow. The Ion AmpliSeq Colon and Lung cancer panel was used to analyze hotspot and targeted regions of 22 genes implicated in colon and lung cancers, including the EGFR gene mutations mentioned. The amplified products were used to prepare libraries and were sequenced using the Ion PGM system. Quantitative real time PCR and digital PCR assays were used to confirm selected results. *Results and Discussion:* Tumor derived genetic alterations could be identified in as little as 10ng of cfDNA. EGFR alterations identified in cfDNA mirrored the alterations identified in all tumor biopsies. EGFR alterations with allelic frequencies as low as 3% could be detected in cfDNA. Additionally, in case of samples collected after therapy, a clear decrease in the cfDNA allelic frequency of the EGFR mutation, that made the patient eligible for therapy, was observed. Furthermore, the screening of a larger panel of genes allowed the identification in two cases of additional gene mutations (e.g. MET and KRAS) that may have impact in the clinical management of patients. In this study, we demonstrate the capacity to identify clinically relevant somatic EGFR mutations in plasma. The possibility to assess information from a larger panel of genes makes this strategy attractive for further optimization of treatment options. The strategy is now being extended to a larger cohort of patients to push forward the concept of liquid biopsy in the clinical management of cancer patients.

No conflict of interest.

SI 4. Autonomous electrochemical biosensor for cancer screening

Lúcia Brandão[1], Carolina Hora[1], Goreti Sales[1]

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Introduction State-of-art electrical biosensors for cancer biomarkers present electrical dependency features, in order to transduce the biorecognition event into an electrical signal. The electrical dependency of such biosensor can be eliminated by coupling the bioreceptor to a transducer that is capable of autonomous energy production, for instance a solar cell. Such strategy would allow the production of a cancer screening sensor fully independent, simple and low cost. In this work, a dye sensitized solar cell (DSSC) was used as the autonomous transducer of an electrochemical biosensor for the carcinoembryonic antigen (CEA) cancer biomarker. *Materials and Methods* Surface imprinting was used for preparing molecularly imprinted polymers (MIP) for carcinoembryonic antigen (CEA) on the DSSC counter-electrode. A monolayer of the template protein was adsorbed on the Pt surface and surface imprinting was performed by electropolymerization of phenol red. Different electropolymerization times were considered to control film thickness in order to not overlay the template protein and sterically hinder its removal for creating the negative imprinted sites. Film thicknesses were controlled by the charge passed through the electrode and by ellipsometry. The template protein was removed from the imprinted sites by potential sweep in acidic media. Initial characterization of the biosensor was made by electrochemical impedance spectroscopy (EIS) in a three electrode configuration cell, using I-/I3- in PBS as redox probe. The biosensor was incubated with increasing concentrations of the target biomarker and the signal obtained by EIS during probe reduction was plotted against protein concentration. Following, a DSSC was assembled with the MIP modified counter electrode and the autonomous biosensor was tested with increasing concentrations of CEA. After each incubation step the DSSC was closed and the energy conversion characterization performed under 1 sun illumination equivalent. *Results and Discussion* Preliminary results indicate that the electrical power produced by the DSSC decreased with concentration of CEA. This effect was related to the rebinding of the CEA biomarker on the imprinted molds that hindered by steric hindrance the counter electrode below. Because of that the catalytic actives sites are not available to catalyze the I3- reduction what decreases light conversion. Acknowledgments Authors acknowledge funding from the ERC Starting Grant project "3P´s".

No conflict of interest.

SI 5. Identification of rare CDH1 non-coding variants in Hereditary Diffuse Gastric Cancer

H. Pinheiro[1*], P. Oliveira[1*], S. Sousa[1], K. Shumansky[2], J. Senz[3], G. Almeida[1], D. Ferreira[1], J. Carvalho[1], D. Huntsman[3], C. Oliveira[1,4]

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Introduction: Hereditary diffuse gastric cancer (HDGC) syndrome, although rare, is severe, highly penetrant, difficult to diagnose and incurable. Forty-five percent of HDGC-families are associated with germline CDH1/E-cadherin coding alterations. We recently implicated other gastric cancer (GC) susceptibility genes in ~5% of CDH1-negative families. Of the 50% that remain without molecular diagnosis, 2/3 present a germline phenotype of monoallelic CDH1 downregulation and >90% display loss of E-cadherin protein expression in tumours. This led us to hypothesize that HDGC families lacking CDH1-coding mutations may harbour germline alterations in non-coding regions. Our aim is to identify rare CDH1 non-coding variants (NCVs) as the potential cause of HDGC. *Material and Methods*: The entire CDH1 locus was divided into 90 overlapping fragments, which were amplified from germline DNA of 90 HDGC probands lacking mutations in the classically screened CDH1 regions. The complete set of amplicons from each proband was barcoded and submitted to targeted re-sequencing. Novel NCVs found were further selected using stringent quality standards and only those present in at least 25% of the reads for each amplicon were considered further. Bioinformatics criteria were used to

prioritize the NCVs to be evaluated from a biological standpoint: 1) chromatin status of the adjoining region; 2) occurrence in putatively transcribed regions; 3) vertebrate conservation level; and 4) predicted creation/deletion of repressors/enhancers consensus sequences. *Results and Discussion*: Thirty rare heterozygous germline CDH1 NCVs were sorted-out as putatively disease causative for 25% of mutation negative HDGC probands. Considering the chromatin status features alone, the selected NCVs were clustered into two different classes suggesting the existence of two different pathways to impact CDH1 gene expression: 1) promoter/enhancer-related variants that create binding sites for CDH1 expression repressors or that eliminate binding sites for crucial CDH1 expression enhancers; and 2) transcription-related variants that may impair the normal expression pattern of the CDH1 locus. This hypothesis will be biologically addressed in order to confirm their impact in gene expression. Any novel pathogenic alteration identified will warrant a complete redefinition of the screening methodology currently applied to HDGC families, providing simultaneously important insights for the understanding of CDH1 expression regulation. Acknowledgements: COMPETE/FEDER and Portuguese Foundation for Science and Technology (FCT): Project FCT PTDC/SAU-GMG/110785/2009; Post-doc grants to HP (SFRH/BPD/79499/2011), PO (SFRH/BPD/89764/2012), GA (SFRH/BPD/87257/2012) and JC (SFRH/BPD/86543/2012).

No conflict of interest.

Loss of Slit-Robo signalling in the pancreas increases the severity of pancreatitis

A. V. Pinho[1], M. Giry-Laterriere[1], L. Chantrill[1], A. Mawson[1], M. Rees[1], J. Youkhana[2], J. Wu[1], P. Phillips[2], A. V. Biankin[1,3], I. Rooman[1,4]

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Genomic analysis of pancreatic tumours revealed that Slit-Robo pathway genes are frequently mutated, hypermethylated or present copy number loss in human pancreatic ductal adenocarcinoma (PDAC) [1,2]. Our aim is to evaluate if Slit-Robo signalling is implicated in pancreatitis, a condition that predisposes for PDAC. Expression of Slit-Robo genes was analysed in normal mouse pancreas, caerulein-induced acute pancreatitis and Kras/p53 mutant tumours. Mice with loss of critical genes in the Slit-Robo pathway were used for experimental analysis. Pancreatic cells isolated from these animals were cultured using an *in vitro* method that mimics the events occurring in pancreatitis [3] and acute pancreatitis was induced *in vivo* by treatment with caerulein. Robo2 is expressed in the exocrine tissue of the normal mouse pancreas, whilst significantly decreased in pancreatitis and nearly lost in PDAC. Exocrine cell cultures with deficiency of Robo2 or its ligand Slit1 present with altered cell adhesion characteristics, upregulation of PDAC precursor markers and activation of pathways involved in pancreatic cancer development such as epithelial to mesenchymal transition, Wnt and Tgfβ signalling. Slit1 deficient animals treated with caerulein present with a more severe acute pancreatitis and altered microenvironment, showing an increase in Desmin+ stellate cells and CD3+ positive T lymphocytes. In conclusion, we have found that pancreatic cells with impaired Slit-Robo signalling, upon exposure to stress, either by *in vitro* culture or *in vivo* induction of pancreatitis, activate mechanisms that lead to a more severe pancreatitis and that are known to predispose to PDAC. These results constitute the first evidence that loss of Slit-Robo genes may be involved in the early stages of pancreatic cancer development. 1. Biankin AV et al. Nature, 2012. 491(7424): 399-405. 2. Nones K et al. Int J Cancer, 2014. 135(5): 1110-8 3. Pinho AV et al. Gut, 2011. 60(7): 958-66.

No conflict of interest.

Cancer Exosomes Perform Cell-Independent MicroRNA Biogenesis and Promote Tumorigenesis

Sonia A.Melo[1,2], Hikaru Sugimoto[1,2], Joyce T. O'Connell[2], Noritoshi Kato[2], Alberto Villanueva[3], August Vidal[4], Carlos A. Melo[5,6], Cristina Ivan[7], George A. Calin[8] and Raghu Kalluri[1, 2]

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Exosomes are secreted by all cell types and contain proteins and nucleic acids. Here, we report that breast cancer associated exosomes contain microRNAs (miRNAs) associated with the RISC Loading Complex (RLC) and display cell-independent capacity to process precursor microRNAs (pre-miRNAs) into mature miRNAs. Pre-miRNAs, along with Dicer, AGO2, and TRBP, are present in exosomes of cancer cells. CD43 mediates the accumulation of Dicer specifically in cancer exosomes. Cancer exosomes mediate an efficient and rapid silencing of mRNAs to reprogram the target cell transcriptome. Exosomes derived from cells and sera of patients with breast cancer instigate non-tumorigenic epithelial cells to form tumors in a Dicer-dependent manner. This study identifies a mechanism whereby cancer cells impart an oncogenic field effect by manipulating the surrounding cells via exosomes. These findings offer opportunities for the development of exosomes based biomarkers and therapies.

No conflict of interest.

ABSTRACTS

INVITED SPEAKER

SII 1. Pre-clinical and clinical data on the efficacy of anti-Notch therapies for lung adenocarcinomas

<u>Manuel Serrano</u>

The Notch signaling pathway can be oncogenic or tumor suppressive depending on the cancer type. We have investigated the role of the Notch pathway in the generation and maintenance of KrasG12V-driven lung adenocarcinomas. We have demonstrated by genetic means that gamma- secretase and RBPJ are essential in the formation of lung adenocarcinomas Importantly, pharmacologic treatment of mice carrying autochthonous lung adenocarcinomas with a gamma-secretase inhibitor (GSI) blocks the growth of already established cancers. Treated carcinomas present reduced HES1 levels and, interestingly, reduced phosphorylated ERK without changes in phosphorylated MEK. Mechanistically, we show that HES1 directly binds and represses the promoter of DUSP1, encoding a dual phosphatase active against phospho- ERK. Accordingly, GSI treatment upregulates DUSP1 and decreases phospho-ERK. These data provide proof for the in vivo therapeutic potential of gamma-secretase inhibitors in primary lung adenocarcinomas. We have extended these findings to other therapeutic agents that also inhibit the Notch pathway, in particular, anti-DLL4 monoclonal antibodies. I will present data on a Phase I clinical trial using anti-DLL4 antibodies in human non-small cell lung carcinomas.

In contrast to lung adenocarcinomas, the Notch pathway is tumor suppressive in several squamous cell carcinomas (SCCs) of different origins. However, little was known about bladder carcinomas. We have found that human bladder tumors harbor loss-of-function mutations in NOTCH1 and NOTCH2. To support the relevance of this finding, we have demonstrated that ablation of the Notch pathway in mice accelerates bladder tumorigenesis and promotes the aggressive SCC subtype. Mechanistically, the Notch effector HES1 downregulates master inducers of epithelial-mesenchymal transition and, therefore, loss of Notch activity unleashes EMT and promotes aggressive tumor behavior. We conclude that Notch is a relevant bladder tumor suppressor.

M.S. is a researcher at the Spanish National Cancer Research Centre (CNIO), in Madrid, and Director of the Molecular Oncology Program of CNIO. After completing his studies and PhD in Madrid, M.S. joined the laboratory of David Beach, at Cold Spring Harbor Laboratory, NY, USA, as postdoctoral fellow. During this time, M.S. made one of his most important contributions with the discovery of the tumor suppressor p16. In 1997, M.S. established his research group in Madrid. The main contributions of the Serrano´s laboratory are related to the concept of oncogene-induced senescence and the anti-aging activity of tumor suppressors. More recently, Serrano´s group has worked on the relevance of tumor suppressors during reprogramming, and it has demonstrated the feasibility of embryonic reprogramming within live adult organisms. In addition, Serrano works on the role of the Notch pathway in lung cancer.

INVITED SPEAKER

SII 2. Telomerase promoter mutations in cancer: an emerging molecular biomarker?

<u>Paula Soares</u>

Cell immortalization has been considered for a long time as a classic hallmark of cancer cells. Besides telomere maintenance due to the "alternative mechanism of telomere lengthening" it was advanced that such immortalization could be due to telomerase reactivation, but the mechanisms underlying such reactivation remained elusive.

Mutations in the coding region of telomerase gene are very rare in the cancer setting, despite being associated with some degenerative diseases. Recently, mutations in telomerase (*TERT*) gene promoter were found in sporadic and familial melanoma and subsequently in several cancer models, notably in gliomas, thyroid cancer and bladder cancer. The importance of these findings has been reinforced by the association of *TERT* mutations in some cancer types with tumour

aggressiveness and patient survival. In particular in differentiated thyroid cancer TERT promoter mutations are an indicator of clinically aggressive tumours, being correlated with worse outcome and disease-specific mortality. TERT promoter mutations have an independent prognostic value in differentiated thyroid cancer and, notably, in papillary thyroid carcinoma, the most common type of endocrine cancer. We will review the information on telomerase genetic alterations in the most relevant types of cancer and discuss the value of telomerase as a new biomarker with impact on the prognosis and survival of the patients and as a putative therapeutic target.

Paula Soares is Assistant Professor of Biopathology at the Medical Faculty of Porto and coordinates the Group of Cancer Biology at the Ipatimup. She received her Biology degree in Science Faculty, University of Porto and MSc and PhD degrees at the Medical Faculty, University of Porto.
Her main research interests include oncobiology of thyroid and other neuroendocrine tumors, mainly addressing the cellular biology and molecular genetics of sporadic and famillial thyroid tumors.

INVITED SPEAKER

SII 3. New biosensors to monitor cancer biomarkers.

Goreti Sales

Cancer diseases remain a major public health concern, with breast, cervical and colorectal cancers the most frequent forms of the disease in the EU. Evidence-based strategies for early recognition and management of patients with cancer throughout the population have been successfully implemented, aiming ultimately at cancer detection in early stages. Substantial improvements are however required to design innovative devices that become readily available for point-of-care use.

Cancer is the phenotypic end point of numerous genomic and/or epigenomic changes accumulated in cells. Within the course of each cancer disease, there are some biochemical alterations that may be followed in body fluids, recognized as cancer biomarkers. Screening such biomarkers may turn out a valuable tool for screening the disease, most especially when these biomolecules are circulating in blood, urine, or saliva, allowing non-invasive procedures. There are few devices described so far for targeting cancer biomarkers, relying mostly in electrical signals and employing antibody biomaterials as biorecognition layer, producing label-free and sensitive determinations.

The activities under the scope of the Starting Grant 3P's, currently in action, are meant to create a new device that (i) employs plastic antibodies instead of the corresponding natural biomaterials, leading lower cost and higher stability operations; (ii) and makes use of photovoltaics to confer full autonomy to the electrical device. This is the first attempt to design a biosensing electrical device linked to a photovoltaic cell. It has been successfully achieved in a first approach for carcinogenic embryonic antigen (CEA), a cancer biomarker. The device is currently subject of further improvements.

Professor at the Chemical Engineering Department of the School of Engineering of the Polytechnique School (ISEP, since 1998), as adjunct professor since 2006. She integrates the Scientific board of ISEP (since July 2014), is the institutional representative of ISEP Supplementary Diploma student data at the Polytechnique School of Porto (since 2006).
She is the founder and scientific coordinator of the research unit BioMark, *Sensor Research* (since July 2011) that integrated in 2013 the research group CINTESIS of the Faculty of Medicine of the Porto University. Current research targets the development of autonomous devices for monitoring cancer biomarkers, by merging two separate fields of knowledge, biosensors and photovoltaics, a novel approach. She has 84 indexed publications, with 580 citations.

ABSTRACTS

INVITED SPEAKER

SIII 1. The many faces of P53.

Moshe Oren

The *TP53* gene, coding for the p53 tumor suppressor protein, is the most frequently mutated gene in human cancer. In normal cells, the wild type p53 protein serves as a potent defense mechanism against cancer, by maintaining metabolic homeostasis and by orchestrating an effective transcriptional response and controlling cell fate in the face of potentially cancer-promoting stress. In contrast, cancer-associated *TP53* mutations often endow the mutant protein with new, oncogenic gain-of-function activities. In reality, the picture is even more complex. Thus, p53 has tumor suppressor activities not only in cells that are at risk of becoming cancerous, but also in their microenvironment. Furthermore, tumors that retain a wild type *TP53* gene may actually paradoxically gain selective advantages from continued expression of the wild type p53 protein. Examples and implications will be discussed.

M.O. is a Professor at the Weizmann Institute, in Israel, and a chairperson of the Life Sciences Appointments Committee of the same institute. After completing his undergraduation and PhD in Israel, M.O. moved to the US to work as a post-doc, first at the Princeton University, NJ, and then at the Stony Brook University, NY. In 1981, M.O. established his research at the Weizmann Institute where he later became Professor. He has focused his research on the tumor suppressor p53, and has over 250 papers published. He has been awarded numerous international prizes and honors, namely the NIH MERIT award, and he is a member of the editorial board of several high-impact journals, such as the EMBO Journal and Molecular Cell. M.O. was President of EACR from 2012 to 2014.

INVITED SPEAKER

SIII 2. Li-Fraumeni syndrome: the prime example of pleiotropism in cancer predisposition

Manuel Teixeira

The Li-Fraumeni syndrome is caused by germline mutations in the *TP53* gene. Several clinical criteria have been proposed for *TP53* mutation screening since this syndrome was first described. The clinical impact of a germline *TP53* mutation is often dramatic, being characterized by a wide range of cancers predominantly diagnosed in children and young adults. Although the heterogeneous clinical presentation and the high rate of *de novo* mutations makes genetic testing essential for its correct diagnosis, offering predictive testing is challenging due to the predisposition to multiorgan tumorigenesis and the lack of proven cancer risk management programs for most cancers. Furthermore, recent developments involving next generation sequencing are also changing the way we diagnose the Li-Fraumeni syndrome, allowing identification of patients with a even wider range of clinical presentations. The clinical challenges originated by this prime example of pleiotropism in cancer predisposition will be discussed.

· Manuel Teixeira is the Director of the Department of Genetics and of the Research Center of the Portuguese Oncology Institute – Porto (IPO-Porto), where his research and diagnostic activities are integrated. The diagnostic work in the area of cancer genetics ranges from identification of hereditary cancer predisposition to cytogenetic and molecular genetic analysis of hematological malignancies and solid tumors. He is also responsible for genetic counseling of families with suspected or confirmed inherited cancer predisposition at IPO-Porto and is a guest Full Professor of Pathology and Genetics at the Biomedical Sciences Institute of the University of Porto. He is a visiting professor at the Center for Cancer Biomedicine, University of Oslo, Norway. Manuel Teixeira has been actively involved in the field of cancer genetics since 1994 and has published more than 180 peer-reviewed international publications.

SIII 3. Identification of familial cancer susceptibility genes – novel genomic approaches.

<u>Branca Cavaco</u>

In the last two decades, major progresses have been made in the identification of genes that contribute to familial cancer risk. However, the molecular pathogenesis underlying several familial cancer syndromes is yet to be clarified. The identification of novel familial cancer susceptibility genes enables the early identification of mutation carriers at risk for developing the disease, supports the genetic counselling, and helps to define prophylactic treatment strategies. In addition, it may also increase the current knowledge on the molecular mechanisms governing tumourigenesis, allowing the generation of new in vitro and in vivo disease models, and improving the search for new drug-targetable pathways.

Linkage studies have successfully lead to the discovery of genetic loci that increase disease risk. High density Single Nucleotide Polymorphisms (SNP)-arrays represent a rapid and powerful tool for discerning linkage between specific genomic loci and cancer predisposition. Recently, the advent of next generation sequencing (NGS), and in particular of whole-exome sequencing (WES), which allows an unbiased analysis of approximately 1% of the human genome, containing protein-coding exons and splice-sites (the location of the vast majority of disease causing mutations), may greatly speed up the identification of cancer predisposing genes. It is possible to combine WES with genome-wide linkage studies (using SNP-arrays) to identify rare causal variants that contribute to the linkage signals, facilitating the identification of candidate genes for targeted NGS in large series of patients with a specific disease.

Familial thyroid cancer is a disease with high genetic heterogeneity, for which the underlying molecular basis remains essentially unknown. Therefore, it represents an excellent model to develop studies, using the above mentioned genomic approaches. Using this strategy, our group has recently identified potential novel familial thyroid cancer susceptibility genes.

Branca Cavaco is the Principal Investigator of the Molecular Endocrinology Group, at the Molecular Pathobiology Research Unit (UIPM), from the Portuguese Institute of Oncology - Lisbon (IPOLFG), and Chronic Diseases Research Center (CEDOC). Since 2012 she is the Director of UIPM. She is an Invited Professor in the Integrated Master Course in Medicine (Endocrinology specialty), from the Nova Medical School, Lisbon. Her research interests focus on the molecular mechanisms involved in the aetiology and progression of sporadic and familial forms of thyroid tumours and familial predisposition to parathyroid-related endocrine diseases that principally affect calcium homeostasis. She authored nearly 40 publications in international refereed journals, including Nature Genetics, Journal of Clinical Endocrinology and Metabolism and British Journal of Cancer.

SIII 4. HOXA9 Promotes Glioblastoma Initiation, Aggressiveness and Resistance to Therapy

<u>Marta Pojo</u>, MSc[1,2], Céline S. Gonçalves, MSc[1,2], Ana Xavier-Magalhães, MSc[1,2], Ana Isabel Oliveira, MSc[1,2], Sandra Costa, PhD[1,2], Luísa Pinto, PhD[1,2], Rui M. Reis, PhD[1,2,3], Miguel Rocha, PhD[4], Nuno Sousa, MD, PhD[1,2], Bruno M. Costa, PhD[1,2]

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Glioblastoma is the most common and malignant subtype of glioma, exhibiting remarkable resistance to treatment. Here we investigated the oncogenic potential of HOXA9 in gliomagenesis, the molecular and cellular mechanisms by which HOXA9 may render glioblastoma more aggressive, and how HOXA9 affects response to chemotherapy and prognosis. Expression microarrays were used to identify HOXA9 target genes. Stable glioblastoma cell lines with ectopic HOXA9 overexpression or shRNA-mediated knockdown of HOXA9 were established to evaluate the roles of HOXA9 in cell viability, death, invasion, and response to temozolomide. Subcutaneous and orthotopic intracranial xenograft models of glioblastoma were established to evaluate the oncogenic potential of HOXA9 *in vivo*, and its role in response to temozolomide and overall survival. Transcriptomic analyses identified novel HOXA9-target genes that have key roles in critical cancer processes, including cell proliferation, adhesion, DNA metabolism and repair, and stem cell maintenance. Functional assays with a variety of glioblastoma cells revealed that HOXA9 promotes cell viability, stemness, and invasion; conversely, HOXA9 displayed anti-apoptotic functions. Additionally, ectopic expression of HOXA9 promoted the malignant transformation of human immortalized astrocytes in an intracranial orthotopic mouse model of

glioblastoma, and caused tumor-associated death. HOXA9 also mediated resistance to temozolomide treatment both *in vitro* and *in vivo*. Mechanistically, BCL2 was identified as a novel HOXA9 target that may be therapeutically targeted. Indeed, the pharmacological inhibition of BCL2 with ABT-737 specifically reverted temozolomide resistance in HOXA9-positive cells. These data establish HOXA9 as a critical driver of glioma initiation, aggressiveness and resistance to therapy.

No conflict of interest.

SIII 5. Flexible IL-7-mediated regulation of autophagy promotes T-cell acute lymphoblastic leukemia cell viability

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Introduction: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive subset of ALL, the most frequent childhood malignancy. Despite considerable treatment success, there is a clear need for more effective, specific and less toxic therapies. Interleukin-7 (IL-7) is essential for normal T-cell development and there is significant evidence that it can also promote leukemogenesis. Previously, we showed that IL-7 induces T-ALL proliferation, survival and metabolic activation in a majority of T-ALL cases via PI3K/Akt/mTOR pathway. Autophagy is a normal cellular homeostatic process. It is upregulated during starvation or in rapidly dividing cells, such as cancer cells, for reuse of intracellular nutrients. When persistent, its protective role may shift to what is called autophagic cell death. mTOR is recognized as the master negative regulator of this process. Since IL-7 regulates mTOR activation we decided to explore whether and how IL-7 regulates autophagy in T-ALL cells. Understanding this modulation may lead to the identification of new molecular targets with therapeutic value. *Materials and Methods*: We used an IL-7-dependent T-ALL cell line, TAIL7, and PI3K, mTOR, MEK1/2 and STAT5 small molecule inhibitors. We assessed the autophagic rate using hydroxychloroquine and assessed LC3 cleavage, puncta and autophagossome formation by western blot, confocal microscopy, electron microscopy and flow cytometry. Cell viability was evaluated by flow cytometry. *Results and Discussion*: Our data suggest that in optimal conditions (complete media) IL-7 inhibits autophagy in T-ALL, albeit in a complex manner that involves triggering both pro- (via MEK/Erk) and anti- (via PI3K/Akt/mTOR) autophagic signaling pathways. Under these conditions inhibition of autophagy contributes to IL-7-mediated T-ALL cell survival. In contrast, under serum starvation IL-7-mediated survival partially relies on autophagy activation. Since autophagy is often associated with cell death but also chemoresistance and higher viability in low nutrient conditions (such as in some tumor contexts), our results may reflect the ability of IL-7 to make use of a 'flexible strategy' to promote T-ALL cell viability by recruiting both pro- and anti-autophagic pathways which prevent tumour cell death in opposing scenarios (e.g. low versus high nutrient microenvironments; or steady state versus chemotherapy). This knowledge may open new therapeutic opportunities for IL-7-dependent T-ALL cases.

No conflict of interest.

SIII 6. Establishment of an inducible cdx2 ko/ki model using zinc finger nucleases: impact on the intestinal phenotype.

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Introduction: Expression of the CDX2 homeobox transcription factor is normally restricted to the intestine, being considered a "master regulator" of intestinal differentiation genes, such as MUC2 and CDX2 itself. It is further implicated in cellular proliferation, cell-adhesion and migration. However, CDX2 becomes ectopically expressed in pre-neoplastic and neoplastic lesions of other organs, namely in the stomach. On the other hand,

CDX2 has been proposed as a tumor suppressor in colorectal cancers but its role is still controversial, since CDX2 expression is rarely lost, despite it can be down-regulated at the invasive front and in tumor buddings. In this study, we attempted to clarify the role of CDX2 in intestinal cells through controlled expression of CDX2, based on specific zinc finger nucleases (ZFNs) targeting and a modified 3G Tet-On system. *Materials and Methods*: LS174T human intestinal cell line was simultaneously transfected with ZFNs to target CDX2 exon 1 and a "reverse" Tet repressor (rTetR) under the control of a CMV promoter. Targeted integration of the repressor into CDX2 locus was achieved by ObLiGaRe system. Resultant clones were screened for targeted integration, constitutive rTetR expression and absence of CDX2 (knock-out (KO) clones). A KO clone was then transfected with ZFNs to target the human genome safe harbor AAVS1, together with a vector containing a codon optimized CDX2 ORF under the control of a Tet responsive element. Clones with correct integration and CDX2 expression upon doxycycline addition (knock-in (KI) clones), were further studied. *Results and Discussion*: CDX2 ablation on LS174T cells was accompanied by a decrease in MUC2 mRNA and protein expression. Cell morphology and ultrastructure was maintained in the absence of CDX2. However, contrary to the parental cell line, KO clones were not able to form cysts in a 3D system. Apoptosis and proliferation rates were not altered but CDX2 KO cells showed increased motility. When CDX2 expression is restored in the KI clones, MUC2 expression is also reconstituted. This model allows the study of CDX2 regulation in intestinal cells, its impact in cell phenotype, as well as evaluation of protein turnover under controlled conditions. We are currently performing a RNA-seq analysis on LS174T and derived CDX2 KO cells to elucidate transcript regulation by CDX2.

No conflict of interest.

SIII 7. TRIB2-mediated AKT activation provides melanoma cells with a novel regulatory mechanism underlying drug resistance

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Introduction: Melanoma is the most aggressive form of skin cancer resistant to all standard therapies. Drug resistance is the major cause of treatment failure in melanoma. Our lab has identified TRIB2 as an oncogene which is dramatically overexpressed in malignant melanoma. We previously demonstrated that TRIB2 acts as a suppressor of FOXO transcription factors, the major transcriptional mediators of the PI3K/AKT pathway. As FOXO proteins are involved in the action of several anticancer drugs we hypothesized that TRIB2-mediated FOXO suppression can lead to drug resistance. In this study we show that TRIB2 indeed confers resistance to drugs used to treat melanoma or which are currently tested in clinical trials. Importantly we show that this novel mechanism of drug resistance has clinical relevance *Materials and Methods*: Using paired isogenic cell lines harboring silenced or overexppresed TRIB2, we analyzed the sensitivity to several drugs relevant in the treatment of melanoma such as dacarbacine, gemcitabine, PI3K, AKT and mTOR inhibitors. To examine the functional importance of FOXO in TRIB2-dependent cell line resistance we used RNAi mediated silencing of TRIB2. We also mapped the domain of the TRIB2 protein responsible for drug resistance using several TRIB2 mutants. qPCR was used to determine the expression of p53 and FOXO target genes, Western blot analysis and immunohistochemistry was employed to monitor expression and activation status of components of the PI3K/AKT pathway. Patient samples with full clinical histories were obtained from Department of Dermatology, Julius-Maximilians University, Würzburg, Germany *Results and Discussion*: Our study shows that TRIB2 confers resistance to several drugs relevant for the treatment of melanoma providing a novel regulatory mechanism underlying drug resistance. As intrinsic and acquired resistance to all treatment modalities is the major cause of treatment failure in melanoma, the implications of the current proposal for clinical management of melanoma patients are extremely important. Keywords: Melanoma, drug resistance, FOXO, AKT, PI3K p53.
No conflict of interest.

ABSTRACTS

EACR SPONSORED LECTURE

SIV 1. *Clonal evolution and cancer medicine.*

Samuel Aparicio

The notion that most cancers are ecosystems of evolving clones has implications for biological understanding and clinical application. The evolution of clonal composition has particular significance when evidence of positive or negative selection can be associated with the clonal genotype or epigenotype. Over the last 4 years next generation sequencing of tumours and methods for single cell analysis have opened up this approach for solid epithelial malignancies.

I will discuss the implications of clonal evolution for cancer medicine and biological studies of cancer with reference to breast cancers. We have developed informatics approaches to population level clonal analysis and extended these to single cell measurements of genotypes. I shall discuss our more recent data from single cell sequencing and clonal analysis applied to clonal evolution of patient derived tumour xenografts, to illustrate the impact of clonal evolution on biological studies of cancer in model systems.

Samuel Aparicio is the Nan & Lorraine Robertson Chair in Breast Cancer Research and a Canada Research Chair in Molecular Oncology at the University of British Columbia and the BC Cancer Agency in Vancouver, Canada. He is also Head of the BCCA's Department of Breast and Molecular Oncology, and a Professor in the Department of Pathology and Laboratory Medicine at UBC.
Aparicio's research programme encompasses the fields of cancer genomics, mouse genetic models, high throughput screens, and translational breast cancer research. Aparicio is also interested in tumour heterogeneity, and is involved in developing genomically- and clonally-characterised xenograft models of breast cancer. His contributions to academic research have been published in journals such as Nature, Science, Cell and the New England Journal of Medicine. He was a co-founder of Paradigm Therapeutics (now, Takeda Cambridge). He is the recipient of numerous awards from academic as well as industrial institutions.

INVITED SPEAKER

SIV 2. *Cancer Metastases: is bone a useful paradigm?*

Luis Costa, Sandra Casimiro; Irina Alho; Arlindo Ferreira; Ricardo Pires; Afonso Fernandes.

The capacity to invade and metastasize is one of the six hallmarks of cancer. Although metastases are considered the major event to explain mortality in cancer, until now the mechanisms underlying invasion and metastasis are poorly defined and not targetable.

The molecular characterization and understanding of the different biologic steps until the formation of clinical detectable metastases could be of extreme importance to select the patients at higher risk for metastases and to develop new therapeutic targets. A salient feature of metastasis is the ability of different tumor types to colonize the same or different organ. For example, bone is the major site for metastases in prostate cancer and breast cancer whereas bone is rarely affected in colorectal cancer.

A bone metastasis gene expression signature was for the first time identified using human breast cancer cells in a mouse model (2003, Kang et al). We analyzed the expression of six BM related genes selected from Kang's BM signature in a set of clinical BMs. We provided experimental evidences that all the analyzed genes were overexpressed by tumor cells in BMs, independently of primary tumor type, and that there exists a significant correlation between the expressions of IL11/CTGF, IL11/ADAMTS1, CTGF/CXCR4, CTGF/ADAMTS1, and MMP-1/ADAMTS1, in the clinical setting, supporting the cooperative function of these proteins in the bone microenvironment. This model created opportunities for new therapies. One

interesting option for a near future is c-met inhibition as it has been showed to induce rapidly responses in bone metastases from prostate cancer patients when evaluated through serial bone scans.

Bone collagen fragments have been consistently used in the early development of new bone-targeted agents. The N-terminal cross-linked telopeptide of type I collagen (NTX) is the most often used biomarker in phase 2 and phase 3 studies when new agents to treat bone metastases are tested. Our recent clinical research with these biomarkers suggests that extra-skeletal metastases do influence also bone metabolism. This observation suggests also that we should analyze whether tumor cells can circulate (re-seed) between different metastatic sites in the same patient, and what is the clinical importance of this phenomena to select therapies.

In the adjuvant setting (after surgery), a recent meta-analysis shows a benefit for the use of bisphosphonates to prevent distant relapses in breast cancer women after menopausal, but not before (34% reduction in the risk of bone metastases). This is the first time that a drug directed to the host rather than to the tumor cell reduces cancer mortality. However, we do not know yet why ovarian function influence response to bisphosphonate treatment.

Conclusions: Findings of tumor–bone interactions have uncovered numerous therapeutic opportunities. Hopefully this will continue to yield new and exciting therapeutic targets to treat bone metastases. Furthermore, the recent evidence that bisphosphonates can prevent bone metastases and reduce breast cancer mortality creates an opportunity to explore other treatment strategies to target the role of microenvironment in cancer progression.

Luís Costa is Professor of Medicine at the Lisbon Medical School, University of Lisbon, and he is the head of the Clinical Translational Oncology Research Unit at IMM since 2007. Luis Costa serves also as director of Oncology Division at Hospital de Santa Maria in Lisbon since 2005. At the School of Medicine he is the Coordinator Professor of "Oncobiologia" a new teaching unit that aims to teach the understanding of clinical oncology through molecular medicine, and he acts as member of the Editorial board of Harvard Medical School Portugal Program. Luis Costa is the IMM Portuguese representative of the Global Cancer Genomics Consortium. Luis Costa is an expert for grant reviews at the European Research Council, the Cancer Research UK, and CAIBER (Spanish Clinical Research Network), and the French National Cancer Institute. Costa's clinical research, publications and scientific presentations have primarily focused on bone metastases, related to breast cancer, and other solid tumors. He has published various peer-reviewed papers discussing these topics.

SIV 3. Through the looking glass: the reversion of EMT

Patrícia Oliveira[1], Joana Carvalho[1], André Vieira[1], Sara Rocha[1], Joana Nunes[1], Jorge Lima[1], Fátima Carneiro[1], Joana Paredes[1], David Huntsman[2], Carla Oliveira[1,3]

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Epithelial-mesenchymal-epithelial transitions (EMT/MET) are key mechanisms during development and cancer (1). Invasive cancer cells are thought to undergo EMT, and concomitantly lose cell polarity while increasing self-renewal potential (2,3). Inflammatory cytokines and oncogenic activation are among the factors known to induce EMT and cell plasticity in cancer cell populations (4). Whereas EMT is important for cell detachment and cancer cell local invasion, at least its partial reversion is mandatory for colonization at distant tissues and organs. The mechanisms that underlie this reversion and the features of this reverted cell phenotype are however poorly understood. Our hypothesis is that EMT-reversion in cancer cells is incomplete, generate hybrid cell populations and novel regulatory circuitries are activated. Therefore, the aim of this study was to develop an *in vitro* EMT/MET model in order to disclose the underlying features and mechanisms associated with the EMT reversion. We reproduced EMT and EMT-reversion *in vitro* by treating a near-normal cell line (EpH4, epithelial cells, E-cells) with TGFβ1, thus obtaining Mesenchymal cells (M-cells). After removing TGFβ1 from the culture medium, we allowed M-cells to revert to a epithelial-like phenotype, obtaining Transient State cells (TS-cells). Cell proliferation, migration, metabolism, stemness, focus-forming-ability and tumourigenesis assays were performed. Moreover, whole-transcriptome-sequencing on E-, M- and TS-cells was performed with correspondent bioinformatic analysis and data validation. We confirmed the occurrence of EMT in M-cells and at least partial EMT-reversion in TS cells, via expression analysis of several epithelial/mesenchymal markers. TS-cells, although displaying an epithelial-like morphology were in fact a highly heterogeneous population displaying functional features similar to: 1) E-cells, for proliferation and metabolic signatures; 2) M-

cells, for self-renewal potential; 3) both E and M-cells for collective and single-cell migration and focus-formation ability. TS-cells presented also de novo features, such as increased *in vivo* tumourigenesis ability and transcriptome data analysis revealed de novo activation of Toll-like receptor signalling, that has been confirmed in TS-cell derived tumours. In conclusion, our model demonstrated that EMT reversion creates cells encoding a transcriptional/biological signature not necessarily mirroring that of a strictly-reversible-EMT. Considering the increasing evidences towards tumours as a heterogeneous mixture of cells, our model is a valuable tool for the discovery of novel pathways of relevance for tumour progression. Acknowledgements COMPETE/FEDER and Portuguese Foundation for Science and Technology (FCT): Project FCT PTDC/SAU-GMG/110785/2009; Post-doc grants to PO, JC. Also authors in this work: Mafalda Azevedo, Daniel Ferreira, Inês Reis, João Vinagre, Calvin Roskelley, Valdemar Máximo, Ali Heravi-Moussavi, Angela Burleigh and Nuno Mendes. References 1) Lim R, Thiery JP (2012) "Epithelial-mesenchymal transitions: insights from development" Development 139: 3471–3486 2) Thiery JP, Acloque H, Huang RYJ, Nieto, MA (2009) "Epithelial-Mesenchymal Transitions in Development and Disease" Cell 139:5 3) Thiery JP(2002) "Epithelial-mesenchymal transitions in tumour progression" Nat Rev Cancer 2(6):422-54 4) Kalluri R, Weinberg R (2009) "The basics of epithelial-mesenchymal transition" J Clin Invest. 2009;119(6):1420–1428.

No conflict of interest.

SIV 4. Targeting of the HGF/cMET pathway in metastatic medulloblastoma

Claudia C. Faria[1,2], Brian J. Golbourn[2], Adrian M. Dubuc[2], Marc Remke[2], Marcel Kool[3], Andrey Korshunov[3], Christian A. Smith[2], Michael D. Taylor[2] and James T. Rutka[2]

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Introduction Medulloblastoma is the most common malignant brain tumor in childhood and accounts for around 10% of all pediatric cancer deaths. The presence of metastases at diagnosis confers a poor prognosis despite current therapies that include craniospinal radiation and high-dose chemotherapy. Recently, it has been shown that medulloblastoma comprises four molecular subgroups - wingless (WNT), sonic hedgehog (SHH), Group 3 and Group 4 – with distinct genetics and clinical outcome. The hepatocyte growth factor (HGF)/cMET signaling pathway has been associated with tumor aggressiveness and dissemination. We recently reported the association of high cMET expression with the SHH subgroup and some Group 3 medulloblastomas. Group 3 tumors are known to have high incidence of metastasis and a poor outcome. We hypothesized that targeting cMET could be effective therapy in metastatic medulloblastoma. *Materials and Methods* We performed mRNA expression analysis of cMET in a cohort of 199 medulloblastomas and confirmed the results using a non-overlapping validation cohort of 439 medulloblastomas. Immunostaining of clinically annotated medulloblastoma tissue microarrays with phospho-cMET antibodies was performed and the expression levels were correlated with survival and recurrence. Foretinib, an orally available multikinase inhibitor of cMET, was used for all *in vitro* and *in vivo* experiments. Mouse xenografts and an aggressive transgenic model of metastatic medulloblastoma were used for *in vivo* studies. *Results and Discussion* We determined that cMET is highly expressed, both at the transcriptional and at the protein level, in SHH medulloblastomas. cMET activation correlates with an increased recurrence rate and a shorter progression-free survival in pediatric SHH medulloblastomas, defining a subset of patients that would most likely benefit from cMET targeted therapy. We were able to demonstrate that SHH medulloblastomas are markedly responsive to cMET inhibition by foretinib, an approved drug currently being evaluated in clinical trials for various cancers, both *in vitro* and *in vivo*. Treatment of mouse xenografts and of an aggressive transgenic model of disseminated SHH medulloblastoma with foretinib reduced primary medulloblastoma growth, decreased the incidence of metastases by 36% and increased survival by 45%. Our results provide strong rationale for advancing foretinib into clinical trials for SHH-driven medulloblastomas.

No conflict of interest.

SIV 5. Blocking Jagged1/2 inhibits prostate tumor development in a murine model of prostate adenocarcinoma (TRAMP)

Ana-Rita Pedrosa[1], Alexandre Trindade[1,2], Catarina Carvalho[1], José L. Graça[1], Sandra Carvalho[1], Maria Conceição Peleteiro[1], António Duarte[1,2]

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Introduction- Jagged-1 (Jag1) is a Notch ligand required for embryonic and retina vascular development, while Jagged-2 (Jag2) was first identified as required for craniofacial, limb and T cell development. Jagged1 is known to be expressed in the vasculature, and its increased expression in the glandular epithelium has been associated with cancer development and even considered to be a marker of bad prognosis and high metastatic potential in breast and prostate cancers. Up-regulation of Jagged2 has been described in prostate cancer cells with metastatic potential. The purpose of our study was to better characterize the role of Jagged1 and Jagged2, and the efficacy of blocking these ligands, in prostate tumor progression. *Materials and Methods-* To achieve this purpose we administered blocking anti-Jagged1/2 antibody to TRAMP mice. The mice were sacrificed at 18 and 24 weeks of age, which correspond to early and late stages of tumor development, respectively, and the prostates were collected and evaluated regarding several parameters such as: weight, histopathological classification of the tumor, vascular phenotype, apoptosis, proliferation, changes in luminal and basal cell compartments, epithelial-to- mesenchymal transition, and changes in cancer stem cell-like population. *Results and Discussion-* We have found that blocking Jagged1/2 has an inhibitory effect on the development of prostate tumors, in all stages of development. The tumor vasculature showed decreased density, with the presence of immature, leaky and non-functional blood vessels. We also observed increased apoptosis and decreased proliferation, relative to respective controls. Moreover, anti-Jagged1/2 antibody treatment inhibited the loss of luminal identity and the proliferation of the basal cell compartment and epithelial-to-mesenchymal transition. Additionally, treatment also blocked the ability of prostatic cancer cells to acquire a more stem like phenotype. These results show that blocking Jagged1 and Jagged2 has not only an anti-angiogenic effect, but that it also targets prostatic cancer cells directly, preventing them to proliferate and to acquire a more mesenchymal phenotype, and dramatically reducing the cancer stem cell population. The combination of all these different effects caused a strong inhibition of tumor progression, indicating that targeting Jagged1/2 may be a new promising therapeutic approach to prostate cancer.

No conflict of interest.

SIV 6. Macrophage differentiation is modulated by decellularized human colorectal cancer matrices

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Introduction: Tumour complexity, either referring to other cells types, soluble factors and extracellular matrix (ECM) components, is a topic that has been widely studied in the last decade. The fact that a cancer is more than just tumour cells is being perceived as a great opportunity for cancer prevention and treatment. In this context, macrophages emerged as modulators of cancer progression, regulating breast cancer cell migration, invasion and metastasis. In a rather simplistic vision, macrophages have been described as key elements for carcinogenesis, preventing the establishment and spreading of cancer cells – M1 macrophages – or supporting tumour growth and progression – M2 macrophages. We are particularly interested to elucidate how ECM components modulate macrophage polarization. Therefore, we developed an innovative 3D-organotypic culture mode, by decellularizing human colorectal cancer (CRC) tissue fragments and by repopulating them with monocytes, mimicking more closely the natural tumour microecosystem. *Materials and Methods*: We optimized the decellularization protocol and accessed its efficiency as well as tissue morphology and architecture by light microscopy and Scanning Electron Microscopy (SEM). The effect of tissue decellularization on DNA and Glycosaminoglycans (GAGs) contents were evaluated. These matrices were then repopulated with

freshly isolated primary human monocytes and allowed to differentiate for 7 days. Macrophage differentiation was analyzed by immunohistochemistry, ELISA and zymography. *Results and Discussion*: DNA quantification and DAPI staining confirmed the efficiency of the decellularization method. SEM analysis allowed the visualization of the ECM fiber meshwork while Hematoxylin-Eosin staining revealed that decellularized fragments retained the original tissue histological features. Decellularization reduced significantly the GAGS content in normal and tumours but other ECM components, such as laminin and fibronectin, are retained. Repopulation experiments clearly evidenced that monocytes are able to colonize these decellularized matrices and to differentiate into macrophages within the fiber network. Most interestingly, normal and tumour-derived matrices seem to retain, at least, part of their endogenous biological properties by distinctly modulating macrophage differentiation. Our results consistently reveal that macrophages repopulating normal decellularized matrices secrete higher levels of IL-6 and present higher MMP-9 activity, while expressing more CD163, than macrophages repopulating tumour-derived decellularized matrices. Elucidating the role of tumour cells and of ECM components, derived from the tumour microecosystem, on macrophage differentiation and polarization, opens new perspectives to the design of novel therapeutic strategies targeting macrophages.

No conflict of interest.

A1. Human UPF1: a cap-independent translation initiation mechanism and a cryptic promoter regulate its expression in cancer cells

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Cervical (CC) and colorectal (CRC) cancers are among the leading causes of death worldwide and their development and progression is dependent on regulation of gene expression. Eukaryotic cells possess a variety of post-transcriptional mechanisms by which they regulate gene expression, namely at translation initiation level. Although in most cases it occurs via the cap-dependent mechanism, several oncogenes, growth factors or proteins involved in the regulation of programmed cell death, among others, have a different translation initiation mechanism. It involves the direct recruitment of the ribosome to the vicinity of the initiation codon without the involvement of the cap structure. This allows the maintenance of protein synthesis under several cellular stresses and promotes tumourigenesis. Apart from its role in nonsense-mediated decay, the human up-frameshift 1 (UPF1) DNA and RNA helicase protein plays a crucial role in telomere replication and homeostasis, and in cell cycle progression. In addition, its expression levels are maintained in every phase of the cell cycle, thus indicating that its translation may occur via a cap-independent mechanism. To test this hypothesis, we cloned the human UPF1 5'UTR in a dicistronic vector and transfected CC and CRC cell lines with either this construct or the control counterparts. We observed a 15- to 25-fold increase in relative luciferase activity of the UPF1 5'UTR-containing construct compared to the levels obtained from the empty counterpart in all tested cell lines, suggesting a cap-independent translation initiation. To control whether these levels of luciferase activity were due to the presence of a cryptic promoter, we transfected cells with promoterless plasmids and observed the same result, demonstrating that UPF1 5'UTR contains a cryptic promoter. To check the cap-independent translation activity alone, we transfected cells with *in vitro* transcribed, capped and polyadenylated mRNAs and observed a 2-fold increase in protein levels, which shows that translation can occur in a cap-independent way. This is maintained under conditions of global protein synthesis inhibition. Deletional analysis of UPF1 5'UTR revealed that the first 50 nucleotides are essential for both cryptic promoter and cap-independent activities. These results provide new insights on the regulation of UPF1 expression in human cancer cells.

No conflict of interest.

A2. TERT promoter mutations: a possible worse prognosis marker in cutaneous melanoma

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Introduction: The reactivation or re-expression of telomerase (TERT) is a widespread feature of neoplasms. TERT promoter mutations were recently reported in cutaneous melanoma and were advanced to result from UV-radiation. This study aimed to evaluate the role of the presence of TERT promoter mutation in the etiopathogenesis of melanoma. *Material and Methods*: We assessed TERT promoter mutations in 4 cutaneous melanoma cell lines, 21 nevi, 116 primary cutaneous melanomas, 6 uveal melanoma cell lines and 26 primary ocular melanomas (21 uveal melanomas and 5 conjunctival melanomas). In all of the aforementioned samples we had previously obtained data of the BRAF status. We examined the TERT protein expression by immunohistochemistry in cutaneous melanomas. We looked at the relationship between the presence of TERT promoter mutation and the immunohistochemical data, and explore the putative association between TERT promoter mutation and the clinico-pathological and prognostic parameters in cutaneous melanoma. *Results and Discussion*: TERT promoter mutations were detected in the 4 cutaneous melanoma cell lines analyzed and in 22% of the 116 cutaneous melanomas. No alterations were found in nevi, uveal melanoma cell lines and ocular melanomas. In cutaneous melanomas, TERT promoter mutations were generally restricted to intermittent sun-exposed areas and significantly associated with nodular and superficial spreading subtypes, increased thickness, ulceration, increased mitotic rate, shorter disease-free and overall survival. Moreover, TERT promoter mutations significantly associated with the presence of BRAFV600E mutation, since 58% melanomas with TERT promoter mutations also harbored the BRAFV600E mutation and BRAF mutation was present in 25% melanomas without TERT mutations. Our results suggest that TERT promoter mutation plays a role in melanoma development. In cutaneous melanoma, TERT promoter mutations are associated with the presence of BRAFV600E mutations and poorer prognosis of the patients.

No conflict of interest.

A3. The estrogenic regulation of c-KIT and SCF in rat prostate supports the protective role of estrogens in prostate cancer

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Introduction: Development and progression of prostate cancer (PCa), the most common type of oncological disorder in men, has been highly related with the circulating and intraprostatic hormonal milieu. Despite the classical role of androgens as stimulating agents in PCa growth, currently estrogens also have been implicated in the onset and progression of PCa. However, a duality for the possible role of estrogens in prostate cells has been gaining consistency over the last years. If some studies defend that estrogens are potential causative agents of PCa, other strong evidences indicate that these steroids may be protective against PCa. The tyrosine kinase receptor c-KIT and its ligand, the stem cell factor (SCF), stimulate cell proliferation in a broad range of tissues, and the SCF/c-KIT interaction seems to play a crucial role in carcinogenesis. Moreover, the expression of SCF/c-KIT system seems to be regulated by estrogens in several tissues. The present work aims to evaluate the role of estrogens regulating the SCF/c-KIT expression in rat prostate *in vivo* and the consequent effect in proliferation and apoptosis of prostatic cells. *Materials and Methods*: Adult male Wistar rats were daily injected with vehicle (control) or with 17β-estradiol (E2, 250 mg/day/kg) for 5 days. After treatment, animals were euthanized under anesthesia and prostates were removed, weighted and either fixed in 4% paraformaldehyde or snap frozen in liquid nitrogen. The expression analysis of SCF and c-KIT in response to E2 was performed by means of real-time PCR, Western Blot and immunohistochemistry. The proliferation was estimated via fluorescent immunohistochemistry of Ki67. The protein ratio of Bax(proapoptotic)/Bcl-2 (antiapoptotic), the expression of caspase-9, Fas and Fas-L, the enzymatic activity of caspase-3 and a TUNEL assay were used to evaluate apoptosis. *Results and Discussion*: The results obtained showed a decreased expression of both SCF and c-KIT in E2-treated rats, suggesting a restricted proliferation of prostate cells in response to estrogens. This fact was confirmed by the diminished prostate weight and reduced Ki67 proliferation index observed in the treated group. Although the Bax/Bcl-2 ratio was decreased in animals treated with E2, the enzymatic activity of caspase-3 was increased, which suggests that estrogen treatment induced apoptosis. The enhanced expression of the Fas system in E2-treated animals indicates the involvement of the extrinsic pathway of apoptosis. The present findings demonstrated that estrogens have anti-proliferative and apoptosis-inducer effects in rat prostate *in vivo* likely by the down-regulation of the SCF/c-KIT system, which supports their protective role in development of PCa. *No conflict of interest.*

A4. P-cadherin expression: its role in the regulation of metabolic properties of breast cancer stem cells

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Introduction: Constitutive upregulation of glycolysis is likely to be a cellular adaption to hypoxic conditions, being recently recognized as a hallmark of cancer [1, 2]. In fact, hypoxia and consequent metabolic alterations have already been implicated in breast cancer progression [3]; additionally, it has been demonstrated that breast cancer stem cells use preferentially glycolysis over oxidative phosphorylation as their main source of energy [4], which make them resistant to hypoxia, as well as protect them from damage induced by mitochondrial-produced ROS (reactive oxygen species). P-cadherin, a biomarker of basal-like breast cancer and a poor prognostic factor in this disease [5], mediates stem-like properties, as well as resistance to radiation therapy [6]. Moreover, we have previously demonstrated that P--cadherin aberrant expression is associated with hypoxic, glycolytic and acidosis markers in breast carcinomas, that HIF-1α stabilization increases membrane P-cadherin expression and that P-cadherin enriched cell populations show increased GLUT1 and CAIX expression. These populations also comprise high mammosphere forming efficiency, suggesting that P--cadherin overexpressing breast cancer cells are more likely to exhibit increased glycolysis and to survive to metabolic--driven pH alterations [7]. In this work, our aim was to understand if P--cadherin expression could be regulating breast cancer stem cell's metabolic properties, which may explain its association with radiotherapy resistance and tumor aggressiveness. *Materials and Methods*: Using a siRNA-mediated approach, we silenced the expression of P-cadherin in breast cancer cells and evaluated the OCR/ECAR rate as a measure of OXPHOS and glycolysis, using the SEAHORSE technology. ATP measurements were performed using CellTiter-Glo Luminescent Cell Viability Assay kit and ROS content was evaluated by luminescence after incubation with DCHF-DA probe. Western blot and zymography was used to evaluate the state of ROS scavenging systems. *Results and Discussion*: We found, for the first time, that P-cadherin silencing was able to modulate cellular bioenergetics of breast cancer cells by allowing an increased OCR/ECAR rate and cellular ATP content. Furthermore, we demonstrated that P-cadherin expression is associated with the production of low ROS levels, by inducing the upregulation of ROS scavenging systems, such as SOD1 and SOD2 (superoxide dismutase 1 and 2). Although preliminary, we believe that the obtained data is demonstrating that P-cadherin might be a player of therapeutic resistance through the metabolic regulation of breast cancer stem cells.

No conflict of interest.

A5. Expression of ST3GAL4 leads to SLeX expression and induces c-Met activation and an invasive phenotype in gastric carcinoma cells

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Introduction: Sialyl-Lewis X (SLeX) is a sialylated glycan antigen frequently expressed on the cell surface during malignant cell transformation and is associated with cancer progression, aggressiveness and poor prognosis for the patients. Increased expression of SLeX antigen in cancer has been associated to alterations in the expression of sialyltransferases, though the exact molecular mechanism has not been completely understood. *Materials and Methods*: In this study we evaluated the capacity of two sialyltransferases to synthesize SLeX antigens in gastric carcinoma, by overexpressing ST3GAL3 and ST3GAL4 in MKN45 cells. In addition, we determined the role of SLeX expression in gastric cancer cell behavior both *in vitro* and *in vivo* using the chicken chorioallantoic membrane (CAM) model. Furthermore, we evaluated the activation of tyrosine kinase receptors and downstream molecular targets and described a possible molecular signaling mechanism involved in SLeX-induced behavior. Results: Our results showed that the expression of ST3GAL4 in MKN45 gastric cancer cells leads to the synthesis of SLeX antigens and to an increased invasive phenotype both *in vitro* and in the *in vivo*

CAM model. Analysis of phosphorylation of tyrosine kinase receptors showed a specific increase in c-Met activation. The characterization of downstream molecular targets of c-Met activation, involved in the invasive phenotype, revealed increased phosphorylation of FAK and Src proteins and activation of Cdc42, Rac1 and RhoA GTPases. The SLeX-induced invasive phenotype was associated to c-Met signaling since inhibition of c-Met and Src activation abolished the observed increased cell invasive phenotype. *Discussion:* In conclusion, the overexpression of ST3GAL4 leads to SLeX antigen expression in gastric cancer cells and induces an increased invasive phenotype through the activation of c-Met and downstream signaling activation. Reference: Gomes C, Osório H, Pinto MT, Campos D, Oliveira MJ, Reis CA. Expression of ST3GAL4 leads to SLe(x) expression and induces c-Met activation and an invasive phenotype in gastric carcinoma cells. PLoS One. 2013 Jun 14;8(6):e66737. doi: 10.1371/journal.pone.0066737. *Acknowledgements:* This work was supported by a grant from the Portuguese Foundation for Science and Technology (FCT), project grant PTDC/BBB-EBI/0786/2012, "financiado no âmbito do Programa Operacional Temático Factores de Competitividade (COMPETE) e comparticipado pelo fundo Comunitário Europeu FEDER". IPATIMUP is an Associate Laboratory of the Portuguese Ministry of Science, Technology and Higher Education, and is partially supported by FCT.

No conflict of interest

A6. P-cadherin represses cell-cell adhesion mediated by E-cadherin in breast cancer cells: an AFM analysis

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Introduction: E- and P-cadherins are cell-cell adhesion molecules crucial for the maintenance of normal epithelial tissue architecture. In epithelial cancers, E-cadherin acts as an invasion suppressor protein, being lost in highly infiltrative tumours. In contrast, P-cadherin is overexpressed in a significant fraction of carcinomas endogenously expressing E-cadherin, inducing collective cell invasion and acting as a repressor of the normal adhesion complex. Recently, we found that P-cadherin-induced invasion is dependent on Src-activity, which regulates the expression and trafficking of E-cadherin. Based on this data, our goals were: 1) to characterize the morphological and mechanical properties induced by P-cadherin overexpression in E-cadherin-positive cancer cells; and 2) to evaluate these properties in P-cadherin-overexpressing cells upon Dasatinib treatment, an inhibitor of Src activity. *Materials and Methods:* Two E-cadherin-positive breast cancer models have been used: MCF-7/AZ cell line, retrovirally transduced to encode P-cadherin cDNA (MCF.7/AZ.Mock and MCF-7/AZ.P-cad); and BT-20 cell line, endogenously expressing high levels of P-cadherin. BT20 were transfected with small interfering RNA (siRNA) against P-cadherin. Dasatininb treatment was applied with a final concentration of 100nM. Atomic force microscopy (AFM) was performed in both cell models, with and without Dasatinib treatment. Cells were scanned and AFM images were analysed, yielding maximum height, area and volume values for the different cell. Differences on cell stiffness and cell-cell adhesion forces were evaluated by AFM-based force spectroscopy. *Results and Discussion:* By AFM, we found that MCF-7/AZ.Pcad cells present significantly higher area and volume, as well as a decrease height. Accordingly, BT20 transfection with P-cadherin siRNA lead to a significant reduction of the cell's area and volume, and an increased height. MCF-7/AZ. Pcad cells also presented a lower Young's Modulus, which indicates higher cell elasticity, whereas P-cadherin silencing in BT-20 induced a significant increase in the Young's Modulus value, revealing a decreased elasticity. Interestingly, the treatment of P-cadherin-overexpressing cells with Dasatinib induced the same results in both models: a reduction in the cell's area and volume, as well as an increase in the cellular height and Young's Modulus (less elastic cells). Concerning cell-cell adhesion, we found that the work necessary to separate MCF-7/AZ.P-cad cells was significantly lower than for MCF-7/AZ.Mock cells. Accordingly, P-cadherin silencing in BT-20 cells induced increased values of work relative to the respective control. The same trend was obtained after Dasatinib treatment in both P-cadherin-overexpressing cell models. *Conclusions:* AFM measurements demonstrated that P-cadherin represses the normal cell-cell adhesion mediated by E-cadherin in cancer cells, justifying the increased invasive phenotype besides the cell-cell adhesion maintenance. These results are supported by the morphological characteristics adopted by P-cadherin-overexpressing cells, which present an increased cell volume and elasticity, parameters usually found in invasive cancer cells. The treatment with Dasatinib reverts the P-cadherin-induced phenotype, allowing cancer cells to adopt a more "epithelial-like" behaviour and being less invasive. References Albergaria, A., A. S. Ribeiro, A. F. Vieira, B. Sousa,

A. R. Nobre, R. Seruca, F. Schmitt and J. Paredes (2011). "P-cadherin role in normal breast development and cancer." Int J Dev Biol 55(7-9): 811-822. Allison, D. P., N. P. Mortensen, C. J. Sullivan and M. J. Doktycz (2010). "Atomic force microscopy of biological samples." Wiley Interdiscip Rev Nanomed Nanobiotechnol 2(6): 618-634. Carvalho, F. A., I. C. Martins and N. C. Santos (2013). "Atomic force microscopy and force spectroscopy on the assessment of protein folding and functionality." Arch Biochem Biophys 531(1-2): 116-127. Carvalho, F. A. and N. C. Santos (2012). "Atomic force microscopy-based force spectroscopy--biological and biomedical applications." IUBMB Life 64(6): 465-472. Paredes, J., A. L. Correia, A. S. Ribeiro, A. Albergaria, F. Milanezi and F. C. Schmitt (2007). "P-cadherin expression in breast cancer: a review." Breast Cancer Res 9(5): 214. Paredes, J., J. Figueiredo, A. Albergaria, P. Oliveira, J. Carvalho, A. S. Ribeiro, J. Caldeira, A. M. Costa, J. Simoes-Correia, M. J. Oliveira, H. Pinheiro, S. S. Pinho, R. Mateus, C. A. Reis, M. Leite, M. S. Fernandes, F. Schmitt, F. Carneiro, C. Figueiredo, C. Oliveira and R. Seruca (2012). "Epithelial E- and P-cadherins: role and clinical significance in cancer." Biochim Biophys Acta 1826(2): 297-311. Ribeiro, A. S., B. Sousa, L. Carreto, N. Mendes, A. R. Nobre, S. Ricardo, A. Albergaria, J. F. Cameselle-Teijeiro, R. Gerhard, O. Soderberg, R. Seruca, M. A. Santos, F. Schmitt and J. Paredes (2013). "P-cadherin functional role is dependent on E-cadherin cellular context: a proof of concept using the breast cancer model." J Pathol 229(5): 705-718. van Roy, F. and G. Berx (2008). "The cell-cell adhesion molecule E-cadherin." Cell Mol Life Sci 65(23): 3756-3788.

No conflict of interest

A7. The mechanisms underlying the regulation of normal breast epithelial architecture mediated by P-cadherin expression

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Introduction P-cadherin has important tumour promoting properties in breast cancer. However, in the normal breast, P-cadherin behaves as a classic cell-cell adhesion molecule, being important for the maintenance of normal tissue architecture, especially in the myoepithelial cell compartment. Studies in P-cadherin knockout mice have shown that loss of P-cadherin causes hyperplasia and dysplasia, as well as precocious branching morphogenesis of the mammary gland; though, the specific mechanisms underlying the regulation of normal breast epithelial architecture mediated by P-cadherin expression have not been explored. *Materials and Methods* 3D matrigel cultures were used to access the organization of the organoids derived from normal breast cell lines. siRNA transfection was used to knock-down P-cadherin. Western blot was used to evaluate the expression of P-cadherin, tight junction proteins and polarity determinants. Nocodazole and taxol were used as microtubule (MT) perturbing drugs that alter cell polarity. *Results and Discussion* Using 2D and 3D cultures of normal breast cell lines (MCF10A and 226L), we have found that P-cadherin expression is implicated in the control of cell-cell and cell-matrix adhesion, as well as with epithelial cell polarity. Both MCF10A and 226L cells form spherical and compact colonies in 3D matrigel cultures. However, P-cadherin knock-down increased the disorganization and the size of the organoids that were formed by these cells. Our data still showed that P-cadherin silencing reduces Zona 0ccludens (ZO)-1 and ZO-2 expression, as well as Claudin-3 expression. Additionally, we found that P-cadherin inhibition decreased pAKt and pSrc signaling as well as a6b4 integrin expression, an heterodimer that is crucial for the interaction of the normal epithelial cells with the basement membrane components. In fact, we found that a6 integrin knock-down caused acini disorganization in 3D matrigel. Analysis of the polarity determinants Par3, aPKC and Scribble showed no difference in the expression of after P-cadherin inhibition. Furthermore, preliminary data show that there is a link between P-cadherin expression and the MT network, since the treatment with MT perturbing drugs (nocodazole or taxol) significantly increased P-cadherin and Claudin 3 expression in MCF10A cells. Further studies are needed to clarify the link between P-cadherin/tight junctions/a6b4 integrin/basement membrane, and possibly the MT network, in determining epithelial cell polarity and tissue architecture of normal breast. Understanding the role of P-cadherin expression in the basal layer of the normal breast may help clarify the progression of pre-neoplastic lesions, such as hyperplasia, as well as the alterations in the stem cell niche that may lead to the precocious differentiation of the mammary gland in the P-cadherin knockout mice.

No conflict of interest.

A8. Patterns of protein expression and cytogenetic alterations in meningiomas

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Introduction: Meningiomas are central nervous system tumors known to contain different types of infiltrating cells. The aim of this study is to investigate the cellular composition of meningiomas, as well as the impact of cytogenetic alterations on the protein expression profile of those individual cell populations coexisting within the tumor, in order to better understand its biology at both the tumor cell and microenvironmental levels. *Material and Methods*: Multiparameter flow cytometry (MFC) was used to evaluate the immunophenotypic profile of individual cell populations, using a large panel of markers together with MFC sorting as well as morphologic, cytogenetic and phagocytic/endocytic analyses, and the Affymetrix U133A chip was applied for gene expression profiling (GEP); all meningioma samples (n=75) were cytogenetically characterized by interphase fluorescence in situ hybridization. *Results and Discussion*: Overall, coexistence of CD45- neoplastic cells and CD45+ immune infiltrating cells was systematically detected among the meningioma samples. Infiltrating cells included a major population of tissue macrophages (TiMa), with an HLA-DR+CD14+CD45+CD68+CD16-/+CD33-/+ phenotype and high phagocytic/endocytic activity, together with lymphocytes (mostly T CD8+- and NK-cells) at lower levels. Our results further showed a close association between these infiltrating immune cells and both the cytogenetic patterns and GEP of tumor cells. Accordingly, meningiomas with isolated monosomy 22/del(22q) had greater numbers of TiMa (with a more activated and functionally mature phenotype), NK cells and (recently)-activated CD69+ lymphocytes, together with a unique GEP characterized by an increased expression of genes involved in inflammatory/immune responses, which may contribute to explain the better outcome of this cytogenetic subgroup of meningiomas. Of note, high levels of TiMa with a polarization towards an M2-phenotype (CD206+ cells) in association with infiltration by regulatory T cells was found in some meningiomas with complex karyotypes, which might help to explain the greater recurrence rate of these tumors. In addition, the cytogenetic profile of meningiomas was also closely associated with the pattern of protein expression of tumor cells. Thus, diploid meningiomas displayed higher levels of expression of the CD55 complement regulatory protein, tumors carrying isolated monosomy 22/del(22q) showed greater levels of bcl2 and PDGFRβ and meningiomas with complex karyotypes displayed a greater proliferation index as well as decreased expression of the CD13 ectoenzyme, the CD9 and CD81 tetraspanins, and the Her2/neu growth factor receptor. In conclusion, here we propose a MFC-based strategy to identify and characterize the different cell populations coexisting in meningiomas, and their patterns of protein expression, both parameters being closely associated with tumor cytogenetics, suggesting the involvement of different signaling pathways in the distinct cytogenetic subgroups of meningiomas and contributing to explain the close association between tumor cytogenetics and patient outcome.

No conflict of interest.

A9. CCR7 expression drives migration and metastization of leukemic T cells

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Introduction: T-cell acute lymphoblastic leukemia (T-ALL) is caused by the malignant transformation of immature T-cells (thymocytes), which become arrested in differentiation, proliferate and disseminate to several body locations. Chemokines are secreted peptides that form gradients in tissues and thus recruit migrating cells expressing the cognate receptors. The CCR7 chemokine receptor plays important roles not only in T-cell development and function but also in cancer cell survival, migration, invasion and metastization. The aim of this study was to investigate the role of CCR7 in T-ALL. *Materials and Methods*: T-ALL cell lines; RT-qPCR; Flow cytometry; Transwell migration assays; TEL-JAK2 transgenic mice; CCR7 KO mice. *Results and Discussion*:

To verify whether CCR7 is involved in T-cell malignant transformation, we have assessed the CCR7 mRNA expression levels by quantitative RT-PCR in several T-ALL cell lines. By doing so, we have found that about 50% of the cell lines tested expressed CCR7 transcripts. Cell surface CCR7 protein expression was confirmed by flow cytometry. Supporting the notion that CCR7 is functional in T-ALL cells, we found that these cells migrated *in vitro* in response to recombinant CCL19 or CCL21, the CCR7 ligands. To study the role of CCR7 *in vivo*, we bred the TEL-JAK2 transgenic mouse model of T-ALL with CCR7 KO mice. Follow-up of double transgenic cohorts showed that CCR7 inactivation did not prevent leukemia onset. However, CCR7-deficient TEL-JAK2 mice presented larger thymic lymphomas and reduced splenic and lymph node involvement, as compared to littermates. These results indicate that CCR7 expression is important for dissemination of leukemic cells from the organ of origin, the thymus, to other organs. Altogether, these results indicate that CCR7 should be considered as a molecular therapeutic target to prevent leukemic cell metastization.

No conflict of interest.

A10. P-cadherin and resistance to DNA damage in normal and breast cancer cells

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Introduction: Our group has shown that P-cadherin is a cancer stem cell biomarker with direct relevance in the stem cell activity of basal like breast cancer cells. Importantly, we found that P-cadherin expression confers resistance of the stem cells to X-ray-induced cell death, however the mechanisms involved in this resistance are not yet clarified (Vieira et al., 2012). *Materials and Methods*: In an attempt to unravel the role of P-cadherin in the DNA damage resistance, we performed the downregulation of this protein using siRNA and exposed MCF10A (normal-like) cells and BT-20 (breast cancer) cells to different DNA stress-inducing stimuli, namely, hydrogen peroxide (H_2O_2), UV-C light (UV) and Taxol. We performed the mammosphere assay in order to study the impact of concomitant DNA damage and P-cadherin in stem cell activity. We analyzed the DNA-damage response/repair pathway (DDR/R) through the evaluation of the expression of Chk1 and Chk2 proteins by WB, as well as the DNA breaks with the gamma-H2AX by IF and with the alkaline single cell gel electrophoresis assay (comet assay). To further confirm the role of P-cadherin in cell death resistance we performed the PI / Annexin V assay. *Results and Discussion* Both cell lines were very sensitive to UV irradiation exhibiting elevated cell death. The treatments with H202 and Taxol showed a significant reduction in the stem cell population (measured by the Mammosphere assay), which after P-cadherin knock-down we found a tendency to decrease further in both cell lines, MCF10A and BT-20. We found that H202, UV light and - to a lower extent - Taxol were able to induce several players of the DDR/R pathway by WB analysis and we observed that P-cadherin inhibition had an impact in the expression of these molecules. Importantly, the three DNA stress-inducing agents increased DNA damage (y-H2AX nuclear foci and comet assay), which was further increased by P-cadherin silencing in both cell lines MCF10A and BT-20. Cell death was increased by P-cadherin silencing in the presence of H202 and Taxol in both cell lines MCF10A and BT-20 and in the absence of any stimuli in normal-like cell line - MCF10A. Herewith, we show that P-cadherin has a role in the DDR/R pathway, promoting DNA damage resistance and consequently cell death resistance. Although further experiments need to be performed, our work points to the idea that breast cancer patients undergoing radiation therapy could potentially benefit from a P-cadherin inhibition strategy.

No conflict of interest.

A11. Proteome analysis in laryngeal squamous cell carcinoma

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Introduction: Laryngeal cancer represents about 25% of malignant tumors affecting this area and 2% of malignancies. It occurs preferentially in women, being one of the most frequents tumors of the head and neck. It can occur in the: supraglottis, glottis and subglottis. Approximately two thirds of the tumors arising in the true vocal cord, located at the glottis and supraglottic can done third is located above the vocal cords. The most common histological type and achieves more than 90% of patients, is the squamous cell carcinoma (SCC). The SCC is classified according to the degree of cell differentiation. Gene expression studies have been conducted in various tumors to allow some of the proteins involved in tumor development as well as to determine severity markers. The objective of the present study was verify the proteomic context in tumor and normal cells of SCC larynx patients. *Materials and Methods*: After to collect the cancer sample, the cancer confirmation was made by hematoxylin-eosin. There after the cells were selected and captured in Laser Mircodissection and the proteomic analysis was performed using the mass spectrometer coupled in liquid chromatograph. Statistical test (t test) was applied and used a software to process the analysis; standardization was made in another software and compared to the database of the Human UniProt, which has 88429 reads and 35,079,223 residues. *Results and Discussion*: Eleven patients were evaluated (normal and tumor tissue) and were matched for age and sex. In the group of males with an average age of 56 without chemotherapy and radiotherapy, were isolate 1057 proteins in normal tissue and 1088 in tumor tissue. After clustering analysis, with p <0.05, were isolated 81 proteins, 38 with higher expression in tumor tissue and 43 with lower expression in the tumor tissue. In the future, these proteins should be analyzed by their function, and could be candidates for biological markers in laryngeal squamous cell carcinoma.

No conflict of interest.

A12. TCTEX1D4: a PPP1-interacting protein possibly involved in prostate carcinogenesis

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Introduction: Prostate cancer (PCa) is one of the most prevalent disorders in elderly men worldwide. Alterations in several cell signalling pathways (e.g.: TGFβ, EGF, and PDGF pathways) represent key aspects of PCa development and sustainability. Phosphoprotein phosphatase 1 (PPP1) is a major serine/threonine protein phosphatase which regulates numerous cellular processes. PPP1 activity is mainly regulated by its interacting proteins (PIPs), which may regulate its subcellular localization and substrate affinity. T-complex testis expressed protein 1 domain containing 4 (TCTEX1D4) is a dynein light chain protein that was recently identified as a PIP. TCTEX1D4 was firstly identified as an interactor of several mediators of the TGFβ signalling pathway and it may also play a role in cell-to-cell junctions, microtubule dynamics, cell proliferation, and cell differentiation. Evidences suggest that TCTEX1D4 dephosphorylation is critical for its regulation but the functions of TCTEX1D4 itself and the TCTEX1D4/PPP1 complex remain to be elucidated. In this study, we characterized TCTEX1D4 and the complex that it forms with PPP1 in human normal prostate and PCa cells, as well as analysed the role of TCTEX1D4/PPP1 complex in cell proliferation and migration. Methods: The experiments performed in this study were conducted in 4 human prostate cell lines: RWPE-1, normal-like cells; PNT-2, preneoplasic cell; LNCaP, androgen-dependent PCa cells; and, PC-3, androgen-independent PCa cells. The expression levels of PPP1 and TCTEX1D4 were evaluated by Western blotting and their subcellular localization by immunocytochemistry. Wild-type and mutant plasmids (with impaired PPP1 binding site) were transfected in order to determine the role of the complex TCTEX1D4/PPP1 in cell proliferation and migration. *Results and Discussion*: The expression levels of both TCTEX1D4 and PPP1 increase with malignancy. Alterations in their subcellular localization were also identified. In normal cells—RWPE-1 and PNT2—TCTEX1D4 and PPP1 are dispersed throughout the cytoplasm and nucleus, showing a fine punctuate pattern, whereas in malignant cells—LNCaP and PC3—TCTEX1D4 is heavily restricted to large clusters within the cytoplasm and PPP1 is mostly confined to the nucleus. TCTEX1D4 overexpression decreases the proliferation of PNT-2 and LNCaP cells and this effect seems to be dependent on its interaction with PPP1. No significant data was obtained for cell migration.
No conflict of interest.

A13. Analysis of the estrogen receptor interactome in tamoxifen sensitive and resistant mouse mammary M05 carcinoma

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Introduction: Two-thirds of all breast cancers diagnosed are estrogen receptor alpha (ERα) positive which is the primary target for endocrine therapies. Currently, tamoxifen (TAM), a partial antagonist of ERα, is the most successful targeted antiestrogen treatment accessible to early and advanced breast cancer patients. Despite that, it has been observed high rates of TAM relapse and also its ineffectiveness as a first line treatment in breast cancer, due to acquired TAM resistance. Thus, we aim to investigate the nature of the proteins which interact with ERs and as result, model the mechanisms of action involved not only in the promotion and progression of breast cancer, but also in the development of the endocrine resistance to therapy. *Materials and Methods*: ERα activity and expression was evaluated by western blot, qPCR and immunofluorescence on the spontaneous hormone-dependent mouse mammary tumour (M05), which is positive for ERα, 17β-estradiol (E2)-dependent and sensitive to TAM (M05-S) which develops TAM resistance (M05-R) from passage 10. Human T47-D breast cancer cells were also used. ERα interacting proteins were identified following estradiol affinity isolation by LC-MS/MS. *Results and Discussion*: ERα expression in M05-S and M05-R tumours at both protein and transcript level were higher relative in M05-S (n=6, p<0,05). Surprisingly, the transcriptional activity of ERα measured by the expression of its target genes, complement C3 and progesterone receptor (PR), was not significantly different between M05-S and M05-R tumours. Suggesting that even though M05-R tumours have lower ERα levels, it is more transcriptionally active. This was confirmed by co-culturing T47-D breast cancer cells in the prescence of M05-S and M05-R extracellular matrix (ECM), which showed more ERα activating phosphorylations in cells grown in M05-R ECM. Finally, 42 proteins were found associated to ERα in M05-S and 10 proteins in M05-R tumours. In M05-S, ERα was associated to proteins involved in: regulation of fibril organization and DNA methylation, ribosome biosynthesis, fatty acids beta-oxidation and in general with RNA processing and translation. In M05-R, the ERα interactome was mainly associated to chromatin remodelling and translational elongation. The results obtained indicate that TAM resistance is accompanied by proteins regulating different signalling pathways associated with ERα.

No conflict of interest.

A14. Immunohistochemical molecular phenotypes of gastric cancer based on SOX2 and CDX2 predict patient outcome

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Introduction: Gastric cancer remains a serious health concern worldwide. Patients would greatly benefit from the discovery of new biomarkers that predict outcome more accurately and allow better treatment and follow-up decisions. Here, we used a retrospective, observational study to assess the expression and prognostic value of the transcription factors SOX2 and CDX2 in gastric cancer. *Materials and Methods*: SOX2, CDX2, MUC5AC and MUC2 expression were assessed in 201 gastric tumors by immunohistochemistry. SOX2 and CDX2 expression were crossed with clinicopathological and follow-up data to determine their impact on tumor behavior and outcome. Moreover, SOX2 locus copy number status was assessed by FISH (N=21) and Copy Number Variation Assay (N=62). Results: SOX2 was expressed in 52% of the gastric tumors and was significantly associated with male gender, T stage and N stage. Moreover, SOX2 expression predicted poorer patient

survival, and the combination with CDX2 defined two molecular phenotypes, SOX2+CDX2- versus SOX2-CDX2+, that predict the worst and the best long-term patients' outcome. These profiles combined with clinicopathological parameters stratify the prognosis of patients with intestinal and expanding tumors and in those without signs of venous invasion. Finally, SOX2 locus copy number gains were found in 93% of the samples reaching the amplification threshold in 14% and significantly associating with protein expression. Discussion: We showed, for the first time, that SOX2 combined with CDX2 expression profile in gastric cancer segregate patients into different prognostic groups, complementing the clinicopathological information. We further demonstrate a molecular mechanism for SOX2 expression in a subset of gastric cancer cases.

No conflict of interest.

A15. Cross-talk between the Src proto-oncogene and F-actin in the acquisition of the pre-malignant phenotype.

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Introduction The c-Src non-receptor tyrosine kinase is one of the most investigated proto-oncogenes implicated in a large number of cancers, including those of the breast. However, we still have an incomplete picture of how misregulation of Src contributes to tumour development and progression. Basal Src activity that occurs early during tumour progression is believed to promote cell proliferation and survival, further in later stages Src activation may facilitate cell migration, adhesion and invasion via the control of F-actin. We have reported that in Drosophila epithelia, Src activation affects F-actin levels, which in turn, control Src-dependent cell proliferation and apoptosis. *Materials and Methods* To explore the role of the actin cytoskeleton in the early stages of tumourigenesis, we searched for actin regulators controlled by Src activation that might be relevant for the acquisition of the pre-malignant phenotype of breast tumours. We used microarray data from pre-malignant Estrogen-positive (ER+) breast tumour samples and from a cell line derived from normal mammary epithelial cells, in which Src activation was induced (MCF10A-ER-Src). After normalization of microarray raw data using fRMA and ComBat methods and statistical comparison, we classified differentially expressed genes into pathways using Pathway-Express and screened for actin regulators (ABPs) whose expression was affected in both. Because ABPs are functionally conserved between species and Src activation shows similar effects in Drosophila epithelia and in mammalian cell culture systems, we investigated the effect of knocking down or overexpressing each Drosophila orthologs of our candidate ABPs in Drosophila epithelia that contained higher Src levels. *Results and Discussion* Strikingly, regulation of the actin cytoskeleton is one of the most significant biological processes affected in pre-malignant cells, suggesting that changes in the actin cytoskeleton is a key step toward the acquisition of the pre-malignant phenotypes. Moreover, non-invasive and invasive breast tumours show distinct F-actin machinery. Interestingly, among the 27 ABPs dysregulated in non-invasive breast tumours, ARPC5L, ACTR3, DST, TPM2 and EVL are also misregulated by Src induction in MCF10A cells and affect the ability of Src to promote tissue growth in Drosophila epithelia, with the Drosophila orthologs of ARPC5L and ACTR3 acting as tumour suppressors, while Drosophila DST, TPM2 and EVL have oncogenic abilities. Taken together, our findings argue that in cancer cells, the control of ARPC5L, ACTR3, DST, TPM2 and EVL by Src activation regulates cell proliferation and survival of pre-malignant cells via F-actin regulation, suggesting that a specialized F-actin machinery built by Src triggers and sustains tumourigenesis.

No conflict of interest.

A16. IL1B signaling leads to increased cell survival of gastric carcinoma cells

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Introduction: Polymorphisms in inflammation-related genes have been associated with risk to gastric carcinoma (GC). However, the biological mechanisms underlying these associations are still elusive. Our objective was to determine whether chronic inflammation-associated IL1B signalling, as seen in the context of Helicobacter pylori infection, can be linked to gastric carcinogenesis by modulating the behaviour of gastric epithelial cells. *Materials and Methods*: The effect of IL1B was assessed by studying the expression and activation status of the IL1B-activated transcription factors C/EBPβ and CREB in GC cell lines. Interaction between CREB and C/EBPβ was explored through interference RNA, chromatin immunoprecipitation and chemical inhibition. CREB and C/EBPβ expression was analysed in 66 samples of primary GC and in normal gastric mucosa. GC cells growth was analysed *in vitro* by BrdU incorporation and *in vivo* employing a chicken embryo chorioallantoic membrane model. *Results and Discussion*: We found that IL1B regulates the expression/activation status of both C/EBPβ and CREB in GC cells through an ERK1/2-dependent mechanism. Our results show that CREB is a direct transactivator of CEBPB, acting as an upstream effector in this regulatory mechanism. Furthermore, we found CREB to be over-expressed in 94% of GC samples and significantly associated with C/EBPβ expression (P<0.05). Finally, we demonstrate both *in vitro* and *in vivo* that CREB can mediate IL1B-induced GC cell proliferation. Our results support the hypothesis that the effect of chronic inflammation on gastric carcinogenesis, as seen in the context of genetically susceptible individuals infected with H. pylori, includes modulation of signalling pathways that regulate survival mechanisms in epithelial cells.

No conflict of interest.

A17. Effect of 2D and 3D growth conditions on expression of signalling GTPase Rac1b in colorectal cells

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Introduction: Rac1 is a member of the Rho-family of small GTPases, which stimulates signalling pathways involved in the control of actin filament dynamics and transcriptional activation. We previously identified that Rac1b, an alternative splicing variant containing an extra exon, is overexpressed in a subset of B-Raf-mutated colorectal tumours. Although the Rac1b protein is expressed at low levels in cells, it is mostly in its active GTP-bound state and stimulates the transcription factor NFkB without affecting other classical Rac1 signalling pathways (such as lamellipodium formation or activation of the kinases PAK or JNK). Experimental depletion of Rac1b revealed an essential role in cell cycle progression and survival of colorectal cancer cells when cultured under 2D conditions. *Materials and Methods* Caco-2 colorectal cells were grown as either monolayers on plastic dishes, as polarized epithelia on filter inserts or as 3D spheroids in Matrigel. Rac1b expression was analysed by RT-PCR, Western blot and confocal fluorescence microscopy. *Results and Discussion*: Different cell culture conditions have been described to affect signalling pathway organization. Therefore, we compared mRNA and protein expression levels and subcellular localization of Rac1b in Caco-2 cells grown as monolayers, polarized epithelia or 3D spheroids. Whereas cell polarization did not affect overall Rac1b levels, the presence of fibroblasts in co-culture with polarized Caco-2 revealed progressive loss of epithelial organization, a transient increase in Rac1b protein levels and release from the plasma membrane. Furthermore, growth in 3D revealed significantly lower Rac1b protein expression. These data indicate that extracellular signals can regulate Rac1b production and intracellular localization in colorectal cancer cells.

No conflict of interest.

A18. Hypoxia mediated up-regulation of the plasminogen receptor S100A10 in cancer cells

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Introduction: A key characteristic of a cancer cell is its ability to escape the constraints imposed by the neighboring cells, invade the surrounding tissue and metastasize to distant sites. During initial tumor

development cancer cells adapt to survive in a hypoxic environment as a consequence of their uncontrolled proliferation in conjunction with a restricted blood supply limiting nutrients and oxygen. The hypoxia response leads to the activation of multiple signalling pathways that promote cancer cell invasion and metastasis. The serine protease plasmin is a key protease that participates in fibrinolysis, extracellular matrix degradation, invasion and angiogenesis (the development of blood vessels). As a component of the annexin A2 heterotetramer (AIIt), S100A10 is an important plasminogen receptor that contributes significantly to plasmin activation at the surface of a number of different cell types, including cancer cells. However, the regulation of S100A10 in cancer cells during hypoxia has not been investigated. *Materials and Methods*: I have used several cancer cell lines, namely MDA MB231 breast cancer cells, HT1080 fibrosarcoma cells and A549 lung cancer cells and compared the protein expression levels of S100A10 in normoxic versus hypoxic conditions by western blotting. I have analysed the cell surface expression of S100A10 protein in MDA MB231 cells under normoxic versus hypoxic conditions using immunofluorescence microscopy. I have investigated annexin A2 and S100A10 gene transcription by qRT-PCR in order to discern how S100A10 is regulated resulting in an increased expression of S100A10 during hypoxia. Finally I have investigated the capacity of hypoxic cancer cells to activate plasmin in the presence or absence of S100A10, using S100A10 knockdown cells. *Results and Discussion*: My results revealed that when exposed to a hypoxic environment there is an increase in transcription of S100A10 gene that resulted in the up-regulation of S100A10 protein expression and the translocation of the AIIt complex to the cell surface. My plasmin assays showed an increase in plasmin activation in the control cancer cells (expressing normal levels of S100A10) which was not observed when cells were depleted of S100A10; these results indicate that S100A10 plays a significant role in the production of plasmin at the surface of cancer cells during hypoxia. Keywords: Annexin A2 heterotetramer, hypoxia, plasmin, S100A10. *This research work has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° PCOFUND-GA-2009-246542 and from the Foundation for Science and Technology of Portugal (FCT).*

No conflict of interest.

A19. Decrease Lactic acid export via basigin disruption sensitizes A549 cells to phenformin

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Introduction: Most cancer and particularly those programmed for rapid growth rely on aerobic glycolysis to generate the energy needed, a phenomenon termed "Warburg effect". To maintain high glycolytic rates, cells must efficiently export lactic acid through members of a family of proton-coupled monocarboxylate transporters (MCT1/4). These transports require a chaperone, Basigin (Bsg), for trafficking to the plasma membrane and exert their activity. There are some reports showing upregulation of MCTs in lung tumours and experimental evidence point at MCTs as potential targets for cancer therapy. Therefore, we would like to understand the functional role of MCTs in lung tumours. Thus, we propose a series of experiments aiming to understand the effect of MCTs inhibition by disrupting Bsg. *Materials and Methods*: MCT1, MCT4 and Bsg expression levels were assessed by immunohistochemistry in a series of 50 lung cancer samples. A549 lung carcinoma cell line was transfected with Zinc finger nuclease (ZFN)-mediated Bsg Knock-out. Bsg negative cells were selected by FACS and characterized by Western Blot. The effect of Bsg disruption was assessed using *in vitro* and *in vivo* models, *Results and Discussion*: Firstly, we analysed the expression of MCT1, MCT4 and Bsg in human lung cancer samples and we observed that all proteins were upregulated in tumour tissues when compared to non-tumoural tissue. Targeting bsg in A549 cells, via ZFNs, we successfully isolated cells knocked out for Bsg, decreasing the expression and activity of both MCT1 and MCT4 by respectively 95 and 80%. We noticed that A549 cells grown in normoxia have a moderate rate of glycolysis, which is reduced by 2-fold in Bsg-null cells. Both wild type and Bsg-null are extremely sensitive to the mitochondria inhibitor phenformin in normoxia. However, only Bsg-null cells remain sensitive to phenformin in hypoxia (1% O2) for cell proliferation

in vitro, and tumour growth in nude mice. Our results demonstrated that inhibiting MCTs via Bsg disruption sensitized A459 tumour cells to phenformin-induced cell death. *No conflict of interest.*

A20. 3-Bromopyruvate induces citotoxicity and inhibits glycolysis and migratory capacity in glioblastomas and breast cancer

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Introduction: The majority of tumor cells presents a metabolic reprogramming, changing to a glycolytic phenotype, even under aerobic conditions, which is named "Warburg effect". 3-bromopyruvate (3-BP) is an antitumor drug, targeting glucose metabolism and inhibiting energy production in tumor cells, both at glycolytic and mitochondrial level. Monocarboxylate transporters (MCTs) maintain these glycolytic rates, mediating the efflux of lactate and protons, being overexpressed in several cancer cell types. 3-BP cytotoxic effect was assessed and correlated with the metabolic activity of the cells and with MCTs expression/activity/ genetic variability. The cytotoxic effect of 3-BP was described for several different types of cancer, like pancreatic, hepatic or melanoma, among others. We investigated the effect of 3-BP in glioblastoma, a very agressive cancer, with a high glycolytic rate and in breast cancer, the most common cancer type in women. *Materials and Methods* In the present study, we aimed at determining the effect of 3-BP in cell viability (using SRB assay), migratory capacity (using wound healing assay) and metabolism (determining lactate production and glucose consumption) in gliomas and breast cancer cells. We also aim to evaluate the putative role of MCTs genetic variability and activity on 3-BP effect, by HRM and expression assays. *Results and Discussion*: Our results showed that the cells lines used presented different sensitivities to 3-BP, being more oxidative cells more resistant to the compound. We also demonstrated that 3-BP reduced glucose consumption, lactate production and migratory capacity in the cell lines. Regarding genetic variability, different genotypes were observed on the tested cell lines, concerning polymorphisms 1470T>A in the gene SLC16A1and 44C>T in the gene SLC16A3, reported as having influence in MCT1 and MCT4 activity, respectively [1-2]. Expression studies to associate MCTs expression with their genotype and with 3-BP toxic effect are underway. This work was supported by the CESPU project 02-GBMC--CICS-2011 References: 1.Lean, Choo Bee and Lee, Edmund Jon Deoon, Genetic Variations of the MCT4 (SLC16A3) Gene in the Chinese and Indian Populations of Singapore. Drug Metabolism and Pharmacokinetics, vol. 27 (2012) no. 4 p. 456-464 2.Sawczuk, M and Banting, LK et al, MCT1 A1470T: A novel polymorphism for sprint performance, 2014 Jan 1. pii: S1440-2440(13)00525-2

No conflict of interest.

A21. Targeting P-cadherin/Src induced mechanotransduction signaling:dasatinib as a promising therapeutic approach in breast cancer

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INTRODUCTION: P-cadherin (Pcad) belongs to the classical cadherin family of proteins and interacts intracellularly with the catenin family proteins. Its expression is mostly found in basal–like tumors, which is a subgroup of breast carcinomas of high histological grade and poor patient survival and with no specific target therapy to date. Pcad has a key role in acquired cancer hallmarks, since its overexpression in breast cancer cells promotes *in vitro* cell migration, invasion and increased self-renew potential. These *in vitro* effects are in part due to the inhibition of the Ecad suppressive invasive function, by inducing the disruption of the Ecad/p120catenin complex at the cell membrane. It is well known that Src activity regulates the expression and trafficking of Ecad, as well as p120ctn delocalization to the cytoplasm, being strongly implicated in cancer cell invasion, tumor initiation and metastasis in several cancer models. Based on these, we hypothesized that the mechanism behind Pcad`s function in breast cancer progression depends on Src kinase activation. *MATERIALS*

AND METHODS: Two different breast cancer cell models were used where Pcad expression was manipulated (MCF-7/AZ and BT20. Immunofluorescence and Western blot were used to assess proteins localization and expression. *In vitro* functional assays were performed to measure cell migration and invasion capacity. Protein-protein interactions between cadherins and catenins were evaluated by in situ proximity-ligation assay (PLA). Atomic force microscopy was used to characterize the biomechanical properties of breast cancer cells. *In vivo* experiments included BT20 cells inoculation in nude mice treated with dasatinib. *RESULTS AND DISCUSSION*: We demonstrated that Pcad overexpressing cells show an increased pSrc expression, delocalization of p120ctn to the cytoplasm and an increase in Rac1 activity. Along with the activation of this pathway, striking changes in the biomechanical properties were observed, as Pcad expression turned cells more flat, elastic and less cohesive, in accordance with the invasive behavior of cancer cells. Moreover, the functional changes observed in Pcad overexpressing cells were counteracted after Src inhibition with dasatinib, leading to a decrease in cell migration, cell invasion, self-new potential, but also in a full recovery of the biomechanical properties of these cells. Additionally, using PLA, a concomitant stabilization of the Ecad/p120ctn complex to the cell membrane was observed, promoting a very significant recover of a more epithelial-like phenotype, further confirmed in tumors from nude mice treated with dasatinib. This work presents a new cellular mechanism that explains why Pcad expression is associated with a poor patient survival in breast cancer, and opens new therapeutic approaches, using dasatinib, to treat these tumors.

No conflict of interest.

A22. Role of beta-blockers in ovarian cancer cells biology

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Human ovarian cancer is the seventh most common tumour in women worldwide and the leading cause of death from gynaecological cancers. Nowadays, there is strong evidence that stress affect cancer progression and patient survival. However, the underlying mechanisms of this association are poorly understood. The catecholamines (CA), adrenaline (AD) and noradrenaline (NA), are released in response to stress, exerting their effects through interaction with adrenergic receptors (AR) termed α and β. β-AR expression has been identified on several ovarian cancer cells. The activation of these receptors triggers several pathways that alter tumour microenvironment, being associated to carcinogenesis and tumour progression. β-blockers have a long history of use for treatment of arrhythmia, hypertension, anxiety, and heart failure treatment, among others. Epidemiological studies show that β-blockers are associated with a decrease of cancer mortality and studies about the efficiency of β-AR as a possible treatment for cancer are acquiring strength. The effects of several β-blockers with distinct intracellular target profiles in human ovarian cancer cells SKOV-3 biology were investigated. Cellular proliferation and migration ability after exposure to the agonists AD, NA and isoprenaline (ISO) and the antagonists propranolol, carvedilol, atenolol and ICI 118,551 per se, or combined with each other, was investigated. AD was able to significantly increase the proliferation of SKOV-3 cells, ISO induced a tendency to increase proliferation, whereas NA had no effect. Our results suggest that β-blockers reduce SKOV-3 cells proliferation, but, similarly to the tested agonists, have no effect on SKOV-3 migration. This study might contribute to elucidate which are the most effective β-blockers in reverting CA induced proliferative effects in ovarian cancer cells and, consequently, to be used as promising strategies in cancer treatment. Supported by: RES4MED – Aprender medicina através da investigação, (EXPL/IVC-PEC/1302/2013), funded by national funds through FCT/MEC (PIDDAC) and cofunded by Fundo Europeu de Desenvolvimento Regional (FEDER) através do COMPETE – Programa Operacional Fatores de Competitividade (POFC), referenced FCOMP-01-0124-FEDER-041872. Marisa Coelho is a recipient of a grant from Liga Portuguesa contra o Cancro (LPCC/GPECO 2013)

No conflict of interest.

A23. Targeting lactate transport suppresses *in vivo* breast tumor growth

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Introduction Acidification of tumour microenvironment by lactate extrusion, performed by up-regulation of lactate transporters (MCTs) has been associated with higher cell proliferation, migration, invasion, angiogenesis and increased cell survival. Previous results from our group showed up-regulation of MCT1 in breast carcinoma samples and demonstrated the importance of *in vitro* MCT inhibition, however additional studies are needed to validate the potential of lactate transport inhibition for cancer therapy. Therefore, this work aimed to evaluate the effect of MCT silencing in *in vitro* and *in vivo* breast cancer models. *Materials and Methods* The effect of MCT1, MCT4 or MCT1 plus MCT4 silencing on lactate efflux, proliferation, cell biomass, migration and invasion was evaluated in MDA-MB-468, MDA-MB-231 and BT20 breast cancer cell lines, under normoxia and hypoxia conditions. Also, silenced cells were used to induce tumor xenografts in nude mice. Results Our results showed a decrease in *in vitro* tumour cell aggressiveness reducing lactate transport, cell proliferation, migration and invasion and importantly inhibited *in vivo* tumour formation and growth. Discussion This work demonstrates the contribution of MCTs for cancer cell aggressiveness and, more importantly, shows, for the first time, the disruption of *in vivo* breast tumour growth by targeting lactate transport. This work was supported by FCT fellowships (SFRH/BPD/69479/2010 and SFRH/BD/87139/2012) and FCT grant (PTDC/SAU-FCF/104347/2008, under the scope of "Programa Operacional Temático Factores de Competitividade" (COMPETE) of "Quadro Comunitário de Apoio III" and co-financed by Fundo Comunitário Europeu FEDER).

No conflict of interest.

A24. Expression of ALDH and p53 in breast cancer stem cells

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

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downregulated but maintains a similar expression in MM-derived cells. The upregulation of ALDH in MM generations in both cell lines prove that it was possible to isolate a population of CSC. The higher expression of ALDH in TN parental cell line comparing to HR+ confirms the poor prognosis of TN tumors. The downregulation of p53 in CSC shows that they might be able to avoid apoptosis, corroborating the resistant phenotype of these cells.

No conflict of interest.

A25. Rac1b expression reverts B-Raf-V600E-induced senescence in colorectal cells

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Introduction: Colorectal cancer (CRC) represents one of the leading causes of cancer mortality in the Western world. It is a heterogeneous disease that can evolve through different pathways, one of which is termed the serrated pathway. Serrated tumor development is most frequently initiated by mutations in the MAP kinase B-Raf. However, the most frequent BRaf mutation (BRAF-V600E) exhibits a much lower transforming activity than conventional oncogenic K-Ras mutations. In fact, expression of BRaf-V600E leads to oncogene-induced senescence (OIS), a stress response that cells undergo upon constitutive oncogene activation by increasing the expression of cell cycle inhibitors. The consequent arrest in cell growth and proliferation represents an important cancer suppression mechanism. The recent discovery in CRC that BRaf-V600E and the hyperactive splice variant of Rac1 - Rac1b - functionally cooperate to sustain CRC cells viability prompted us to investigate whether the transformation of colorectal cells initiated by BRaf-V600E would trigger a subsequent increase of Rac1b expression as a way to overcome senescence, allowing for tumor progression. *Material and Methods*: A cellular model of non-transformed colonocytes (NCM460 cell line) was used to induce Rac1b and BRaf-V600E expression. Cell senescence was evaluated through a β-galactosidase assay and the expression of the cell cycle inhibitors p14ARF, p15INK4B, p16INK4A and p21CIP1 analyzed by qPCR and Western Blot. *Results and Discussion*: Using both BRaf-V600E-directed RNAi and overexpression we demonstrate that this mutation does not directly lead to Rac1b overexpression, indicating the latter as an independent event during tumor progression. Nonetheless, we observed that expression of BRaf-V600E in non-transformed colonocytes increased both the transcript and protein levels of p14ARF, p15INK4b and p21CIP1 and led to increased expression of β-galactosidase, all indicators of OIS induction. Interestingly, whereas the protein levels of these markers were reduced upon Rac1b overexpression, the levels of their respective transcripts remained unchanged. Importantly, the co-expression of Rac1b with B-Raf-V600E reverted the OIS phenotype, reducing the expression levels of the cell cycle inhibitors and β-galactosidase to those of control cells. These results suggest that Rac1b overexpression is selected in B-Raf-V600E initiated cells to overcome OIS and promote tumor progression.

No conflict of interest.

A26. Effects of exemestane metabolites in sensitive and resistant breast cancer cells and in bone homeostasis

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Introduction: Breast cancer is the most common cause of cancer death in Women Worldwide, being 80% of cancers hormone-dependent (ER+). There are several therapeutic approaches for ER+ breast cancer, being one of them the use of aromatase inhibitors (AIs), which inhibit the enzyme aromatase and block the conversion of

androgens to estrogens. Exemestane is a third-generation steroidal AI that binds covalently and irreversibly aromatase [1]. Recently, our group showed that Exemestane induces apoptosis and autophagy in MCF-7aro cells, being autophagy a pro-survival process [2], that may be involved in exemestane-acquired resistance [3]. In order to better understand the biological mechanisms of exemestane in cancer cells, it was investigated the effects of active exemestane metabolites, 17β-hydroxyexemestane (17-βHE), 6-hydroxymethylexemestane (6-HME) and 6α/β-sprirooxiranandrosta-1,4-diene-3,17-dione (C32), in sensitive and resistant breast cancer cells. *Materials and Methods*: It was explored the anti-proliferative effects of exemestane metabolites in sensitive (MCF-7aro) and resistant (LTEDaro) breast cancer cells, as well as in an osteoblast-like cell line (MG-63). It was also evaluated in MCF-7aro cells the effects on cell cycle progression, Annexin V-PE labelling, mitochondrial membrane depolarization, caspases activities, production of ROS and formation of acid vesicular organelles (AVOs). The effects of an autophagic inhibitor (3-methyladenine, 3-MA) in sensitive and resistant treated cells were also investigated. *Results and Discussion*: Our results indicate that exemestane metabolites inhibit MCF-7aro cell proliferation, promote cell cycle arrest and induce cell death by apoptosis, since it was observed translocation of phosphatidylserine (PS) to the cell surface, mitochondrial transmembrane potential loss and activation of caspases. These metabolites can also sensitize resistant cancer cells and stimulate proliferation of osteoblasts. In addition, the results obtained with 3-MA suggest that autophagy is a promoter mechanism of apoptosis. Together our results indicate that exemestane after metabolization originates active metabolites that inhibit aromatase and proliferation of hormone-dependent breast cancer cells, have the ability to prevent AIs-acquired-resistance and bone loss, being, in that way, more effective in breast cancer cells as anti-cancer drugs than exemestane. This work was funded by a financed FCT project FCOMP-01-0124-FEDER-020970 (PTDC/QUI-BIQ/120319/2010). We thank Dr. Shiuan Chen, from Beckman Research Institute, City of Hope, USA, for kindly supplying MCF-7aro and LTEDaro cells. References: [1] Macedo LF. (2009). Ann N Y Acad Sci; 1155: 162-173. [2] Amaral C. (2012). PLoS ONE; 7(8): e42398. [3] Amaral C. (2013). J Steroid Biochem Mol Biol.; 135:51-9.

No conflict of interest.

A27. Hydrogen peroxide-mediated oxidation of the 37 kDa laminin receptor precursor promotes tumor metastasis.

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Introduction: Metastasis formation is considered a critical step in cancer development since the colonization of organs by tumor cells is the main cause of death among cancer patients. To become metastatic, a tumor cell must acquire new adhesion properties that allow migration into the surrounding connective tissue, transmigration across endothelial cells to reach the blood stream and, at the site of metastasis, adhesion to endothelial cells and transmigration to colonize a new tissue. Hydrogen peroxide (H_2O_2) is a redox signaling molecule produced in the tumor cell microenvironment with importance in cancer progression. However, very few thiol-regulated proteins have been identified and the consequences of protein oxidation *in vivo* are still poorly understood. *Materials and Methods* We treated cells with H_2O_2 using a steady-state delivery method and identified proteins which are H_2O_2 targets by mass spectrometry. We then analyzed the role of oxidation of one of the identified targets using molecular dynamics simulations, cell adhesion assays, immunofluorescence and Western blot to determine its subcellular localization or co-localization with different adhesion molecules, and extravasation assays in zebrafish. *Results and Discussion* We identified 37 kDa laminin receptor precursor (37LRP) as a direct target of H_2O_2 and investigated the importance of this oxidation. Molecular dynamics simulations showed that the two conserved cysteine residues of human 37LRP might form an intramolecular disulfide without compromising the structural stability of the protein, but inducing conformational changes that might account for the regulation of protein function. 37LRP in the oxidized state associated with the cell membrane in clusters and promoted the signaling activation of specific adhesion molecules. Furthermore, 37LRP oxidation induced tumor cell adhesion to laminin *in vitro* and tumor cell extravasation *in vivo*. Thus, our results unraveled a new mechanism for H_2O_2-dependent increase of tumor cell

metastatic potential and indicate that high levels of 37LRP expression might confer a selective advantage to tumor cells in an oxidative environment. *No conflict of interest.*

A28. Hydrogen peroxide modulation of tumor angiogenesis

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Introduction: The development and dissemination of solid tumors depends upon their capacity to recruit blood vessels that supply the tumor mass with oxygen and nutrients for growth and allow tumor cells to enter the blood stream and create metastasis, a process known as tumor angiogenesis. Our study focuses on the effects of altered levels of hydrogen peroxide (H2O2) in cancer cells. H2O2 is a reactive oxygen species found to be elevated in tumor cells and known to play a key signaling role in the angiogenic process. However, the cellular mechanisms modulated by H2O2 and overall effects of discrete changes in H2O2 levels are still poorly understood. *Materials and Methods* We lowered levels of H2O2 in 4T1 mouse mammary tumor cell cultures by overexpressing catalase in a subpopulation of cells and studied its effect *in vivo* in a Zebrafish xenotransplant model. Using fluorescence microscopy to visualize early tumor angiogenesis, we analyzed the effect of lower levels of H2O2 in tumor cells in both endothelial cell recruitment and tumor vessel invasion. Putative H2O2 molecular targets were identified using PCR-array analysis to determine changes in mRNA expression of angiogenesis related factors. We then used immunocytochemistry and western blotting to study the protein levels of some of the targets previously identified. These methods were also used to study the effect of several antioxidants, e.g. selenium and tocopherol (vitamin E), on the expression of some putative H2O2 target proteins. *Results and Discussion*: Lower levels of H2O2 in tumor cells leads to a decrease in tumor induced angiogenesis *in vivo* with an observed endothelial cell recruitment angiogenesis of approximately 50% when compared to controls. Where tumor vessels were formed, a decrease in tumor vessel invasion was apparent. To understand the molecular pathways responsible for our *in vivo* observations we analyzed gene expression changes due to lower H2O2 in cancer cells in 94 angiogenesis related factors. We found several significant alterations involving, among others, Notch pathway effectors and several antiagiogenic molecules including Thrombospondin 1 (Thbs1) and Tissue inhibitor of metalloproteinase 3 (Timp3). The protein expression of Timp3 and Thbs1 was found to be regulated by H2O2 in distinct ways: autonomously and non-autonomously, respectively. While Timp3 was upregulated specifically in cells overexpressing catalase, Thbs1 expression did not correlate in an autonomous way with catalase overexpression but presented a higher percentage of Thbs1 positive cells in catalase overexpressing cell cultures. Cell autonomous expression of timp3 was studied in different tumor cell lines to confirm that this was not a cell-specific effect, with the U251 glioblastoma-derived human cell line showing a similar timp3 modulation by H2O2. Considering the very specific changes in Timp3 in tumor cells overexpressing catalase, we studied the effect of antioxidants and found an increase of Timp3 levels in the presence of non-toxic concentrations of selenium. Due to the multiple antiangiogenic effects of Timp3 including inhibiting metalloproteinases, blocking the binding of VEGF to VEGF receptor-2 and inhibiting the TNF-converting enzyme, the modulation of this molecule by antioxidants via H2O2 is an exciting potential therapeutic tool. The effects of modulating H2O2 levels in tumor cells and the tumor microenvironment continue to be the focus of our work, which will contribute to a better understanding of H2O2 dependent molecular mechanisms and, subsequently, the development of more efficient antitumor therapies.

No conflict of interest.

A29. Dll4 blockade reduces tumour metastasization in a murine xenograft model

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Introduction Current anti-angiogenic therapies have been shown to prolong the disease progression free period but without significant increase in overall survival. This has been attributed to molecular pathway redundancy in angiogenesis regulation, onset of drug resistance, recurrence to vascular mimicry or vessel cooption for tumour growth and, most importantly, possibly favoring the selection of more invasive cancer cell clones by

increasing hypoxia. Dll4 blocking therapies were shown to be efficacious in preclinical models resistant to VEGF-based anti-angiogenic therapies. However, its impact on tumour metastasization has not been addressed and there is the possibility that the observed disruption of the tumor vasculature could facilitate it. In this preliminary study we focused on effect of Dll4 blockade on tumour metastasization using a xenografted metastasis mouse model. *Materials and Methods* In this experiment metastasis frequency in xenografted endothelial-specific Dll4 loss-of-function mouse mutants, eDll4cKO, was compared with that of wild type control mice. They were subcutaneously injected with a single cell suspension of Lewis Lung Carcinoma cells (106 cells per mouse), which preferentially metastasize to the lung. Xenographted tumour measurements started seven days after the injection and were performed twice weekly for 6 weeks. At the end-point half the mice were injected under anesthesia with Lycopersicon esculentum biotinylated-lectin and the other half with Evans' Blue. One hour later they were sacrificed, the lungs resected for fixation in Bouin's fixative and macro-metastases counted under a dissection microscope, and tumor samples collected for histological vascular analysis. *Results and Discussion* Analysis of the primary tumour at endpoint revealed a reduction in tumour volume in the eDll4cKO group. We observed that eDll4cKO mice primary tumours had an increase in vascular density, with reduced perfusion and increased extravasation and endothelial regression. We also found a significant reduction in macro-metastases both number and volume in the eDll4cKO mouse mutants. These results were unexpected since other studies suggested that current anti-angiogenic therapies could be inducing a proinvasive/metastatic tumour phenotype and offer renewed strength for targeting DLL4 in cancer treatment.

No conflict of interest.

A30. May our diet interfere with colon cancer aggressiveness?

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

No conflict of interest.

A31. Influence of extracellular pH modulation on the bioenergetics of glioblastoma cells

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Introduction: Most cancer cells exhibit high glycolytic activity, even in the presence of oxygen, a phenomenon known as the Warburg effect. Continuous activation of glycolysis results in higher lactate production, leading to acidification of the tumor microenvironment (TME), whereas the intracellular milieu has alkaline pH. This 'reversed' pH gradient enables cancer progression, multidrug resistance (MDR) phenotype and is associated with a metabolic switch. The differences in metabolism between cancer and normal tissues offer new cancer therapy opportunities. Most proteins involved in the MDR and cancer aggressiveness, such as ABC transporters and pH regulators, involved in extracellular environment acidification, are ATP dependent. Thus, inhibition of the main energy producing pathways in cancer cells, will probably overcome drug resistance by depletion of cellular ATP necessary for the activity of efflux pumps. *Materials and Methods*: To associate the influence of tumor acidification with MDR, we evaluate the pH regulator expression by quantitative PCR. To assess the effect of metabolic inhibition, 4 metabolic modulators (glycolytic inhibitors: 2-deoxyglucose, iodoacetate and dichloroacetate and an OXPHOS inhibitor, phenformin), were used. Cancer cell lines were exposed to the modulators and the IC_{50} values were determined by sulforhodamine B assay. The cell metabolic status was also analyzed in the presence of modulators IC_{50}, at different extracellular pH (pHe) (6.6 and 7.4). Trying to correlate the cell bioenergetic *status* with chemoresistance, cells were pre-treated with modulators, followed by temozolomide. Results/ Discussion: Cell treatment with modulators reduced cell viability, at different pHe and led to changes in lactic acid production and glucose consumption, but the differences observed are dependent of the type of bioenergetic modulator used. The IC_{50} values determined are dependent of the pHe used. The pH regulators, namely vacuolar-ATPase, were expressed in all cell lines. The analysis of the modulators pre-treatment effect in temozolomide resistance is underway. The use of bioenergetic modulators probably leads to ATP depletion, important for cell proliferation, and presumably potentiate the cytotoxic effect of antitumor agents. On the other hand, these compounds reverts the reverse pH gradient, contributing to a decrease of the cancer cells aggressiveness.This work was supported by an internal CESPU project 02-GBMC-CICS-2011.

No conflict of interest.

A32. High systemic cholesterol increases the number of CTCs and endothelial blood vessel permeability

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Introduction It is now clear that tumor progression is not only dependent on tumor cell intrinsic characteristics but is also greatly affected by the environment that surrounds malignant cells. This refers to the so-called tumor microenvironment that is composed of several non-malignant cells, but also to what can be designated by "tumor macroenvironemnt", that comprehends the general state of the organism and factors that circulate systemically. We and others have demonstrated that high levels of cholesterol in circulation lead to increased breast tumor growth and metastasis formation. While the mechanisms through which cholesterol boosts tumor growth are starting to be understood, little is known about how it may affect metastasis progression. Metastasis is a multistep process that involves invasion of surrounding tissues, intravasation into the blood stream, extravasation and growth at secondary organs. *Materials and Methods* In this work we made use of a high cholesterol diet and an orthotopic model of breast cancer, together with *in vitro* assays of endothelial

permeability. *Results and Discussion* We were able to see that high cholesterol increases the presence of circulating tumor cells independently of tumor size. This suggests that cholesterol affects an early event of the metastatic process. Interestingly we also demonstrated that LDL-cholesterol increases endothelial permeability *in vitro*. As more permeable blood vessels are more likely to be crossed by tumor cells, we propose that a mechanism through which high cholesterol facilitates the formation of CTCs, is by turning tumor blood vessels more permeable. We are now testing this hypothesis *in vivo*. This study may lead to the development of new therapies to treat and prevent metastasis, the main cause of deaths due to cancer.

No conflict of interest.

A33. 7α-allylandrostanes as new aromatase inhibitors: biological effects in MCF-7aro and LTEDaro cells

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Introduction: Aromatase inhibitors (AIs) are one of the standard adjuvant endocrine therapies in post-menopausal women with ER+ breast cancer. They suppress the conversion of androgens to estrogens via inhibition of the aromatase enzyme. Despite their efficacy, AIs frequently present with acquired resistance and bone loss. The aim of our group is to uncover new potent steroidal AIs, which may overcome endocrine resistance. Materials/Methods. Recently, we have described the new steroids 7α-allylandrostanes as potent AIs in placental microsomes: 7α-allylandrost-4-ene-3,17-dione (6), 7α-allylandrost-4-en-17-one (9), 7α-allyl-3-oxoandrosta-1,4-dien-17β-ol (10) and 7α-allylandrosta-1,4-diene-3,17-dione (12). In this work, the anti-aromatase activity and cell viability were studied in an ER+ aromatase-overexpressing human breast cancer cell line (MCF-7aro). The anti-proliferative effects were also explored in an AI-resistant cancer cell line (LTEDaro), an ER- cancer cell line (SK-BR-3) and a non-cancerous cell line (HFF-1). Moreover, in order to understand the involvement of autophagy in AI-acquired resistance, the effects of an autophagic inhibitor (3-methyladenine, 3MA) were also studied. Results/Discussion. Our results show that AIs 9, 10 and 12 are able to strongly inhibit aromatase and induce a decrease in MCF-7aro cell's viability in a dose- and time-dependent manner, via mechanisms independent of aromatase inhibition. The use of SK-BR-3 cells allowed for the identification of possible ER-dependent effects for AI 10. No effect was detected in non-tumor cells. On the other hand, AI 10 also induces a decrease in LTEDaro cells' viability, indicating that these AI-resistant cells are sensitive to this compound. In addition, the autophagic inhibitor promoted a greater decrease in cell viability observed in LTEDaro cells, for 3 days of treatment. These studies show that steroid 10 is a promising AI and further studies should be undertaken in order to explore its biological effects in bone remodeling, the other major adverse effect of AIs used in clinic. *Acknowledgements:* FEDER Funds – COMPETE and FCT project FCOMP-01-0124-FEDER-020970 (PTDC/QUI-BIQ/120319/2010); Dr. Shiuan Chen (Beckman Research Institute, USA) for providing MCF-7aro and LTEDaro cell lines.

No conflict of interest.

A34. Extracellular vesicles - shuttles of epithelial and mesenchymal properties

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Introduction: Flexible transitions between epithelial-mesenchymal (EMT) and mesenchymal-epithelial (MET) cellular states have been envisioned as dynamic processes, underlying cancer cells' behaviour when in contact with their microenvironment and along with metastatic spreading. Whereas EMT facilitates the initial steps of tumour cell detachment and promotes migration and invasion, the subsequent MET is claimed to be required for tumour cell colonization at distant sites. In this study, we aimed at understanding whether EMT/MET cells

use extracellular vesicles (EVs) to perpetuate aggressive phenotypes that ultimately cause metastasis. *Material and Methods* A TGFβ1-induced EMT/MET model was generated using MCF10A breast cells. This strategy allowed us to derive from the same genotypic background, three distinct phenotypic states: epithelial (E), mesenchymal (M) and mesenchymal-reversed-epithelial (RE), as observed by brightfield microscopy. E, M and RE cells were characterised by qRT-PCR/immunofluorescence for the presence of well-established epithelial (E-cadherin, Occludin) and mesenchymal (Vimentin, Fibronectin, Snail) markers. EVs were isolated from the conditioned medium of E, M and RE cells by differential ultracentrifugation and characterised by TEM and WB analysis of commonly associated exosomal markers. Angiogenic activity of E, M, RE cells and corresponding EVs was assessed by CAM *in vivo* assay. The impact of E, M and RE-derived EVs over recipient E, M and RE cells was evaluated in terms of invasiveness (matrigel transwell invasion assay) and proliferation (BrdU incorporation assay). *Results and Discussion* We confirmed that MCF10A cells that underwent EMT displayed a significant E-cadherin and Occludin loss and concomitant gain of Vimentin, Fibronectin and N-cadherin RNA/protein expression levels, in comparison with parental E cells. These M cells partially recovered the epithelial features after TGFb1 withdraw, and were characterized by membranous E-cadherin expression and Fibronectin loss. EVs isolated from E, M and RE cells-conditioned media displayed a diameter of 30-150nm, and expressed CD9, CD81, Tsg101. E, M and RE cells had similar proliferation rates, but exhibited distinct invasive and angiogenic potentials. M cells were more invasive and recruited more vessels than E and RE cells. However, EVs isolated from M cells were not able to promote invasion of E and RE cells. Further, EVs isolated from E cells showed a lower angiogenic potential than their cells of origin, while EVs from M and RE cells behaved as their cells of origin. These preliminary findings suggest a relative irresponsiveness of E-like cells to M signals transmitted by EVs, that may allow maintaining normal epithelial integrity, as well as the co-existence of dynamic cell populations during cancer progression. They also suggest that EVs recapitulate only some of the functional properties displayed by cells of origin, and shed light into the EVs-mediated influence in the behaviour of recipient cells.

No conflict of interest

A35. Establishment of a 3D model of EMT/MET-induction using molecularly-designed ECM-like hydrogels

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Introduction: Epithelial to mesenchymal transition (EMT) is a biologic process that allows a polarized epithelial cell to undergo biochemical changes that enable it to assume a mesenchymal cell phenotype. EMT has been associated with a concomitant decrease of epithelial markers (e.g. E-cadherin) and increase of mesenchymal markers (e.g. α-SMA), as well as enhanced migratory and invasion capacity, resistance to apoptosis and increased production of extracellular matrix (ECM) components. EMT-cells may undergo a reverse process, mesenchymal to epithelial transition (MET), through which they are able to recover the epithelial phenotype. In some cases, EMT reversion may not be fully accomplished, giving rise to a metastable phenotype characterized by a simultaneous expression of epithelial and mesenchymal features. Several reports suggested that EMT/MET may have a role during cancer progression and metastasis establishment. To elucidate this hypothesis, different EMT models have been implemented using traditional 2D systems. However, such models lack the influence of a proper 3D ECM-like structure that could better mimic the *in vivo* microenvironment, and closely reproduce the EMT transcriptional program. The central aim of work was the establishment of a 3D *in vitro* model of TGF-β1-driven EMT/MET induction in EpH4 epithelial cells cultured on an artificial alginate matrics with tuneable properties. In addition, we aimed to further characterize the 2D *in vitro* model of TGF-β1-driven EMT/MET induction previously established in the group. *Materials and Methods*: EpH4 cells were collected at different stages of EMT/MET processes in order to scrutinise the expression of several epithelial and mesenchymal classical markers both at RNA and protein levels. For that purpose, real-time PCR, immunofluorescence, cytometry and zymography assays where performed. In addition, we measured the metabolic activity of these cells and we characterized the spheroids obtained in the 3D system, both in size and in number. *Results and Discussion* Our results demonstrated that the 2D EMT-derived cells have enhanced stem-like features (enrichment of CD29+/CD44+ subpopulation) and produce increased levels of MMP9. Regarding the 3D EMT/MET model, our findings showed a TGF-β1-mediated EMT program characterized by E-

cadherin impairment and increased mesenchymal markers, both at RNA and protein level (e.g. Vimentin and α-SMA). Moreover, the removal of TGF-β1 stimulus enabled the establishment of a metastable phenotype with concomitant E-cadherin presence at cell membrane and α-SMA expression in the cell cytoplasm. In conclusion, we created a 3D *in vitro* TGF-β1-mediated EMT/MET system that makes use of RGD-modified alginate hydrogels that provide a well-defined and tuneable environment. The insights gained with this work, may ultimate be useful for the establishment of a high-throughput system for the study of cancer progression and metastasis.

No conflict of interest.

A36. CDH3/P-cadherin is negatively regulated by TAp63 in a p53-dependent manner in breast cancer cells

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Introduction: P-cadherin, a cell-cell adhesion molecule codified by the CDH3 gene, is frequently expressed in high-grade breast cancer, being a well-established indicator of poor patient prognosis and has been reported as an important inducer of cancer cell migration and invasion. P-cadherin also confers stem cell features to breast tumorigenic cells that could be linked to the aggressive behaviour of basal-like breast cancers. P-cadherin has been associated with already described stem cell markers, such as p63, which was recently demonstrated to transcriptionally regulate CDH3 in a context of the developmental biology. In fact, the parallelism between p63 and P-cadherin interestingly involves the cancer and the developmental setting. In cancer, however, the relationship between p63 and P-cadherin was only explored in a pathological perspective.*Material and Methods*: Breast cancer cell lines and luciferase report assays were used to demonstrate the transcriptional regulatory effect of p63 isoforms on CDH3 promoter. *In vitro* functional assays were used to demonstrate the impact of this transcription factor in P-cadherin-mediated effects. *Results and Discussion*:We demonstrate that TAp63 isoforms transcriptionally represses CDH3 promoter, downregulating P-cadherin protein expression in MCF7 breast cancer cells.This repression is functionally reflected on P-cadherin-induced breast cancer cellular invasion and mammosphere-forming efficiency. Interestingly, we also observed that this effect of TAp63 isoform on CDH3/P-cadherin was not replicated in cells harbouring p53 mutations, and that the induction of p53 hotspot mutations on p53 wild-type cells restored CDH3 promoter activation. These results suggest that the repressive effect of TAP63γ isoform onto CDH3 promoter is disabled by the p53 mutants. The validation of these observations in human breast cancer samples revealed that breast tumours expressing TAp63γ isoform, but harbouring some type of known pathogenic p53 mutations were positive for P-cadherin expression, while the only case negative for P-cadherin expression was the one where no p53 mutations were detected. Taken together, our data reveal previously unknown molecular functions of TAp63γ isoforms on CDH3/P cadherin where TAp63γ is able to represses CDH3 promoter activity and P-cadherin expression levels, being this regulation dependent of p53 mutational status.

No conflict of interest.

A37. Synergetic activity of proteasome inhibitor and nanoparticles in pancreatic adenocarcinoma cells

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Introduction: Proteasome inhibitors and their associated mechanism of action foresee large-scale effects in the organism. Proteasomes are crucial regulatory complexes where protein degradation and recirculation occurs. Gold nanoparticles (AuNPs) are promise structures for biomedical applications as drug delivery systems (DDS). They exhibit high monodispersity, non-cytotoxicity and good biocompatibility. AuNPs have been investigated to

enhance the delivery and efficacy of anticancer drugs minimizing drug toxicity in normal tissues and multi-drug resistance. Polymeric nanoparticles (NPs) are suitable drug nanocarriers due to their high capacity of encapsulation of hydrophilic and hydrophobic molecules and endocytosis efficiency by enhanced permeation and retention (EPR) effect.These properties improve drug delivery and reduce undesirable side-effects. Bortezomib (BTZ) is a FDA approved inhibitor of 26S proteasome. The aim of the present work is to evaluate *in vitro* cytotoxic studies of BTZ when combined with nanocarriers in the pancreatic cancer cells, S2-013s. Our strategy is to design nanocarriers based on AuNPs and chitosan-gum Arabic (Ch-GA) NPs for DDS improvement, minimizing the drug side-effects and increasing their half-life time, which will promote new opportunities in cancer therapy research. *Materials and Methods*: AuNPs are prepared by the Turkevitch method and stabilized with a poly(ethylene glycol) (PEG) layer. Ch-GA NPs are prepared by complex coacervation. The initial concentrations of chitosan 0.4 % (w/v) and GA/Ch weight ratio of 1.2. The final PEGAuNPs concentration into Ch–GA–PEGAuNPs was 2 nM. Both nanosystems are combined with BTZ. *Results and Discussion*: In vitro cytotoxic studies of the effects of BTZ alone, Ch–GA–BTZ NPs and PEGAuNPs-BTZ are performed with S2-013s for 48 h at 37 ºC. The cells are exposed to a range of experimental concentration of BTZ from 0.01 to 100.0 nM loaded NPs. Ch–GA NPs with Ch concentration up to 3.3x10-3 mg/mL and RGA/Ch of 1.2 do not show any cytotoxicity. Also, PEGAuNPs show no cytotoxicity for concentrations up to 0.5 nM. Analyzing the effect of BTZ at the concentrations in the range 0.1 - 1.0 nM, a significant difference in the cell growth is evident, 60% for BTZ alone and about 40% for BTZ+PEGAuNPsIn the presence of BTZ combined with Ch–GA-BTZ NPs the growth rate of the S2-013s decreased when compared with BTZ alone, particularly at 0.1 and 1.0nM of BTZ concentration. Also for 0.1 nM BTZ loaded in Ch–GA–PEGAuNPs reduced the cell growth to about 70 % compared with 96 % to BTZ alone. *Conclusions:* This work demonstrates Ch–GA nanosystem efficacy might improve through the EPR effect. Also, the significant increase of BTZ toxicity with PEGAuNPs at low concentration levels can be explained by the BTZ surface adsorption to NPs.These nanosystems can be responsible for higher drug mass transfer rate across cell membrane and subsequently for increased drug diffusion in the cytoplasm.

No conflict of interest.

A38. Involvement of Monocarboxylate Transporter 1 (MCT1) in glioblastoma aggressiveness

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Introduction: Glioblastoma is the brain tumor with the highest prevalence and lethality, being important to identify new molecular therapeutic targets. Hypoxia is a common feature in malignancy, which contributes to increased glycolytic metabolism. The high glycolytic rates of tumor cells increase lactate production, which is transported to the microenvironment through monocarboxylate transporters (MCTs), important in the maintenance of physiological intracellular pH. Glioblastomas present a high heterogeneity with high levels of hypoxia and glycolysis, which for instance confer resistance to therapy. Thus, it is important to characterize the distribution of metabolism-related proteins, particularly MCTs, in the response to hypoxia. *Material and Methods*: Evaluation of metabolic marker expressions (MCT1, MCT4, CD147 and GLUT-1) and hypoxic markers (CAIX and HIF-1α) expression was performed in a series of 50 glioblastomas by immunohistochemistry. The role of hypoxia on MCT, CD147 and metabolic marker expressions was also evaluated in U251 and SW1088 cell lines by immunocytochemistry and Western blot. Additionally, the effect of MCTs downregulation on cell proliferation, cell invasion and cellular metabolism was also evaluated. **Results:** In glioblastoma tissues, the expression of MCTs, CD147 and other metabolic markers increased significantly in hypoxic compared to normoxic regions. The plasma membrane of GLUT-1 and CAIX, as well as, the nuclear HIF-1α expressions were restricted to areas adjacent to necrosis (hypoxia). Importantly, a significant increase in the plasma membrane expression of MCT1 and CD147 was found in hypoxic regions. The same association was not found for MCT4, being present in the cytoplasm in normoxic and hypoxic regions analyzed. In glioma cell lines, hypoxia induced an increase in MCT1 and CD147, but not MCT4, plasma membrane expression in the most oxidative cell line (SW1088) when compared to normoxic conditions. Additionally, we observed that MCT1 downregulation, but not MCT4, decreased lactate production, leading to a decrease in cell proliferation and invasion. *Conclusion:* MCT1 is responsible for lactate efflux in hypoxia-induced glycolytic phenotype, being an important mediator in

cell proliferation and survival of glioblastomas. Thus, MCT1 activity may contribute to the aggressiveness and therapy resistance in glioblastomas, thereby representing a promising therapeutic target.

No conflict of interest.

A39. Variability in centriole number and size is a hallmark of cancer

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A century ago, Theodor Boveri suggested that abnormalities in the major microtubule organising center of animal cells, the centrosome, lead to abnormal cell division and tumorigenesis. Those abnormalities have since been observed in cancer in vivo, but their incidence and causes remain poorly characterised. To address those important questions, we implemented a two-step screen and developed a state-of-the-art algorithm to score centriole number and length in 3D in the NCI-60 panel of cancer cell lines that represents cancer diversity. Importantly, we observed that the majority of these cell lines shows increase in both centriole number and length, and uncovered an important link between centrosome amplification and invasive capacity. Our work also suggests a novel origin of centriole amplification in cancer, which we subsequently validated with complementary approaches: as centriole length control is deregulated in cancer cells, centrioles can grow more than 3 fold and form an abnormal structure that fragments and generates smaller, functional centrioles. Centriole fragments then behave as microtubule organising centers leading to abnormal mitosis. The fact that centriole abnormalities are widespread in cancer, but not in normal cells, and the identification of novel causes of those abnormalities solidifies and suggests new avenues to the use of centrosomes in the clinic as diagnostic, prognostic and therapeutic tools.

No conflict of interest.

A40. A p53 loss leads to centrosome amplification in tumor initiation

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Centrosome abnormalities are a hallmark of human cancer. However, the causes and consequences of such abnormalities in tumorigenesis remain poorly understood. As the major microtubule-organizing centre in animal cells, the centrosome has key roles in signaling, polarity, motility and bipolar mitotic spindle formation. Deviation from normal centrosome numbers and/or structure can lead to aberrant mitotic spindles associated with chromosome segregation errors and/or cell death, and have long been proposed to contribute to aneuploidy and the development of cancer. Centrosome numerical amplification has been recently shown to lead to altered invasive and migratory behavior in cultured cells. However, though centrosome abnormalities have been found in a variety of human solid tumors, little is known on how centrioles change in human tumor progression. It is crucial to examine centrosome abnormalities in a well-established and genetically well-defined cancer model that allows the study of tumor initiation and progression within the same patient. In addition, in order to further understand the causes of centrosome changes it is important to be able to manipulate the different stages of tumor initiation and progression. This is possible in some tumor models where cell lines that mirror tumor progression are available. Barrett's esophagus (BE) carcinogenesis presents several advantages as a human cancer model. It is a multistep process from metaplasia (premalignant

condition) to dysplasia/intra-epithelial neoplasia (premalignant lesion) and adenocarcinoma (invasive neoplasia). Also, BE malignant transformation is accompanied by a progressive accumulation of well-characterized genetic lesions that are observed in many other solid tumors. To investigate centrosome defects in BE tumorigenesis we established a method to unequivocally identify true centrosomes in paraffin-embedded tissue samples and validated a panel of cell lines that represent all stages of progression of BE tumorigenesis as a model to test the origin of such centriole abnormalities and how they contribute to tumor progression. We found that centriole number aberrations arise early in BE progression. Strikingly, these were more prominent in intra-epithelial neoplasia and were dependent on p53 loss of function.

No conflict of interest.

A41. Anti-influenza drug, oseltamivir phosphate, increases canine mammary cancer aggressiveness

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Oseltamivir phosphate is a widely used anti-influenza sialidase inhibitor. Sialylation, governed by sialyltransferases and sialidases, is strongly implicated in the oncogenesis and progression of breast cancer. In this study we evaluated the biological behavior of canine mammary cell tumors upon oseltamivir phosphate treatment (a sialidase inhibitor) *in vitro* and *in vivo*. Our *in vitro* results showed that oseltamivir phosphate impairs sialidase activity in a dose-dependent manner leading to increased sialylation in CMA07 and CMT-U27 canine mammary cancer cells. Surprisingly, oseltamivir phosphate stimulated, in a dose-dependent manner, CMT-U27 cell migration and invasion capacity *in vitro*. CMT-U27 xenograft tumors of oseltamivir phosphate treated nude mice showed increased sialylation, namely α2,6 terminal structures and SLe(x) expression. Remarkably, a trend towards increased lung metastases was observed in oseltamivir phosphate-treated nude mice. Taken together, our findings revealed that oseltamivir impairs canine mammary cancer cell sialidase activity, altering the sialylation pattern of canine mammary tumors, and leading, surprisingly, to *in vitro* and *in vivo* increased mammary tumor aggressiveness.

No conflict of interest.

A42. Identification of a site-specific modification of E-cadherin N-glycans with key roles in its functional regulation in cancer

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Introduction: E-cadherin is a cell-cell adhesion molecule and the major component of the adherens-junctions in epithelial cells. The dysregulation of E-cadherin is a common feature of more than 70% of invasive carcinomas,

including gastric cancer. N-glycosylation has been described as a fundamental mechanism underlying the dysregulation of E-cadherin in gastric cancer cells [1-3]. We have recently demonstrated that E-cadherin glycosylated with the β1,6 GlcNAc branched N-glycan structure, catalyzed by GnT-V, induces a deleterious effect on E-cadherin biological functions with destabilization of adherens junctions, being associated with tumour cell invasion and progression [4]. Interestingly, gastric cancer patients displaying E-cadherin loss of function (not explained at genetic level) exhibit a significant glycosylation with the β1,6 GlcNAc branched N-glycan structure, which was found to underlie its functional impairment in gastric cancer [4]. In line with this, it is critical to further determine the site-specific modification of the branched N-glycans on E-cadherin, catalyzed by GnT-V. E-cadherin ectodomain has different potential N-glycan sites, and its occupancy and functional relevance remains to be identified. *Materials and Methods*: We have performed an in silico bioinformatics analysis combined with site-directed mutagenesis of the potential N-glycosylation sites of E-cadherin, followed by the structural and functional characterization of each E-cadherin N-glycan in a gastric cancer context. *Results and Discussion*: We have demonstrated that some specific sites are more important for the regulation of E-cadherin biological functions than others that appear to be irrelevant for protein functions. In addition, we have identified the key site that whenever occupied with responsible for the defective functions of E-cadherin in cancer. In this study, we have mapped the E-cadherin N-glycans in terms of site-occupancy, structure and function in gastric cancer envisioning potential translational clinical applications.

1.Pinho, S.S., et al., Gastric cancer: adding glycosylation to the equation. Trends Mol Med, 2013. 19(11): p. 664-76.
2.Pinho, S.S., et al., The role of N-acetylglucosaminyltransferase III and V in the post transcriptional modifications of E-cadherin. Hum Mol Genet, 2009.18(14): p. 2599-608.
3.Pinho, S., et al., Modulation of E-cadherin function and dysfunction by N-glycosylation. Cell Mol Life Sci, 2011. 68(6): p. 1011-20.
4.Pinho, S.S., et al., E-cadherin and adherens-junctions stability in gastric carcinoma: functional implications of glycosyltransferases involving N-glycan branching biosynthesis, N-acetylglucosaminyltransferases III and V. Biochim Biophys Acta, 2013. 1830(3): p. 2690-700.

No conflict of interest.

A43. Galectin-3 is part of the cell response to stressfulconditions in canine mammary tumors

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The tumor microenvironment encompasses several stressful conditions for cells such as hypoxia, oxidative stress and pH alterations. Galectin-3, a well-studied member of the beta-galactoside-binding animal family of lectins, has been implicated in tumor progression and metastasis by promoting cell-cell and cell-extracellular matrix adhesion, angiogenesis, cell proliferation and preventing tumor cell apoptosis. Its abnormal up- and down-regulated expression has been observed in several types of cancer. However, the mechanisms that regulate galectin-3 expression in neoplastic settings are not clear. In order to demonstrate the putative role of hypoxia in regulating the galectin-3 expression by canine mammary tumors (CMT), *in vitro* and *in vivo* studies were performed to evaluate its expression under hypoxic conditions. In malignant canine mammary cells, hypoxia was observed to induce an increased expression of galectin-3, a characteristic that is almost completely prevented when cells are treated with catalase. The protein increased expression was confirmed at the mRNA level. Under hypoxic conditions the expression of galectin-3 shifts from a predominant nuclear location to cytoplasmic and membrane expressions. In *in vivo* studies, galectin-3 was overexpressed in hypoxic areas of primary tumor and well-established metastases. Thus tumor hypoxia up-regulates the expression of galectin-3 that may increase tumor aggressiveness.

No conflict of interest.

B1. Identification of a novel histone H2A variant with roles in proliferation and differentiation of the mammary epithelium

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Introduction: Replacement of histone variants regulates transcription and gene silencing. Altered expression of H2A.X and H2A.Z histone variants has been described in cancer; yet there are still several non-canonical H2A variants with unknown function. In this work, we carried out a wide screening comparing expression of histone variants in undifferentiated and differentiated mammary epithelial cells (MECs) and identified a novel H2A variant highly expressed in undifferentiated cells. *Materials and Methods*: Screening of histone varians was carried out using nanoLC-MS/MS. The expression of H2A2C was confirmed using real-time PCR and immunocyto/histochemistry in MEC and human breast cancer (BC) cell lines, mouse mammary gland and human BC cases. H2A2C biological function was studied by silencing its expression with shRNA and analysis of proliferation, differentiation and apoptosis carried out with colorimetric assays and confirmation of genes and proteins in these pathways using real-time PCR and immunoblot. *Results and Discussion*: Up-regulation of H2A2C mRNA and protein levels was observed in proliferating/undifferentiated MECs undergoing epithelial-mesenchymal transition (EMT). Results were confirmed in mouse mammary glands throughout the reproductive cycle, where H2A2C was highly expressed in pregnant stage. Activation of Ras/Raf/MAPK and PI3K/AKT pathways by epidermal growth factor (EGF) was necessary to induce H2A2C expression. A preliminary screening of human BC cases showed that H2A2C mRNA levels were highest in luminal A and basal-like subtypes. Silencing of H2A2C variant induced epithelial differentiation through downregulation of Sox-2 and Zeb-1 and upregulation of E-cadherin expression. In addition, H2A2C silencing inhibited proliferation stimulated through MAPK or PI3K activation in MECS, in mouse mammary carcinoma cells and in human T47-D breast cancer cells. In summary, in this study, we report for the first time the role of non-canonical variant H2A2C in proliferation and in maintenance of an undifferentiated phenotype in the mammary epithelium. We describe that H2A2C expression is regulated by EGF and is necessary to maintain EGF stimulation of proliferation and EMT.

No conflict of interest.

B2. SLC23A2-05 and KRAS-LCS6 polymorphisms in patients with head and neck squamous cell carcinoma

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Introduction: Cancer is a genetic disease that is influenced by environmental factors. To determine the risk factors in head and neck squamous cell carcinoma, two polymorphisms - solute carrier family 23 member 2 [SLC23A2-05 (rs4987219)] and v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog [KRAS-LCS6 (rs61764370)],- and environmental factors, including smoking and alcohol consumption, were analyzed in a population. *Material and Methods*: The study enrolled 165 males diagnosed with head and neck squamous cell

carcinoma. The control group consisted of 230 healthy male subjects without cancer or family history of cancer. The SLC23A2-05 and KRAS-LCS6 polymorphisms were analyzed by polymerase chain reaction followed by enzymatic digestion. All patients and healthy subjects were assessed with regard to their smoking habit and alcohol consumption, which are risk factors for cancer. *Results and Discussion*: For Kras-LCS6 polymorphism, the allele frequency for the T and G alleles in patients were 0.91 and 0.09 while in the control group was 0.90 and 0.10, respectively. For the SLC23A2-05 polymorphism, the frequency of the C and G alleles were 0.47 and 0.53 respectively in both groups. In the analysis of the logistic regression test, no association was observed between the studied polymorphisms and squamous cell carcinoma of the head and neck, as well as the staging of the disease.

No conflict of interest.

B3. Quantitative analysis of clonal evolution driving relapse of acute myeloid leukemia escape variants

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Introduction: Post-therapeutic relapse severely limits the success of treatments against acute myeloid leukemia (AML). Clinical studies comparing matched primary and chemotherapy-resistant AML genomes indicate that tumor escape variants arise through clonal evolution. Preclinical *in vivo* systems evaluating (sub)clonal evolution of cancer during treatment/recurrence are however lacking. Methods: We describe a novel *in vivo* model system to analyze (sub)clonal dynamics and evolution of human AML (hAML) in response to therapy. This is based on: 1) Cellular barcoding technology, i.e. transduction with a lentiviral library allowing quantitative longitudinal tracking of the progeny of numerous individual disease-founding hAML cells; 2) Intra-bone-marrow (IBM)-based mouse xenotransplantation, recapitulating primary hAML disease development and response to clinically relevant therapies; 3) Molecular characterization of different (sub)clones relapsing after various treatments. *Results and Discussion*: We barcoded hAML cell lines and IBM-injected these in immunodeficient mice. We observed robust gradual disease progression *in vivo*. Importantly, standard chemotherapeutic treatment significantly decreased tumor burden *in vivo* and increased survival, compared to untreated mice, but failed to completely eradicate all tumor cells – thus leading to relapse in all animals. Relapsed hAML cells exhibited increased aggressiveness and chemoresistance upon re-transplantation into secondary hosts, as compared hAML cells isolated from untreated primary hosts. Furthermore we were able to amplify genome-integrated barcodes from hAML cells isolated from the leukemic mice. Finally we readily barcoded primary hAML from untreated patients and successfully established these cells *in vivo* through IBM-injection, thus generating a xenograft model to study (sub)clonal dynamics in clinically relevant hAML samples. The comparison of the barcode composition in untreated and relapsed hAML cells will clarify the nature of the relapse phenomena after different therapies. Recurring barcode patterns in replicate animals indicates that pre-existing populations drive relapse, while diverse barcodes argues that stochastic events are responsible. Furthermore, molecular characterization of relapsing cells, together with the (sub)clonal lineage tracing resolution, will reveal how (sub)clonal evolution provides the patient's hAML bulk tumor with the qualitative properties that drive its *in vivo* relapse.

No conflict of interest.

B4. Uncovering PRMT6 deregulation effect in prostate cancer

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Prostate cancer (PCa) is one of the most incident and prevalent cancers in men worldwide, and a leading cause of cancer-related morbidity and mortality. The limited knowledge about its biology hinders the development of new diagnostic and prognostic markers, able to improve management and therapeutic of this malignancy. Epigenetics plays an important role in prostate carcinogenesis and in fact, abnormal expression of histone modifier enzymes,such as histone methyltransferases (HMTs) and demethylases (HDMs), and its chromatin modifications are related to PCa. Notwithstanding, the specific role of deregulated activity/expression of several members of both HMTs andHDMs is still poorly understood. Previously, we have found in a small number of clinical samples that PRMT6 was overexpressed in PCa compared to normal prostate tissue samples (NPTs) and demonstrated a promising capacity to distinguish NPTs from PCa. Based on these previous results, the main goals of this work were to validate the overexpression of PRMT6 in PCa and further explore its putative oncogenic role in prostate carcinogenesis, using *in vitro* models. Using a large series of prostatic samples we found that PRMT6 was overexpressed in PCa, at both transcript and protein level. Intriguingly, PIN lesions displayed significantly higher PRMT6 expression levels, compared to PCa. Furthermore, PRMT6 mRNA levels are able to discriminate cancerous from non-cancerous prostate tissues. Contrarily to LNCaP, stable PRMT6 knockdown in PC-3, attenuated of the malignant phenotype, in which the increased apoptosis as well as the decreased viability levels, migration and invasion ability was observed. Moreover, at molecular level, PRMT6 silencing was associated with decreased H3R2me2a levels and increased MLL complex and SMYD3 expression, although no global H3K4me3 levels were found. The silencing of PRMT6 significantly associated with increased expression levels of p21, p27 and CD44, as well as decreased expression of MMP-9. Regarding signaling pathways, PRMT6 knockdown related with a reduction of the PI3K / AKT / mTOR pathway and an increase in AR, supporting an oncogenic role for this enzyme. Nevertheless, to confirm the observed alterations as a direct consequence of PRMT6 activity further experiments are needed. Similarly, additional in vitro studies should be performed to elucidate the specific therapeutic utility of AR re-expression in PC-3 cell line. Indeed, restoration of AR expression in Sh-PRMT6 PC-3 cells, might be of clinical relevance as it may re-sensitize androgen-insensitive neoplastic cells to ADT.

No conflict of interest.

B5. Sirtuin gene expression in endometrial carcinoma

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Introduction: Sirtuins are a family of NAD(+)-dependent deacetylases that play a key role in the regulation of several important cellular processes, including metabolism, cell division and aging. Their role in cancer is just starting to emerge. In this study we aimed to characterize the expression of sirtuins in endometrial carcinomas. *Materials and Methods*:Total RNA was extracted from 77 endometrial carcinomas (64 Type 1 and 13 Type 2) and 30 normal endometrium samples previously collected fresh from hysterectomy specimens and snap-frozen in liquid nitrogen as part of CHSJ and IPO-Porto tumor tissue banques. Expression levels of SIRT1-7 mRNA were determined using quantitative real-time polymerase chain reaction. Clinical-pathological data was reviewed. Wilcoxon rank-sum test and Kruskal-Wallis test were used to compare differences in expression between groups. Results: Endometrial carcinomas compared to normal endometrium showed SIRT7 overexpression, whereas SIRT1 (p<0.001), SIRT2 (p<0.001), SIRT4 (p<0.001) and SIRT5 (p<0.001) were underexpressed (p<0.001). Moreover, no significant differences in expression levels were observed for SIRT3 (p=0.42) and SIRT6 (p=0.45). Interestingly, Type 2 endometrial carcinomas displayed significantly lowerexpression levels of SIRT1 (p=0.03) and SIRT3 (p=0.02) than to Type 1 carcinomas. No significant associations were found between expression levels of any SIRT and histological grade, lymphovascular invasion or stage. Discussion: Our data shows that sirtuins are misregulated in endometrial cancer. The diversity of expression levels observed suggests that the role of each of these enzymes might be different in endometrial cancer. Indeed, diverse putative oncogenic and tumor-suppressive functions have been described in the

literature for some sirtuins in other cancer models. Additional studies, including immunohistochemical sirtuin expression, are ongoing to support our gene expression findings.

No conflict of interest.

B6.The Functional Role of Histone Methylation Deregulation in Renal Tumorigenesis

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Renal cell tumors (RCTs) are the most lethal of the common urological cancers. Currently with the widespread use of imagiology, the incidence of renal small masses is increasing, which emphasizing the need of an accurate distinction between benign and malignant RCTs, which nowadays represents a challenge in clinical practice. In fact, owing to the risk of tumor progression, in case of doubt between benign and malignant RCT, the option mostly includes an invasive approach, which in the case of some benign tumors represent an overtreatment as they could be safely monitored by imaging, precluding nephrectomy. Although histone methylation, namely the histone modifying enzymes, has been implicated in renal tumorigenesis, there are no feasible biomarkers for assisting in diagnosis neither stratification of patients into clinically meaningful subgroups. Hence, the main goal of this study was to determine which histone methyltransferases (HMTs) and histone demethylases (HDMs) might be relevant for renal tumorigenesis, focusing on the discrimination between oncocytomas and renal cell carcinomas (RCCs), especially chromophobe renal cell carcinomas (chRCCs), and to translate those findings for the clinical management of RCTs patients. It was screened 58 HMTs and 29 HDMs, which resulted in the identification of three altered enzymes of lysines 4 and 36 of histone H3: SMYD2, SETD3 and NO66. Specifically, it was found that *SMYD2, SETD3* and *NO66* were upregulated in RCTs compared to renal normal tissues (RNTs) and their expression levels were higher in chRCCs and oncocytomas compared to clear cell renal cell carcinoma (ccRCC) and papillary renal cell carcinoma (pRCC). Moreover, *SMYD2* expression levels discriminated RCTs from RNTs and chRCCs from oncocytomas, whereas *NO66* expression levels were able to distinguish benign malignant from RCTs. Survival analysis revealed that combined *SETD3* expression levels and Fuhrman grade were independent prognostic factors for disease-free survival, and *NO66* expression predicted metastasis-free survival. Additionally, *SMYD2* and *SETD3* protein expression evaluated by immunohistochemistry correlated with transcript levels and Score A might help in differential diagnosis of benign and malignant RCTs. Overall, these results suggest that *SMYD2*, *SETD3* and *NO66* are putative potential biomarkers for RCTs.

No conflict of interest.

C1. Signaling pathways in human prostate carcinogenesis

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Introduction: Prostate cancer (PCa) incidence has increased in recent years. The successful treatment of PCa depends on its early detection; however, a significant fraction of men are diagnose in late metastatic stage, when treatment is prone to fail. PCa early detection is compromised by the lack of sensitivity and specificity of the currently available markers. In this study we aimed to identify differentially expressed proteins that aid the discrimination between normal and PCa tissues. *Materials and Methods*: Prostate biopsies from 8 patients (malignant and adjacent benign tissue) were pooled per group (normal and tumor). An antibody microarray was employed to analyze the expression and phosphorylation status of 800 signaling proteins in the two pooled samples. Western blot was then applied to analyze the expression levels of six promising proteins in individual prostate biopsies in order to correlate them with patients' clinical data. *Results and Discussion*: From the 40 proteins identified as differentially expressed between the two conditions, 16 were up-regulated in the tumor group, 24 were down-regulated, and 13 revealed alterations in their phosphorylation levels. This study empowers the current knowledge on human PCa proteomics and identifies key molecules in prostate carcinogenesis with potential future application in PCa early detection.

No conflict of interest.

C2. Alternative splicing of tumour-related Rac1b in colorectal cells is regulated by phosphorylation of splicing factor SRSF1

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Introduction The pre-messenger RNA of the majority of human genes can generate various transcripts through alternative splicing, and tumours show specific patterns of splicing variants. These patterns depend on the relative concentrations of the splicing factors present in the cell nucleus, either as a consequence of their expression levels or of post-translational modifications, such as protein phosphorylation, which are determined by upstream signal transduction pathways. Splice variant Rac1b is overexpressed in certain tumour types and thus we analyzed the contribution of candidate protein kinases to its regulation. *Materials and Methods* Colorectal HT29 cell or normal NCM460 colonocytes were transfected with shRNA-encoding plasmids targeting a selected panel of protein kinases. Changes in Rac1b alternative splicing were analyzed by RT-PCR and

Western blot. *Results and Discussion* In colorectal cells, we found that depletion of AKT2, AKT3, GSK3β, and SRPK1 significantly decreased endogenous Rac1b levels. Although knockdown of AKT2 and AKT3 affected only Rac1b protein levels suggesting a post-splicing effect, the depletion of GSK3β or SRPK1 decreased Rac1b alternative splicing, an effect mediated through changes in splicing factor SRSF1. In particular, the knockdown of SRPK1 or inhibition of its catalytic activity reduced phosphorylation and subsequent translocation of SRSF1 to the nucleus, limiting its availability to promote the inclusion of alternative exon 3b into the Rac1 pre-mRNA. Altogether, the data identify SRSF1 as a prime regulator of Rac1b expression in colorectal cells and provide further mechanistic insight into how the regulation of alternative splicing events by protein kinases can contribute to sustain tumour cell survival.

C3. Sphingosine Kinase is Required for IL-7-Mediated Signaling in T-cell Acute Lymphoblastic Leukemia

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Introduction: Interleukin 7 (IL-7) and its receptor (IL-7R) are essential for normal T-cell development and homeostasis. However, IL-7 produced in the leukemia milieu can also contribute to the viability and proliferation of T-cell acute lymphoblastic leukemia (T-ALL) cells and accelerate leukemia progression *in vivo*. Moreover, around 9% of T-ALL patients display IL-7Rα gain-of-function mutations that lead to constitutive signaling and are oncogenic. Sphingosine Kinase (SK), a lipid kinase that can be activated by extracellular cues, promotes cell viability by phosphorylating sphingosine, thereby regulating the ceramide/sphingosine 1-phosphate rheostat. Whether SK is involved in IL-7-mediated effects in T-ALL remains to be determined. Methods and Materials: SK expression was determined by qRT-PCR, and SK activity measured by a fluorescence assay. To determine the involvement of SK in IL-7 mediated signaling, we treated D1 (IL-7-dependent, thymocyte-like), TAIL7 (IL-7-dependent, T-ALL), HPB-ALL (IL-7-responsive, T-ALL) and DND41 (IL-7Rα mutant, T-ALL) cell lines with an SK small molecule inhibitor. Cell viability was determined by flow cytometry analysis (FSCxSSC distribution and Annexin V/7AAD staining). Maintenance of mitochondrial membrane potential was measured by TMRE staining. Caspase 3 cleavage and activation of signaling pathways were measured by immunoblot. Cell cycle profile was determined by flow cytometry analysis of PI incorporation and proliferation by 3H-thymidine incorporation. *Results and Discussion*: We show that IL-7 positively regulated SK activity without significantly affecting its expression. Inhibition of SK prevented IL-7-mediated activation of both PI3K/AKT and JAK/STAT pathways, suggesting that SK is mandatory for the activation of IL-7-dependent survival and proliferative pathways. In accordance, IL-7 stimulation was not able to prevent apoptosis or promote cell cycle beyong G0/G1 after SK inhibition. In summary, our study identifies SK as an essential modulator of IL-7-dependent activation of pro-survival and proliferative pathways in T-ALL. Since more than 70% of T-ALL patients respond to IL-7 or have IL7R gain-of-function mutations, our observations open new possible therapeutic avenues in this pathology.

No conflict of interest.

C4. Post-transcriptional modulation of the LKB1-AMPK-mTOR axis by the RNA-binding protein MEX3A in cancer

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INTRODUCTION: As part of a highly dynamic web of messenger ribonucleoprotein complexes that act at the level of transcript processing, localization, translation, and decay, RNA-binding proteins control virtually all aspects of RNA life. Not surprisingly, defects in their expression or activity underlie the onset of diverse pathological conditions, including cancer. We have recently showed that MEX3A, a member of the novel MEX-3 family of RNA-binding proteins, affects intestinal differentiation, polarization, and stemness associated with gastrointestinal carcinogenesis. We are now focusing on a putative role of MEX3A on the regulation of the LKB1-AMPK-mTOR signalling axis, known to be altered in different cancer contexts where it affects polarization

and energy homeostasis. *MATERIAL AND METHODS*: 2D and 3D cell line-based assays with modulation of MEX3A expression and of different elements of this signalling pathway are being employed to determine how this regulation specifically affects cell polarity and the response to induced metabolic stress. Results will be confirmed *in vivo* in gastrointestinal tissue samples. *RESULTS AND DISCUSSION*: Our data shows that MEX3A regulates the LKB1-AMPK-mTOR axis by decreasing STRAD protein expression, an obligate co-activator of the tumour suppressor LKB1. The induced LKB1 kinase activity inhibits the mTOR pathway in an AMPK-dependent manner. In agreement, we observe decreased interaction between LKB1 and STRAD in intestinal cells overexpressing MEX3A, lower activation levels of AMPK, and increased mTOR signalling assessed by the activation of two canonical mTOR targets, phosphorylated S6 ribosomal protein and phosphorylated 4E-BP1. The discovery of MEX3A as a novel LKB1-AMPK-mTOR post-transcriptional regulator provides insights into how control of basic cellular processes like metabolic response and polarity can be more effectively delivered via translational mechanisms and will potentially reveal concealed opportunities for the design of novel cancer therapeutic strategies.

No conflict of interest.

C5. Regulation of REST/NSRF by DYRK1A in GBM

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INTRODUCTION Glioblastomas (GBMs) are the most common and the most aggressive primary brain tumors in adults. The presence of self-renewing GBM cancer stem cells (GSCs) in these tumors could explain their resistance to chemo- and radio-therapy. However the mechanisms that regulate the oncogenic properties of GSCs are only now being unraveled. Transcriptional repressor element 1 silencing transcription factor/neuron restrictive silencing factor (REST/NRSF) is a master repressor of neuronal programs in non-neuronal tissues. Whereas in human epithelial cells REST has been described as a potent suppressor of malignant transformation, it has an oncogenic role in neuroblastoma and medulloblastoma. Moreover it has been shown that REST is highly expressed in GBM and it maintains the self-renewal properties of GSCs. Our group has recently determined that GSC self-renewal and tumorigenicity depend on DYRK1A (Dual-specificity tyrosine(Y)-phosphorylation-Regulated Kinase 1A) activity. Because REST and DYRK1A are coordinately expressed during neurodevelopment and DYRK1A dosage imbalance reduces REST protein stability we sought to determine the relation between both proteins in GBM tumors and GSCs. *MATERIAL AND METHODS* We have used primary GBM lines derived from patients and we have explored the response of these cells to Harmine, a known DYRK1A kinase inhibitor. We have checked REST protein expression after Harmine treatment, in the presence or in the absence of Cycloheximide, by WB, as well as the levels of RIN1, a known REST transcriptional target, by qRT-PCR. In order to determine the relevance of REST in DYRK1A GBM function we have performed rescue experiments in the presence of non-degradable mutant REST isoforms. We have characterized the expression of RIN-1 and DYRK1A in a panel of GBM samples and we have performed a correlation study. *RESULTS AND DISCUSSION* We have observed that DYRK1A inhibition provoked a clear reduction in the half-life of REST protein in some of the primary GBM cell lines. As a result we detected an increase in the expression of RIN1. Moreover nucleofection of a mutant form of REST (E1009A/S1013), that cannot be ubiquitinated, partially rescues this effect. Moreover we have detected a significant inverse correlation between the levels of DYRK1A and RIN1 in patient samples. Therefore our results suggest that DYRK1A could be modulating GSCs self-renewal, at least in part through the modulation of REST stability. More experiments would be necessary to determine the molecular mechanism of this regulation and whether this relation is maintained in other neuronal tumors.

No conflict of interest.

C6. Delta-like 4/Notch signalling has a direct supportive role maintaining intestinal Lgr5+ stem cells in the Apcmin/+ mice tumors.

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Introduction: Mutations in the Adenomatous polyposis coli (Apc) gene are needed for the initiation of the hereditary colorectal cancer familial adenomatous polyposis (FAP) syndrome and most of the sporadic colorectal cancer. It has been demonstrated that Delta like 4 (Dll4)/Notch is a key regulator of tumor development through its role on angiogenesis. Our aim was to examine if this pathway was also implicated in tumor initiation. *Materials and Methods*: Using the Apcmin/+ mouse model of colorectal cancer we assessed the effect of endothelial-specific and ubiquitous Dll4 loss-of-function on tumor initiation and progression. *Results and Discussion*: We observed that both endothelial-specific and ubiquitous Dll4 mutants presented a significant reduction in the average number and volume of the intestinal tumors, displaying immature and dysfunctional tumoral angiogenesis, increased tumoral hypoxia and apoptosis. Moreover, the tumors in the ubiquitous Dll4 knock-out mice displayed a significant reduction of tumor cell proliferation and of Leucine-rich G-protein coupled Receptor 5 (Lgr5) positive stem cells, associated with an increase of secretory cell differentiation. In conclusion, Dll4/Notch seems to have an important role both in the initiation and development of Apc mutant intestinal tumors possibly by contributing to the maintenance of a large pool of Lgr5 positive stem cells.

No conflict of interest.

C7. Investigating the role of annexin A2 in epidermal growth factor (EGF) induced signalling

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Introduction: Over the past decade increasing evidence has shown that the reactive oxygen species (ROS), hydrogen peroxide (H_2O_2) is an important second messenger in cell signal transduction, due to its high diffusion and ability to target reactive cysteine residues in proteins. H_2O_2 is induced by various signalling proteins, including growth factors, cytokines, hormones and neurotransmitters through the activation of NADPH oxidase. Currently, H_2O_2-dependent signalling has been implicated in fundamental processes such as cell proliferation, differentiation, migration and apoptosis. Cancer cells typically exhibit increased ROS levels compared to normal counterparts that provides them a proliferative advantage and promote malignant progression. To balance the advantage of low ROS levels (proliferative signalling pathways) versus its damaging effect (as an oxidant at high concentrations), cancer cells induce the cellular antioxidant response. Our laboratory identified a novel redox regulatory protein, annexin A2 (ANXA2) and showed that ANXA2 antioxidant function plays a crucial role in supporting tumor growth and chemo-resistance. As a logical continuation to this research I investigated the role played by ANXA2 in the regulation of oncogenic signalling pathways induced by the epidermal growth factor, EGF. *Materials and Methods*: I established cancer cell lines (A549 lung cancer, MDA breast cancer and HT1080 fibrosarcoma) knockdown for ANXA2 and respective control cells and investigated by western blotting the activation of signalling pathways upon treatment of these cells with 15 nM EGF at different time points (time courses). I analysed the levels of intracellular ROS in the ANXA2 depleted versus control cancer cells upon treatment with EGF, using the probe 2',7' dichlorodihydrofluorescein diacetate (DCF-DA). *Results and Discussion*: My results showed that ANXA2 knockdown cells have enhanced levels of ROS and over activation of the PI3K signalling pathway (increased phosphorylation of Akt) compared to control cells. The PI3K pathway is negatively regulated by the protein phosphatase and tensin homolog (PTEN). PTEN has two cysteine residues (Cys-124 - Cys-71) within its catalytic domain that can be readily oxydized by ROS, inactivating its phosphatase function. The enhanced levels of ROS observed in the ANXA2 knockdown cells might lead to PTEN oxidation and to the consequent activation of the PI3K pathway observed in these cells. Additional research work needs to be done to prove this hypothesis. Keywords: annexin A2 (ANXA2); reactive oxygen species (ROS); epidermal growth factor (EGF).

No conflict of interest.

C8. Selective eradication of osteosarcoma stem cells by targeting Wnt/β-catenin signalling activity

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Introduction: Canonical Wnt/β-catenin (cWnt) plays a regulatory role in the self-renewal/differentiation of adult stem cells. Several studies demonstrated this pathway is involved in stemness regulation of cancer stem cells (CSCs), but little is known about the specific mechanisms governing CSCs' self-renewal in osteosarcoma. We therefore aimed to explore the role of cWnt signalling in osteosarcoma CSCs. *Materials and Methods*: CSCs were isolated from osteosarcoma cell lines, using a sphere assay. Adherent cells were dissociated and plated in serum-free media, under suspension conditions. cWnt activity was assessed by analysing expression of nuclear β-catenin and expression of specific target genes (AXIN2, DKK1). Expression of genes involved in stem cell-related signalling pathways was analysed using real-time PCR. Cell cycle alterations were analysed using flow cytometry. *Results and Discussion*: Putative CSCs isolated using a sphere assay contained a cellular fraction with nuclear β-catenin-positivity, the hallmark of activated cWnt signalling. Gene expression studies revealed increased AXIN2 in spheres, a specific target gene of activated cWnt, and decreased levels of the Wnt antagonist DKK1 compared to parental cells. Inactivation of cWnt signalling with IWR-1 (a tankyrase inhibitor) was cytotoxic for CSCs, reducing their viability in a dose-dependent manner. IWR-1 diminished AXIN2 mRNA and the expression of key stemness-related genes, such as the pluripotency transcription factors SOX2, NANOG and OCT4 and the mesenchymal-related SPARC and RUNX2. IWR-1 diminished S-phase proliferating cells ≥70% in spheres, while on parental cells by only ≈25%, after 96h. Moreover, caspase 3/7 activity was significantly increased in spheres, indicative of apoptotic cell death. Combination of IWR-1 with doxorubicin diminished CSCs viability demonstrating synergistic effects that were less pronounced in parental cells. In summary, cWnt appears to be specifically activated in CSCs, but not in parental cells. Our results suggest that targeting this pathway can eliminate CSCs, while current chemotherapy can only eradicate bulk tumour mass. Combining chemotherapy with cWnt inhibition in osteosarcoma treatment can contribute to reduce chemotherapy doses and eradicate CSCs, which are thought to be involved in cancer progression and recurrence. Funding: FCT/COMPETE/QREN/FEDER – SFRH/BD/69603/2010 and Pest-C/SAU/UI3282.

No conflict of interest.

C9. TRIB2 promotes resistance to various chemotherapeutics by deregulating the AKT signalling network

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Introduction: Intrinsic and acquired cancer cell resistance to conventional and targeted chemotherapy is the primary reason for treatment failure in many patients. The identification of molecular mechanisms involved in drug resistance that could be targeted is of enormous clinical importance. The constitutive activation of the phosphoinositide 3-kinase (PI3K)/AKT signalling pathway are often found in tumours and as a result, specific PI3K or AKT inhibition has become a key pharmaceutical objective. As such, crucial transcription factors such as forkhead box O (FOXO) proteins and p53 have been shown to mediate the action of multiple anti-cancer drugs, including PI3K pathway inhibitors. *Materials and Methods*: We extensively evaluated cancer cell line sensitivity

to a broad range of chemotherapeutic agents. Using standard molecular biology methodologies including immunoblot analysis, qRT-PCR, MTS viability assays, FACS, immunofluorescence, co-IP and ChIP analysis we examined the stress signalling cascades in cells exposed to various chemotherapeutics including a range of novel PI3K inhibitor compounds that are currently in clinical trials. We also conducted the detailed analysis of ex vivo melanoma, colon and pancreatic patient samples and correlated patient prognosis to tribbles homolog 2 (TRIB2) expression. *Results and Discussion*: Here we reveal that TRIB2 expression ablates FOXO activation, disrupts the p53/MDM2 regulatory axis thus confers resistance to anti-cancer drugs that include PI3K inhibitors. We show that TRIB2 expression is significantly increased in melanoma, colon and pancreatic cancers and correlates with extremely poor clinical prognosis. We report that TRIB2-mediated suppression of FOXO and p53 activity is indirect and rather, is via the activation of AKT. Mechanistically, the TRIB2 protein is stabilized by reduced proteasome-dependent degradation and promotes AKT activation via its COP1 domain. Altogether, our study reveals a novel regulatory mechanism underlying drug resistance in a range of cancers, and suggests that TRIB2 functions as an important component within the AKT signaling network, particularly in cancer cells. Keywords: TRIB2, AKT, p53, FOXO3a, chemotherapy.

No conflict of interest.

C10. Genetic deletion of Dll4 reduces tumor growth and metastasization to the lung in a murine model of Her2+ breast cancer

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Introduction: The Notch ligand Delta-like 4 (Dll4), , is a major regulator of angiogenesis and its expression is increased in a variety of pathological conditions, including cancer, Its role in tumor vessel development and maturation as established it as a potential therapeutic target. In this work, we studied the effect of its deletion in an autochthonous murine model of Her2+ breast cancer. *Materials and Methods*: For this study we used the MMTV-NeuNDL2-5 mouse model, characterized by overexpression of the neu gene under the control of the Mouse Mammary Tumor Virus (MMTV) promoter. MMTV-NeuNDL2-5 females spontaneously develop mammary tumors at 20 to 25 weeks of age, and present lung metastasis. We crossed them with Dll4+/- mice, mutants previously generated in our lab, to understand the effect of reduced Dll4 function in mammary tumor growth and metastasization. The two experimental groups were formed of Dll4 wild-type and of Dll4 hemizygotic MMTV-NeuNDL2-5 females. The animals were sacrificed at 25 weeks and the mammary tumors and lungs were collected and processed for histological analysis and counting of metastasis. *Results and Discussion*: Partial deletion of Dll4 resulted in increased tumor vascular density but with poorly matured new blood vessels, that were weakly perfused and displayed high levels of leakage, causing reduced blood supply to the tumor. This appears to be responsible for a significant reduction in tumor growth when compared to wild-type controls. Significantly there was also a large reduction in the number of lung metastasis in the Dll4+/- animals. These findings indicate that Dll4 signaling may constitute a good target for breast cancer therapy.

No conflict of interest.

C11. Expression of misreading Serine tRNAs in mammalian cells accelerate tumor growth *in vivo*

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Introduction: Expression deregulation of translation machinery-related factors has been reported in cancer, and raises the hypothesis that translational accuracy is compromised in this setting. This hypothesis is supported by the recurrent observation of protein misfolding, proteotoxic stress, overexpression of molecular chaperones and activation of the unfolded protein response (UPR) in many tumor types [1]. Protein misfolding

leads to endoplasmic reticulum (ER) stress, activating several pathways to alleviate damage and to promote cellular adaptation. Although prolonged ER stress and consequent UPR activation ultimately leads to apoptosis, cancer cells, along with their unique features, highjack the ER adaptive measures in order to thrive [2]. To test if translation accuracy is involved in tumor progression, we expressed misreading tRNAs in a near-normal cell line and studied UPR and cancer-associated signaling pathways. *Materials and Methods*: NIH3T3 cell line was stably transfected with pIRES2-DsRED plasmids containing misreading tRNAs altered in the anticodon, that misincorporate Serine (Ser) at Alanine (Ala)-GCU or Leucine (Leu)-CUU codons throughout the proteome. An empty plasmid and a cell line overexpressing the WT Ser tRNA were used as controls. Cell lines were injected in mice and their tumorigenic potential was evaluated. Levels of tRNA expression both in cell lines and in tumors were determined by SNaPshot sequencing. Alterations in UPR and cancer related pathways were accessed by western blot and RT-PCR. *Results and Discussion*: Here we report an unexpected role for overexpression of WT and misreading tRNAs in tumor progression. Our data show that expression of misreading tRNAs produce tumors *in vivo* with a fast growing phenotype that is similar to that of K-Ras-induced tumors. Remarkably, cells expressing the misreading tRNAs were selected *in vivo*, suggesting advantageous features of this phenotype. Our results also showed that the *in vivo* microenvironment drives cells to activate the Akt pathway and induce the UPR, as these pathways were not activated *in vitro*. Accumulating evidence has demonstrated that UPR activation and decrease in translation fidelity are required for cancer cells to maintain malignancy and acquire therapy resistance, suggesting that compromising translation fidelity may select adaptative mutations. Our results support the hypothesis that mistranslation phenotypes have a role in tumor growth, worth to explore further.

No conflict of interest.

D1. LTβR in microenvironmental cells modulates surface lymphotoxin expression on leukemic T cells and promotes leukemogenesis

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Introduction: The lymphotoxin-β receptor (LTβR) has been implicated in several physiological and pathological processes, including lymphoid organ development, T-cell maturation and cancer. T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) is an aggressive hematological malignancy that arises not only from the combination of genetic and epigenetic alterations in thymic T-cell precursors (thymocytes) but also extracellular signals provided by the microenvironment. Since the LTα1β2-LTβR signaling axis plays a prominent role in the communication between thymocytes and the thymic stroma, we aimed to determine whether an evolving crosstalk between leukemic thymocytes expressing LTα1β2 and thymic stromal cells expressing LTβR might favor leukemogenesis. *Materials and Methods*: Using the transgenic TEL-JAK2 (TJ2-Tg) mouse model of T-ALL, human T-ALL cell lines and primary samples, we determined LTα1β2 expression by RT-qPCR and flow cytometry in leukemic T cells. To assess the impact of LTβR deletion, TJ2-Tg mice were crossed with Ltbr knockout mice and leukemia development was monitored. In order to study the interaction of leukemic with stromal cells, we co-cultured the former with either mouse embryonic fibroblasts (MEFs) or the bone marrow stromal cell line MS-5. *Results and Discussion*: The LTβR-encoding gene was expressed in TJ2-Tg thymic lymphomas while the genes encoding the respective ligands, lymphotoxin (LT)-α and LTβ, were found to be expressed *in vivo* and in cultured leukemic T cells in an NF-κB-dependent manner. However, surface LTα1β2 heterotrimeric protein was detected on TJ2-Tg leukemic T cells only upon ex vivo culture or mitogenic stimulation. Demonstrating that LTβR-expressing microenvironmental cells are involved in LTα1β2 downmodulation following interaction with the receptor, lymphotoxin was detected on the surface of leukemic cells collected from Ltbr-deficient mice or from co-cultures with Ltbr-/- stroma, but not from respective Ltbr-proficient controls. Importantly, LTβR genetic deficiency delayed TEL-JAK2-induced leukemia onset. Together, these data support the notion that lymphotoxin-expressing leukemic T cells can activate LTβR signaling in stromal cells and thus promote leukemogenesis. LTα and LTβ mRNA expression was also detected in human T-ALL cell lines and primary samples, indicating they may be involved in human disease too. Future studies will assess the utility of blocking LTβR signaling with an LTβR antagonist as a therapeutic approach against mouse and human leukemia.

No conflict of interest.

D2. The role of cancer stem cells in breast cancer metastasis

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Introduction: Breast cancer is the most common malignant disease in western women (1), being metastases the most important cause of death (2). Recent studies have suggested that breast cancer stem cells (BCSCs), a subset of cancer cells with stem cell characteristics, mediate tumorigenic potential as well as distant metastases (3), being resistant to radiation and chemotherapy, and thus contributing to both the heterogeneity of breast cancers and the relapse of tumors after treatment (4). Although several BCSC markers have been already described, it is still unclear whether these proteins are enriched in metastatic lesions compared to primary tumors, as well as if these can be used as relevant targets in untreatable metastatic breast cancer (5). Aim: Thus, the aim of this work was to investigate the expression of BCSC markers in breast cancer metastasis, in order to search for their potential use as clinical targets in advanced breast cancer. *Materials and Methods*: The expression of some BCSC markers was assessed by immunohistochemistry in clinical samples of primary breast carcinomas and matched lymph node metastases, as well as in unmatched brain metastasis. The results were correlated with the clinicopathological data and patient outcomes. BCSC markers were also evaluated in two human brain tropic cancer cell lines obtained from P. Steeg´s laboratory - NIH (National Institute of Health, Bethesda, USA). Mammosphere-forming ability generated from brain tropic and parental cell lines were compared. The expression levels of BCSC markers were evaluated by western blot, RT-PCR and flow cytometry. *Results and Discussion*: Our results showed that the expression of P-cadherin in metastatic lymph nodes was significantly enriched and associated with worse DFS and OS. Furthermore, P-cadherin expression was associated with the expression of the CD44 and CD49f BCSC markers in metastatic lymph nodes. However, no statically significant association was found between the expression of CD49f and CD44 and patient outcome. Additionally, P-cadherin, CD44 and CD49f were highly expressed in brain metastasis. Using the metastatic breast cancer cell model, we verified an enrichment of the CD24 marker in brain tropic cells, potentiating the epithelial-like phenotype of CD44+CD24+ compared to the parental cell line. Additionally, brain tropic breast cancer cells exhibited a higher mammosphere forming efficiency than the parental cell line, as well as an altered profile of the cancer stem cells/metastatic markers EpCAM, CXCR4, CD49f. We still found that Src was hyperactivated in brain tropic cells and significantly expressed in human brain metastases. Mammosphere assay was still performed to explore the effect of the Src inhibitor dasatinib on stem cell activity. Dasatinib markedly reduced the number of mammospheres of brain tropic cell lines, with no effect in the parental cell line. Interestingly, dasatinib treatment also induced a higher rate of cell death in brain tropic cells, contrarily to the parental cell line, which showed substantially more resistance to the dasatinib effect.

No conflict of interest.

D3. SOX2 and CDX2 expression profile impacts oesophageal Barrett carcinogenesis

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Barrett oesophagus (BO), a premalignant condition for oesophageal adenocarcinoma (OA), results from the metaplastic replacement of the normal oesophageal squamous epithelium by a columnar lining. BO initiation relies on the sequential development of gastric and intestinal features, the latest related to the risk of malignant progression. Although the precise mechanism leading to BO is not fully elucidated, it is possible that

the metaplastic microenvironment alters the transcription factor expression profile of stem cells, leading to the production of cell types characteristic of a different tissue. Thus, the balance between transcription factors, namely those involved in intestinal, gastric and esophageal differentiation, such as CDX2 and SOX2 respectively, may play a key role in the onset and maintenance of BO. Here, we sought to characterize the expression profile of SOX2 and CDX2 in BO pathway, since its earliest morphological expression, the columnar-lined esophageal segments (CLEs) without intestinal metaplasia (IM) to OA. We studied 10 CLEs, 18 BO, 3 dysplasias and 8 OA. We observed that SOX2 was always expressed in CLEs whereas CDX2 was rarely present in these segments (1/10). On the other hand, CDX2 was always expressed in areas of IM, whereas SOX2 expression was heterogeneous and frequently associated with MUC5AC. In neoplastic lesions, the expression of these transcription factors was also heterogeneous. This study strengthens the association of SOX2 with oesophageal and gastric differentiation and suggests that altering the transcription factor profile is critical for oesophageal Barrett carcinogenesis.

No conflict of interest.

D4. Mid-Esophagus Columnar Metaplasia – What's the Biopathogenic Pathway?

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The presence of columnar epithelium in the esophagus occurs in two conditions: the heterotopic gastric mucosa (HGM) which is usually of fundic-type, and mainly located at the cervical esophagus and Barrett's Esophagus (BE), a metaplastic columnar lining of the distal esophagus associated with long-standing gastroesophageal reflux (GER). HGM is a condition with low risk of malignant transformation whereas BE is the precursor of esophageal adenocarcinoma, whose incidence increased dramatically in the past 4 decades. BE results from the sequential development of gastric and intestinal phenotypes in the esophageal lining, according to molecular mechanisms that start to be clarified. The presence of a columnar-lined mucosa in the mid-esophagus was only sporadically reported in association with caustic injury reepithelization. We describe the case of a 75-year-old male submitted to esophagectomy due to severe caustic esophageal stenosis, after 3 years of gastrostomy. Macroscopically, a 5 cm stenotic area lined by red velvet mucosa and surrounded by grey-white esophageal mucosa was observed in the mid-esophagus. Microscopically, a columnar-lining, cardiac-type, with no intestinal features was documented in the area of stenosis. The immunohistochemical study with gastric (SOX2, MUC5AC, MUC6) and intestinal (CDX2) markers showed expression of gastric markers and only a small focus of CDX2, confirming the gastric phenotype of the columnar epithelium. Histological features of GER-induced esophagitis were present in the squamous epithelium below the stenotic area. This case suggests that reepithelization of the mid-esophageal segment after caustic injury was probably modulated by the presence of GER which led to transcription factor and phenotypic reprogramming of the regenerative epithelium.

No conflict of interest.

D5. Relevance of MUC1 splice variants in pancreatic carcinogenesis

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MUC1 is a heavily glycosylated transmembrane glycoprotein expressed in low levels in apical surfaces of epithelial cells and overexpressed in more than 80% of pancreatic tumors. This overexpression is well known to be correlated with tumor initiation, tumor progression and poor survival of cancer patients. The MUC1 gene encodes a protein with a large extracellular domain with a tandem repeat region, a self-cleaving domain, a

transmembrane domain and a highly conserved cytoplasmic domain (MUC1-CD) that participates in several oncogenic signaling pathways. Different MUC1 isoforms, generated by alternative splicing events, have been associated with carcinogenesis processes. MUC1/Y, a splice variant that lacks the tandem repeat region was associated with breast cancer and together with similar isoforms (MUC1/X and MUC1/Z) was also associated with malignant ovarian tumors. In contrast, the MUC1/SEC isoform, which lacks the cytoplasmic tail and the transmembrane domain was associated with absence of malignancy. Despite a few reports, the functional significance of the MUC1 splice variants, is not well known. The main objective of this work is to study the relevance of MUC1 splice variants in pancreatic carcinogenesis. We used two *in vitro* models established from human pancreatic duct cells (hTERT-HPNE and hTERT-THPNE cell lines) to reproduce the early stages of pancreatic carcinogenesis. We used a lentiviral system to transduce two different MUC1 splice variants (MUC1/X and MUC1/S2) into both cell lines. A detailed evaluation of MUC1 isoforms overexpression impact in phenotypic characteristics (morphology, proliferation, aggregation, migration and invasion) and in the associated oncogenic signaling pathways is currently being performed.

No conflict of interest.

D6. Regucalcin, as a protective molecule in breast cancer: clinicohistopathological and *in vivo* evidences

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Introduction: Regucalcin (RGN) is a calcium (Ca2+)-binding protein that plays an important role in intracellular Ca2+ homeostasis, and its action influencing cell death and proliferation was also suggested. Moreover, a decreased expression of RGN has been related with neoplastic conditions. This study aimed to evaluate the association of RGN expression with clinicohistopathological features of human breast infiltrating ductal carcinoma (IDC). In addition, the role of RGN in mammary gland carcinogenesis was investigated by determining the susceptibility of transgenic rats overexpressing RGN (Tg-RGN) to develop carcinogen-induced tumors. *Material and Methods*: RGN immunoreactivity was evaluated in 158 cases of human breast IDC and correlated with clinicohistopathological parameters. Virgin female Tg-RGN rats (n=31) and wild-type (Wt, n=10) controls received a single intragastric administration of the carcinogen 7,12-dimethylbenz[α]anthracene (DMBA, 20mg/kg). DMBA-induced tumors were histologically classified and the Ki67 proliferation index was estimated by immunofluorescent staining. The expression of cell-cycle and apoptosis regulators in the mammary gland of Tg-RGN and Wt rats was assessed by real-time-PCR and Western blot. The enzymatic activity of apoptosis effector caspase-3 was determined using a colorimetric assay. *Results and Discussion*: RGN expression was negatively associated with the differentiation grade of human IDC (p<0.0498). Well differentiated breast tumors displayed moderate or high regucalcin immunoreactivity (70%), while only 5% of poorly differentiated tumors showed high levels of RGN, which indicates the loss of RGN with progression of breast cancer. The incidence of DMBA-induced tumors at 44 weeks was 100 % in Wt animals against only 25,8 % in Tg-RGN (p<0.001). Also, the percentage of invasive tumors was significantly higher in Wt animals (45,5% vs. 3,8% in Tg-RGN), and pre-cancerous lesions (19,2%) were only detected in the Tg-RGN group. Moreover, non-invasive tumors of Tg-RGN animals displayed a decreased proliferation index. It was also found that overexpression of RGN is associated with altered expression of key regulators of cell cycle and apoptotic pathways, and with enhanced activity of caspase-3. The present findings showed that progression of human breast cancer is associated with loss of RGN, and that RGN overexpression restricts cell proliferation and fosters apoptotic response protecting mammary gland from carcinogenesis.

No conflict of interest.

D7. Regucalcin, a new androgen target gene involved in the regulation of prostate cell growth

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Introduction: Regucalcin (RGN) is a calcium (Ca2+)-binding protein that plays an important role in the maintenance of intracellular Ca2+, and its action in regulating cell proliferation and apoptosis also has been suggested. Recently, we described a diminished expression of RGN in human prostate cancer, which was correlated with the cellular differentiation of tumors. This highlighted for the importance of RGN in prostate pathophysiology, but its actions over prostate cells remain to be elucidated. The present work aimed to investigate the role of RGN in controlling prostate cell growth. Since androgens are widely associated with prostate carcinogenesis, we also determined whether RGN is an androgen-target gene in prostate. *Materials and Methods*: Orchiectomized male rats were injected with 5 -dihydrotestosterone (DHT 500 µg/Kg.day), and the expression of RGN in prostate was determined by means of real-time PCR (qPCR) and Western Blot (WB). In order to investigate the role of RGN in the regulation of target genes involved in cell proliferation and apoptosis, we made use of transgenic animals overexpressing RGN (Tg-RGN) and performed gene expression analysis, comparatively with wild-type counterparts, by means of qPCR, WB and fluorescent immunohistochemistry (IHC). The enzymatic activity of caspase-3 also was determined. *Results and Discussion*: DHT down-regulated the expression of RGN in rat prostate. Proliferation index determined by means of Ki67 IHC and prostate weight were reduced in Tg-RGN, and accompanied by altered expression of cell-cycle regulators. The expression of the oncogene H-ras was decreased in the prostate of Tg-RGN, while an increased expression of the cell-cycle inhibitor p21 was observed. Tg-RGN rat also displayed increased levels of the anti-apoptotic Bcl-2 protein, as well as, augmented Bcl-2/Bax ratio. The expression of caspase-8 and caspase-3 was decreased in the prostates of Tg-RGN, in accordance with the reduced activity of caspase-3. In sum, overexpression of RGN was associated with the inhibition of cell proliferation and apoptosis, which demonstrated its important action in maintaining the prostate growth balance. The down-regulatory effect of DHT on RGN expression suggested that the effect of androgens promoting prostate cell growth may be linked with the diminution of RGN levels. The present findings, suggest that RGN may play a crucial role restricting proliferation of prostate cells and, thus, preventing development of tumors.

No conflict of interest.

D8. Retinoblastoma: an orthotopic animal model.

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Introduction: Retinoblastoma (RB) is a very aggressive tumor that can affect children mainly at the age of 5 years. Animal models are good tools that can allow understanding tumor biology and evaluating new therapeutic approaches. The purpose of this work was the development of an orthotopic model of Retinoblastoma that mimetize tumor environment and growth. *Materials and Methods*: Y79 human RB cell line was propagated according to standard procedures. Six Rowett Nude Rat (RNU) female rats were used to develop an orthotopic animal model of RB. Animals were anesthetized with a solution 3:1 of ketamine and chlorpromazine. Half a million cells were injected in the vitreal cavity of the left eye. Right eye was used as the control in all studies. After injection, OCT was performed. The animals were monitored daily. Six days after injection X-ray was performed. After six months of follow-up the animals were killed and eyes and brain were collected and preserved for histological analysis. *Results and Discussion*: OCT did not demonstrated alterations in injected eyes immediately after injection and neither X-ray 4 weeks after injection. Macroscopic follow up allowed observing alterations in the eyes comparing to control, a few days after injection. Histological analysis proved existence of tumor in injected eyes and showed its aggressiveness, invasiveness and dependence of its vascularization. It was also observed characteristics of an invasive retinoblastoma like necrotic areas and arrangement of cells in a line and rosettes. This model can allow us to study *in vivo* the characterization of retinoblastoma and to study new therapeutic approaches like photodynamic therapy.

D9. A transgenic mouse model for HPV-associated head-and-neck cancer: anatomical and histological characterization.

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Introduction HPV-associated head-and-neck cancer is a rapidly growing public health problem. HPV+ cancers arise in specific locations and show distinctive morphological and molecular features, as well as a distinct biological behaviour when compared with their HPV- counterparts. Developing adequate animal models is critical for understanding the biopathology of HPV+ oropharyngeal cancer and for developing effective preventive and therapeutic strategies. Here we report on the occurrence, distribution and histology of oropharyngeal pre-neoplastic and neoplastic lesions in K14HPV16 transgenic mice. *Materials and Methods* Hemizygous (HPV+/-, n=13) and wild-type (HPV-/-, n=13) female mice in a FVB/n background were maintained under controlled conditions of light, temperature and humidity, with a standard diet, according to national and international animal welfare regulations. Genotypes were determined for the HPV16 E2 and E6 genes. All animals were sacrificed at 30 weeks-old. Following necropsy, whole head tissues were fixed in 10% neutral buffered formalin for 72 hours, decalcified in a formic acid solution for 12 hours and routinely processed for histological analysis. H&E slides were examined by two researchers and lesions were classified according to their location and histological type. *Results and Discussion* Five out of thirteen HPV+/- animals (38%) died during the study, while all HPV-/- animals survived to 30 weeks-old. Control animals showed normal oropharyngeal histology, while all eight surviving HPV+/- animals (100%) showed diffuse dysplastic lesions of variable severity in the lip, dorsal/ventral tongue, hard/soft palate and pharynx. Invasive lesions were present in four mice (50%), and were restricted to the region between the 6th and 8th palatine ridges, in the caudal area of the hard palate. In this region, cancer cells formed poorly-differentiated nests that invaded the overlying stroma and bone, eliciting a severe scirrhous reaction and extensive bone remodelling. Nuclear pleomorphism and mitotic activity were low. The focal location of HPV-induced cancers in this model, makes it attractive for studying the pathogenesis of HPV+ cancers as well as potential preventive and therapeutic strategies.

D10. Molecular mechanisms of hexavalent chromium toxicity: effects on the stress response

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Introduction: Although the molecular basis of Cr(VI)-induced lung carcinogenesis remains essentially elusive, it is generally accepted that Cr(VI) exposure produces several cellular stresses, namely oxidative and metabolic stresses. These stresses are expected to activate the stress response of the exposed cells, which, in turn, might protect cells from further stresses, namely those encountered during neoplastic transformation. In this study, we determined the effects of Cr(VI) on resistance to acute cold shock, as well as on the intracellular levels of three components of the stress response known to be involved in carcinogenesis: heat shock proteins 90 alpha (Hsp90α) and 70 (Hsp70) and heat shock factor 1 (HSF1). Additionally, we determined the duplication times of cultures that survived Cr(VI) insults that were not overly cytotoxic (0.5-2 μM), to verify previous findings by our group suggesting that their proliferation rates were higher than those of their non-exposed counterparts. *Materials and Methods*: BEAS-2B cells were used throughout this study. Transient cold shock was induced by replacing spent medium with cold medium (4 °C). Intracellular levels of Hsp90α, Hsp70 and HSF1 were

determined using commercial ELISA kits. 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. 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 and Discussion*: Contrary to what might be expected, the intracellular levels of Hsp90α, Hsp70 and HSF1 were not increased upon a 48 h exposure to 1 μM Cr(VI): on the contrary, levels of Hsp90α were decreased, while those of Hsp70 and HSF1 were unaffected (the slight decrease in HSF1 levels was not statistically significant). In fact, the decrease in Hsp90α levels was not totally unexpected, as it was in line with earlier reports for this and other cell lines. On the other hand, our results show that the proliferation of cells exposed to 0.1-2 μM Cr(VI) was less affected by a transient cold shock than that of their non-exposed counterparts. Finally, determination of doubling times confirmed higher proliferation rates for cells that survived Cr(VI) insults for this range of Cr(VI) concentrations. Acknowledgments: This work was supported by grants from CIMAGO (16/12) and FCT (PEst-OE/QUI/UI0070/2011).

No conflict of interest.

E1. Oncogenic TERT promoter mutations are present in small fraction of GISTs.

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Introduction: Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors, which are molecularly characterized by activating KIT/PDGFRA mutations that constitute important predictive biomarkers of imatinib response in these patients. Recently point mutations in the promoter of telomerase reverse transcriptase (TERT) gene, mainly at positions c. − 124 and c. − 146 bp, were described in several human cancers, representing a novel mechanism of telomerase activation in cancer. In GISTs, there is no data on TERT promoter mutations. Herein, we searched for the presence and clinicopathological association of TERT promoter mutations in a series of 130 bona fide GISTs. Furthermore, we investigated the functional importance of the TERT promoter mutations in terms of transcriptional activity in a GIST cell line. *Materials and Methods*: Genomic DNA from 130 paraffin tumor tissues was extracted and the hotspot TERT promoter region was amplified by PCR followed by direct sequencing. In the GIST-T1 cell line, a reporter assay system with the relevant portion (c. − 290 to c. − 47) of the mutant or wild-type TERT core promoter was cloned upstream of the firefly luciferase gene and evaluated its luciferase activity. *Results and Discussion*: We found TERT promoter mutations in 3.8% (5/130) of GISTs. No statistical correlation was found between TERT mutation and GIST clinical or molecular (KIT/PDGFRA/BRAF) features. Yet, TERT mutations appeared in tumors of slightly older patients, and no TERT-mutated cases were detected in benign/very low malignancy risk GISTs. *In vitro*, we showed that in comparison to the wild-type TERT promoter, both mutations conferred increased transcriptional activity. In the present study we showed that TERT promoter mutations are present in a small fraction of GISTs. The mutations identified (c. − 124 and c. − 146 bp) are associated with increase of the TERT transcriptional activity in GIST cell line. Further studies are needed to extend and validate these findings in order to determine its clinical and biological impact in GISTs.

No conflict of interest.

E2. Detection of glyco-mucin profiles improves specificity of MUC16 and MUC1 biomarkers in ovarian serous tumours

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Introduction: The CA125 assay detects circulating MUC16 and is one of the most widely used cancer biomarkers for the follow-up of ovarian cancer. We previously demonstrated that detection of aberrant cancer-associated glycoforms of MUC16 as well as MUC1 in circulation could improve the yield of these serum assays. Our aim was to refine ovarian cancer biomarkers by detection of aberrant glycoforms (Tn, STn, and T) of MUC16 and MUC1 in ovarian cancer tissue using Proximity Ligation Assays (PLA). *Material and Methods*: We studied two series of serous ovarian tumours, a pilot series of 66 ovarian tumours (27 cystadenomas, 16 borderline tumours and 23 adenocarcinomas) from Centro Hospitalar S. João, Porto and a validation series of 89 ovarian tumours (17 cystadenomas, 25 borderline tumours and 47 adenocarcinomas) from the Portuguese Institute of Oncology Francisco Gentil, Lisbon. *Results and Discussion*: PLA reactions for MUC16/Tn, MUC16/STn, MUC1/Tn and MUC1/STn were negative in benign lesions but often positive in borderline and malignant lesions, in both series. An even better yield was obtained based on positivity for any of the four glyco-mucin profiles, further increasing sensitivity to 72% and 83% in the two series, respectively, with 100% specificity. The strategy is designated glyco-mucin profiling and provides strong support for development of PLA-based serum assays for early diagnosis.

No conflict of interest.

E3. *In vitro* generation of tumor lysate-pulsed dendritic cells with immunotherapeutic potential

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Introduction Dendritic cells (DC) are professional antigen-presenting cells which have been shown to initiate anti-tumoral immune responses. Early clinical experiences with DC-based immunotherapy have suggested this to be a promising strategy for malignant glioma patients. The heterogeneity in tumor-associated antigens expression in glioma favors the use of tumor lysates to load the DC. Here we summarize our first experience in the generation of tumor lysate-pulsed DC, which is part of an authorized pre-clinical study with solid tumors we have in course in our institution. *Materials and Methods* Tumor mass, obtained by surgical resection, was immediately snap-frozen in liquid nitrogen without additives. For further preparation, the tissue was thawed, placed in phosphate-buffered saline (PBS), minced into small pieces and lysed by 5 freeze-thaw cycles. The lysed cells were centrifuged and the supernatant was irradiated and filtered. Protein concentration was determined and aliquots were stored until use. Peripheral blood mononuclear cells (PBMC) were isolated from healthy donor's fresh blood by density gradient centrifugation and monocyte fraction enriched using plastic adherence. Isolated PBMC were resuspended in cRPMI (RPMI 1640 2% Albumin 2% L-Glutamine) and cultured in 6-well plates in an atmosphere with 5% CO_2 at 37°C. After 2h, nonadherent cells were gently removed by 3 PBS washes. The adherent cells were cultured in cRPMI containing GM-CSF and IL-4. At day 6, immature DC (iDC) were pulsed with the tumor lysate and a maturation cytokine cocktail (TNF-α, IL-1β, IL-6 and PGE2) was added. After 48h, loaded mature DC (mDC) were harvested and evaluated for cell counting and viability staining. The phenotype and morphology of iDC and mDC were determined by flow cytometry (BD FACSCanto II) and microscopy, respectively. Microphotographs highlighting the DC morphology were taken. *Results and Discussion* The tumor lysate guaranteed a sufficient amount of protein and the separation of PBMC (96%) provided two DC preparations. At day 6 of culture, a typical iDC phenotype CD11c+CD14-CD83+/- was obtained; cells showed to be irregular, semi-detached and clumping up forming colonies. At the end of culture, characteristic maturation markers CD11c+CD83+CD86+ were revealed; a single-cell suspension showed numerous cells presenting extended and multiple cytoplasmic projections (the so-called dendrites). Cellular counts (total: 32,8x10E4) and viability (92%) results were in a very good range. With this methodology we are able to generate dendritic cells *in vitro* from monocytes, and to mature them by pulsing with tumor lysate. Considering the mechanisms involved in the immune modulation using DC-based vaccines, it remains necessary

further *in vitro* functional analysis to ensure their efficacy. We think to have established the first steps for a well-designed clinical trial in the near future. *No conflict of interest.*

E4. Functional characterization of two novel RET variants associated with medullary thyroid carcinoma

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Introduction: Activating germline mutations in the RET proto-oncogene are responsible for about 98% of the familial forms of medullary thyroid carcinoma (MTC), which represent 25% of the cases. The search for germline mutations in this gene is important for the recognition of hereditary forms of MTC and further identification of at risk relatives who may benefit from early clinical intervention. Genotype-phenotype correlations are well-established for most disease causing RET mutations, allowing risk stratification. The association of a new RET variant with the MTC phenotype and familial predisposition requires the assessment of its functional and clinical significance. The aim of this study was to evaluate, through *in vitro* functional experimental approaches, the oncogenic potential of two newly identified RET germline variants associated with late-onset MTC. *Material and Methods*: RET missense variants correspond to changes in codons 515 and 636 (p.C515W and p.T636M located, respectively, in exons 8 and 11), that result in amino acid substitutions in the extracellular region of the receptor tyrosine kinase RET. The transforming potential of these new variants were evaluated by *in vitro* functional assays, by expressing the novel RET variants in non-transformed cells, and comparing their effect with wild-type RET. *Results and Discussion*: The new RET variants were able to increase cell growth and proliferation, induce loss of contact inhibition and stimulate cell migration when compared to wild-type RET. Furthermore, the autophosphorylation status of the novel RET variants is consistent with their ability to stimulate intracellular signalling pathways, including the RAS/MEK/ERK pathway and the PI3K/AKT/mTOR pathway. Nevertheless, the low-grade transforming potential displayed by these new variants, when compared to that of RET MEN2A-causing mutation C634R, probably explains the mild phenotype characterized by late onset and low aggressiveness.

No conflict of interest.

E5. Patient-derived bladder cancer xenografts: a systematic review

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Context: Patient derived tumor xenografts are said to accurately reflect the heterogeneity of human tumors. In the case of human bladder cancer, few studies are available featuring these models. The best methodology to develop and the real value of the model remain unclear. Objective To elucidate the best methodology to establish and use patient derived bladder cancer xenografts to study the characteristics and behavior of human bladder tumors. *Data acquisition:* A comprehensive literature search was performed to identify published studies using xenograft models directly established from human bladder cancer samples into mice. A total of twelve studies were included in the final analysis. Included reports All studies differed in design, the reported take rate varied between 11 and 80%, with the implantation via dorsal incision and with matrigel obtaining the higher take rate. Advanced stage and high grade tumors were associated with increased take rate. Xenografts preserved the original tumor identity in the establishment phase and after serial passages. Although some studies suggest a correlation between engraftment success and clinical prognosis, evidence about the association between the response of xenografts to treatment and the clinical response of the tumor of origin is still missing. *Conclusions:* All methodological approaches resulted in the establishment of tumor xenografts with preservation of the original tumor identity but variable take rate. The time needed to establish the model

and propagate xenografts to a number suitable for drug testing is the main limitation of the model, along with the success rate and lack of consistency in the early passages. Comparison between tumor response in mice and clinical outcome remains to be assessed. *No conflict of interest.*

E6. Dacomitinib, an irreversible EGFR inhibitor, effectively inhibits glioblastoma growth

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INTRODUCTION Glioblastomas (GBMs) are devastating tumors in which there has been little clinical improvement in the last decades. New molecularly-directed therapies are under development being EGFR one of the most promising targets as this receptor is mutated and/or overexpressed in nearly half of the GBMs. However the results obtained with first generation tyrosine-kinase inhibitors have been disappointing with no clear predictive markers of tumor response. Here we have tested the antitumoral efficacy of a second generation inhibitor: dacomitinib (PF299804, Pfizer) that binds in an irreversible way to the receptor. In fact dacomitinib can inhibit other member of the HER family of receptors and is active in erlotinib and gefitinib-resistant nonclinical models of NSCLC. Simultaneously to our pre-clinical research a fase II clinical assay with dacomitinib it is being performed in patients with recurrent GBM with amplification of the EGFR gene and/or presence of the truncated isoform EGFR-VIII (EudraCT: 2011-004671-37; GEINO-11). Our goal was to elucidate its mechanism of action and to determine the molecular features of the different GBM samples in which dacomitinib treatment could be efficient. *MATERIAL AND METHODS* We have performed *in vitro* experiments in GBM primary cell lines in order to determine how dacomitinib affects their viability, self-renewal capacity and proliferation. In parallel to the clinical assay we have used lines with EGFR amplification, with or without the presence of the EGFR-vIII isoform, as well as non-amplified control GBM lines. We have also carried out *in vivo* experiments with dacomitinib to determine if this second generation EGFR inhibitor affects GBM tumor burden. On the one hand with the heterotopic xenografts we have performed tumor growth curves and we have analyzed the tumor tissue looking for changes in the molecules acting downstream of EGFR (by western blot and immunochemistry) after dacomitinib treatment. On the other hand, using the orthotopic models we have carried out survival experiments. *RESULTS AND DISCUSSION* Our results confirm that dacomitinib has an effect on cell viability, self-renewal and proliferation in EGFR amplified +/- EGFRvIII GBM cells. Moreover the *in vivo* tumor growth rate of these EGFR amplified cell lines was also strongly impaired after systemic administration of dacomitinib to the mice, which provoked also a decrease in the expression of stem-cell-related markers. Therefore dacomitinib is the first EGFR inhibitor showing a strong antitumoral effect in EGFR amplified tumors (independently of the presence of the vIII mutant isoform) being the only one that clearly affects receptor signaling *in vivo*. However dacomitinib was not so efficient in the absence of PTEN, suggesting that this pathway should be analyzed to predict the antitumoral effect.

No conflict of interest.

E7. Out of the bag – recovering functional cells from usually discarded bags

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Introduction Bone marrow (BM) is the primary source of progenitor cells in the adult body. First recognised as home to haematopoietic progenitor cells (HPC), it also contains progenitor cells of other lineages, such as mesenchymal and neurological. Although its use in the Haematopoietic Stem Cell Transplant (HSCT) setting has decrease in last decades, its interest in cellular therapy is increasing, with numerous protocols using BM derived mononuclear cells (MNC), such as ex vivo expansion of HPC, neuro and mesenchymal stem cell differentiation. The standard protocol for BM collection is thru multiple aspirations in the iliac crests, added in a collection bag containing an anticoagulation solution (ACD-A). Prior to infusion or manipulation, the BM is filtered to remove bone fragments and cell clumps. In order to obtain a minimum MNC necessary for most protocols, a relatively large volume (>20ml) of BM is collected or removed from BM graft. To overcome this

difficulty, we studied whether cells that remain in the collection bag and filter system, should not be considered clinical waste. *Materials and Methods* From 14 BM collected at our institution, intended for allogeneic HSCT, we compare the amount of MNC obtained from 1ml of the filtered BM (used for clonogenic assays) and from the waste system, and calculated the virtual equivalence in BM volume. The collection bag and filter system were back-washed and rinsed with RPMI under aseptic conditions. MNC for both samples were isolated by density gradient centrifugation. The viability and functionality of the recovered MNC were evaluated by trypan blue exclusion and *in vitro* differentiation into mesenchymal stem cells (MSC), respectively. For obtaining MSC, MNC were cultured in DMEM media supplemented with foetal bovine serum, at 37ºC and 5% CO2. *Results and Discussion* From the 14 BM products we obtained an average of 1.18x108 recovered MNC. After adjusting for the MNC isolated from the 1ml filtered sample, we can estimate this recovery is the equivalent to starting with an average 51 ml of filtered BM. In all cases viability was superior to 90%, and long term MSC cultures were established, as shown by morphology and immunophenotype. With this study we demonstrated that the usually wasted BM collection bag and filter system is a source of MNC to consider in the investigation and clinical settings, capable of yielding large amounts of viable, fully functional and sterile MNC.

No conflict of interest.

E8. HOTAIR is transcriptionally activated by HOXA9 and is an independent prognostic marker in patients with malignant glioma

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Introduction: The lncRNA HOX transcript antisense intergenic RNA (HOTAIR) mediates chromatin epigenetic modifications that regulate expression. Aberrant HOTAIR expression has been implicated in the aggressiveness of several human cancers. Here, we evaluated the molecular alterations and upstream regulatory mechanisms of HOTAIR in glioma, the most common brain tumor type, and its clinical relevance. *Materials and Methods*: HOTAIR gene expression, methylation, and copy-number data were assessed in glioma data from The Cancer Genome Atlas (TCGA). HOTAIR expression was further evaluated in 2 Portuguese datasets and in Oncomine. Chromatin immunoprecipitation, and quantitative PCR were performed in glioblastoma (GBM) cell models. Univariate and multivariate analyses were used to assess HOTAIR prognostic value. *Results and Discussion*: HOTAIR was highly expressed in a subset of GBMs in a gene dosage- and promoter DNA methylation-independent manner, and frequently co-expressed with HOXA9 in high-grade gliomas from TCGA, Oncomine, and our datasets. Mechanistically, we found the homeoprotein HOXA9 directly binds to the promoter region of HOTAIR and induces its transcription. Clinically, malignant glioma patients with high HOTAIR expression had a reduced overall survival, independently of other variables. In summary, we reveal HOXA9 as a novel direct regulator of HOTAIR, and establish HOTAIR as an independent prognostic marker. Our findings may provide new therapeutic opportunities to treat this highly-aggressive cancer.

No conflict of interest.

E9. Prognostic significance of PI3K/Akt/mTOR pathway using phosphoflow in pediatric acute lymphoblastic leukemia–preliminary results

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Introduction: Acute lymphoblastic leukemia (ALL) is the most frequent childhood malignancy, accounting for roughly one quarter of all pediatric cancers and more than 80% of leukemias. Despite significant improvements

in treatment outcome, around 10-20% of patients still relapse, so there is a clear demand for new prognostic factors predicting therapy resistance. Both cell-autonomous lesions and microenvironmental cues, such as IL-7, contribute to the activation of the pro-survival PI3K/Akt/mTOR pathway in ALL. However, it remains to be determined whether the PI3K/Akt/mTOR activation status has prognostic value. We propose to tackle this issue using phospho-flow cytometry, which is potentially applicable to clinical diagnostics, in pediatric ALL. *Materials and Methods*: Bone marrow samples (n=58) were collected from pediatric ALL patients at diagnosis at the Pediatric Department of IPOL, after internal review board approval and obtaining informed consent. We analyzed the levels of phosphorylation of Akt (S473 and T308), and S6 (S235/236), both ex vivo and *in vitro* after stimulation with IL-7 at the single-cell level, by using phospho-flow cytometry. Levels were normalized to those of a reference ALL cell line (NALM6) and compared with clinical parameters, such as age, maturation stage, white blood cell count (WBC), and minimal residual disease (MRD) at the end of induction therapy. Statistical analysis was performed using unpaired two-tailed Student's t test or one-way ANOVA, as appropriate. P values lower than 0.05 were considered significant. *Results and Discussion*: Basal or IL-7-stimulated levels of activation of the PI3K/Akt/mTOR pathway did not correlate with age or maturation stage (EGIL classification). Notably, higher basal levels of phosphorylation of S6 and Akt on S473, but not on T308, associated with higher WBC. This result suggests two independent mechanisms leading to Akt activation in ALL. Interestingly, there is a trend, albeit non-significant, suggesting that the ability to respond to IL-7 may correlate with lower WBC. None of the basal or IL-7-stimulated phospho-protein levels analyzed were significantly associated with MRD status. Overall, our preliminary results suggest that there may be an association of high Akt S473 and S6 phosphorylation levels with high risk but not with poor prognosis.

No conflict of interest.

E10. Regulation of Wnt Signaling by HOXA9 in Glioblastoma: Mechanistic and Prognostic Insights

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Introduction: Glioblastomas (GBMs) are the most common and malignant type of gliomas, a heterogeneous group of neoplasia that account for the majority of primary brain tumors. While the clinical outcome of GBM patients is particularly unpredictable, patients are equally treated with a standardized approach. To overcome this problem of a universal therapy to strikingly heterogeneous tumors, the identification of molecular prognostic factors that allow the stratification of subgroups of GBM patients is crucial. The overexpression of the HOXA9 in GBM is associated with poor prognosis and pro-proliferative properties. Since HOXA9 is a transcription factor, its targets can be the true biological effectors of its aggressiveness. *Material and Methods*: Gene set enrichment analysis (GSEA) was used to query the HOXA9-transcriptome. qRT-PCR, western blot, chromatin immunoprecipitation (ChÎP), methylation specific PCR (MSP; in cell lines and patients from Hospital Santo António), and immunohistochemistry (in xenograft tumors and in a cohort of Brazilian patients) were performed. TCGA and Rembrandt datasets were assessed. *Results and Discussion*: Interestingly, we found that the Wnt pathway, hyper-activated in cancer and associated with higher proliferation and therapy resistance, is over-activated in HOXA9+ cells. Specifically, a key gene of this pathway, WNT6, is a direct transcriptional target of HOXA9 and is overexpressed in a subset of GBM patients from TCGA. Additionally, we observed that WNT6 expression correlates with higher glioma grades and with the GBM proneural subtype, whose patients do not benefit from more intensive therapies. Interestingly, we demonstrated that WNT6 expression is also regulated by methylation in GBM patients. Importantly, we provide the first evidence of the clinical prognostic value of WNT6 in GBM from TCGA and at the protein level in a cohort of Brazilians patients, implicating high levels of WNT6 as a novel independent negative prognostic marker. Together, our findings provide mechanistic and prognostic insights into the role of the Wnt pathway in GBM, creating opportunities to novel therapeutic approaches to treat this highly-aggressive cancer.

No conflict of interest.

E11. Specific alterations in the TCF7L2 gene correlate with colorectal cancer metastases and poor prognosis

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Introduction: The majority of colorectal cancers (CRC) presents mutations in genes coding for specific Wnt pathway components, such as APC or CTNNB1 (β-catenin), thus impairing β-catenin down-regulation. β-catenin forms a complex, together with TCF7L2 transcription factor, that leads to the activation of Wnt target genes and consequently to uncontrolled proliferation and tumor formation. Whereas an oncogenic function has been initially proposed for TCF7L2, lately this gene has been shown to act as a tumor suppressor. However, little is known regarding the role of specific TCF7L2 molecular alterations for colorectal tumor development. Thus, we aimed to study the contribution of specific TCF7L2 molecular alterations for colorectal tumor progression and aggressiveness. *Materials and Methods*: Seventy-three CRC (microsatellite stable -MSS) and 27 adenomas have been analyzed for loss of heterozygosity (LOH) of TCF7L2, and a subset of these have been characterized for copy-number variations (CNVs) in this gene by MS-MLPA, using synthetic probes. Gene expression analysis by RT-qPCR was also performed to further characterize specific alterations. TCF7L2 copy-number and gene expression were also evaluated in 213 and 688 MSS CRC samples, respectively, from the PETACC-3 study group (TNM stage III), and correlated with relapse-free survival and with the occurrence of relapse after surgery. Statistics: Stata 8.0. *Results and Discussion*: LOH of TCF7L2 was detected in 21/73 (29%) CRC and 4/27 (15%) adenomas. Correlation with TNM staging revealed a significant association between LOH and synchronous metastases (stage IV disease) (p <10-3). TCF7L2 CNVs were frequently detected in CRC, especially gains in the 5'UTR or in the 5' region of the gene (19/34;56%). These gains were also detected in normal mucosa, although significantly less frequent (P<0.01). The concomitant presence of these alterations in both tumor and normal mucosa was significantly associated with stage IV disease (P=0.02). A significant increase in gene expression was found in samples presenting 5' TCF7L2 gains. A positive association between increased TCF7L2 expression and the occurrence of relapse after surgery and relapse-free survival was observed in the PETACC-3 group [OR, 1.46; 95%CI, 1.08-1.99; 0.01 and HR, 1.37; 95%CI, 1.08 - 1.75; 0.01, respectively]. These results lead us to suggest specific TCF7L2 alterations as potential biomarkers of poor prognosis and predictors of disease-free survival.

No conflict of interest.

E12. Influence of EGF+61G>A and TGFB+869T>C polymorphisms in renal cell carcinoma development: the link to circulating microRNAs

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Introduction: The epidermal growth factor (EGF) is responsible for the activation of intracellular signal transducers responsible for cell-cycle progression, cell motility, angiogenesis, inhibition of apoptosis. However, cells can block theses effects activating opposite signaling pathways, such as the transforming growth factor beta 1 (TGFβ1) pathway. Thus changes in expression levels of EGF and TGFB1 in renal cells might modulate the

renal cell carcinoma (RCC) development, in consequence of changes in regulatory elements of signaling networks such as the microRNAs (miRNAs). Our purpose was to investigate the synergic role of EGF+61G>A (rs4444903) and TGFB1+869T>C (rs1982073) polymorphisms in RCC development. *Material and Methods*: Genetic polymorphisms were studied by allelic discrimination using real-time PCR in 133 RCC patients vs. 443 healthy individuals. Genotypes of the two polymorphisms analyzed were combined into three profiles considering the functional consequence of the polymorphisms in the modulation of cell proliferation The circulating EGF/EGFR-MAPK-related miR-7, miR-221 and miR-222 expression was analyzed by a quantitative real-time PCR in plasma from 22 RCC patients vs. 27 healthy individuals. *Results and Discussion*: The intermediate/high genetic proliferation profile was associated with RCC progression (OR=6.02, P=0.053), patients carriers of this genetic profile present a significantly reduced time-to-progression and a higher risk of an early relapse compared with the low genetic proliferation profile carriers (HR= 8.8, P= 0.038) with impact in a lower overall survival (Log rank test, P=0.047). The RCC patients presented higher circulating expression levels of miR-7 than healthy individuals (2-$\Delta\Delta$Ct=6.1, P<0.001). Moreover, the intermediate/high genetic proliferation profile carriers present an increase in expression levels of miR-7 and miR-221/222 during the RCC development and this increase is not observed in low genetic proliferation profile. The stimulus to angiogenesis, cell-cycle progression and tumoral cells invasion, through activation of EGFR/MAPK signaling pathway in intermediate/high proliferation profile carriers is associated with an early disease progression, resulting in a poor overall survival. We also demonstrated that the intermediate/high proliferation profile is an unfavorable prognostic factor of RCC and miR-7, miR-221/222 expressions may be useful phenotype biomarkers of EGFR/MAPK activation.

No conflict of interest.

E13. Nucleolin in lung cancer – an emerging target to overcome stroma-mediated anti-angiogenic resistance

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Introduction: Recognizing the limitations associated with chemotherapy and the possibility of interrupting a tumor vascular network, there has been an enormous interest in targeting the tumor vasculature and much effort has been directed towards the development of agents that disrupt angiogenesis. In addition to the target accessibility, the endothelial cells of the tumor-associated vasculature have superior genetic stability than cancer cells. Anti-angiogenic drugs, such as bevacizumab, have become a standard treatment option in lung cancer. Despite some clinical successes with these inhibitors, disappointing results have been reported with a substantial number of cancer patients being or becoming resistant to anti-VEGF therapies. Emphasizing this problem, have been described that beyond to the cancer cells themselves, several host factors and stromal components play an important role in resistance to bevacizumab. Aims: Validate nucleolin as a therapeutic target for a previous developed F3 peptide-targeted liposomes containing doxorubicin (DXR) in human lung cancer models resistant to bevacizumab and ultimately to assess the potential to overcome anti-angiogenic resistance; assess nucleolin expression in patients-derived lung cancer. Experimental design/Results/Discussion: Aiming at confirming the potential application of a previously developed nanoparticle on lung cancer, cellular association studies were carried out using flow cytometry. Cell lines with different types of resistance to the anti-angiogenic drug bevacizumab (A549, H1975 and H441 cells) were incubated with fluorescently-labeled liposomes, either non-targeted (SLpH), or targeted by a non-specific (SLpHNS) or F3 (SLpHF3) peptide, for 1 h at 4 or 37ºC. Data demonstrated binding and internalization of the F3 peptide-targeted liposomes by lung cancer cells, suggesting a ligand-specific interaction, in an extent of association that was 30 to 170-fold higher than the one observed with the other tested controls. Improved association and intracellular delivery of encapsulated DXR enabled by F3 peptide-targeted liposomes, justified a maximum of 9.6-fold increase of DXR cytotoxicity relative to the activity of DXR delivered by the non-targeted counterparts. In order to assess the clinical potential of the developed nucleolin-targeted strategy, immunohistochemistry of human specimens derived from lung cancer patients with surgical staged disease has been performed, aiming at investigating the expression of the mentioned protein. Results generated so far

revealed that nucleolin was highly expressed in different types of cells in the tumor microenvironment of patient-derived lung tumors, in a tumor-specific manner. The generated results render an important indication of the therapeutic potential of the tested nucleolin-targeted strategy against bevacizumab-resistant lung cancer. *Acknowledgements:* This work was supported by grants QREN/FEDER/COMPETE (Ref. 23240) and PEst-C/SAU/LA0001/2013-2014. Ângela Valério-Fernandes is a graduate student from the PhD programme on Biomedicine and Experimental Biology from the Center for Neuroscience and Cell Biology, University of Coimbra (FCT fellowship reference: SFRH/BD/51191/2010).

No conflict of interest.

E14. Lung cancer: reporting the reality of an oncology practice

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Introduction Lung cancer is a worldwide cause of death, estimated to be responsible for 1,59 million deaths each year. Despite recent advances in available therapeutics, it still has a dismal prognosis with less then 15% of advanced lung cancer patients alive at 5 years from diagnosis. In order to improve what we can offer these patients, we need to better know our own reality. Objectives and Methods Therefore, we primarily aim to describe a series of patients referred patients to the oncology department of Centro Hospitalar de Lisboa Central (CHLC) in a 18 months period (between January 2011 and June 2012), reporting frequencies of lung cancer subtypes and patients characteristics. Secondarily, we explore impact of several demographic, histological and clinical factors on survival, using t-test and linear regression for comparisons between groups. The statistical software used was Stata v.13. Results We reviewed 109 lung cancer cases referred to the medical oncology department in CHLC. The mean age at diagnosis was 63 years (SD 10 years) and 80,0% were males. Of 73 patients with available records on smoking history, 49 were current smokers and 20 reported having quit by the time they were diagnosed, with a mean of 64 pack-per-year smoked considering both groups (SD 37 ppy). Regarding histology, 10,1% were small cell carcinomas, 82,6% non small cell carcinomas (NSCLC) and 6,4% had other histologies that did not fit in previous categories. Among NSCLC, 24,7% were squamous cell carcinomas and 75,3% were adenocarcinomas. Staging according to AJCC 7th edition was distributed as follows: 79,5% had advanced disease (stages IIIB and IV), 7,7% IIIA, 6,8% IIB, 1,0% IIA, 2,9% IB and 1,9% IA. In a median follow up period of 35,5 months (min 27,5, max 43,5) 86 deaths occurred. This corresponds to 21% of patients alive at 3 years. The median survival for patients with advanced disease was 5,3 months. Linear regression analysis (crude and multivariate) considering survival as the dependent variable and independent variables histology, age, sex, smoking history and having been treated with chemotherapy only found significance for OS in relation with chemotherapy (p<0.001 in all models). Discussion We have to underline that all patients in this database were referred for oncology: patients are mostly referred from a multidisciplinary meeting, if decision is to do chemotherapy (which selects more advanced AJCC stages). One must also discuss the fact that chemotherapy is associated with survival: only patients with better performance status are proposed for chemotherapy and also those with too aggressive disease may not live long enough from diagnosis to start active treatment, so we report association, not causation. This is a small sample retrospective study, with all inherent limitations, but we believe that reports like this are valuable for oncologists to know what is the reality of their daily practice.

No conflict of interest.

E15. B2-microglobulin predicts the clinical behavior of myelodysplastic syndromes, and has prognostic value in association with LDH

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Introduction: Beta2-microglobulin (B2M) is a major histocompatibility complex class I component, whose serum levels increase with immune activation, rapid cellular turnover and renal failure. It has been suggested that, in myelodysplastic syndromes (MDS), increased B2M levels associate with decreased overall survival (OS) and an increased risk of progression, improving the discriminatory power of the International Prognostic Staging System (IPSS). Despite outcomes studies, the association between B2M levels and clinical characteristics at diagnosis has not been thoroughly described, and it has not been included into any prognostic model. Methods: A prospective study of 83 newly-diagnosed pre-treatment MDS patients, between 2004 and 2014. B2M levels were considered elevated (High-B2M) when >2.53 ug/mL. No patients had renal failure (creatinine >2.0 mg/dL). Results: The median B2M was 2.59 ug/mL; 50.6% of patients had High-B2M, with a mean of 4.32+/-0.28 ug/mL. While there were no differences in B2M between genders (3.26+/-0.30 ug/mL in males vs 3.12+/-0.25, p=NS), the mean age was higher in High-B2M (76.6+/-1.1 vs 71.2+/-1.6 years, p=0.004). Patients with High-B2M had lower hemoglobin levels (10.1+/-0.3 vs 11.0+/-0.3 g/dL, p=0.03); however, there were no differences in platelet (142+/-12.7 vs 132.7+/-17.7 G/L, p=NS), neutrophil (2.5+/-0.3 vs 2.0+/-0.2 G/L, p=NS) or leukocyte (4.7+/-0.4 vs 4.1+/-0.4 G/L, p=NS) counts. High-B2M had a five-fold increase in bone marrow blastosis >5% (4.9 vs 21.4%, p=0.03), while RAEB-1/2 and CMML were three times more likely than other MDS subtypes to present with High-B2M (75 vs 25%, p=0.02). High-B2M had a higher incidence of hypoalbuminemia (25.8 vs 0.0%, p=0.004). There were no associations between B2M and vitamin B12, erythropoietin levels or hyperferritinemia, nor with lactate dehydrogenase (LDH), another marker of cancer bulk and cell turnover. With a median follow-up of 33.7 months, the median OS for Normal-B2M and High-B2M were 57.5 and 54.0 months, respectively (p=NS), with a 2-year OS of 89.3 vs 83.6%, p=NS. The addition of LDH into the survival model resulted in 4 prognostic groups with significantly different survivals: 23.8m with High-B2M/LDH, 36.5m with High-LDH/Normal-B2M, 58.7m with High-B2M/Normal-LDH, and median not reached with Normal-B2M/LDH (p=0.03). Discussion: We have shown that the patients with high B2M at diagnosis are older, with more severe anemia, hipoalbuminemia and a greater incidence of marrow blastosis over 5%; these negative prognosticators, taken together, could help explain the influence of B2M on outcomes. The lack of a correlation between the two biomarkers of bulk and turnover (LDH and B2M) suggest that they evaluate different aspects of neoplastic proliferation, apoptosis and immune response, as confirmed by their ability to stratify 4 prognostic subgroups with differences in survival of over a year between each group.

No conflict of interest.

F1. Vorinostat Impairs Cellular Viability, Differentiation, Redox Homeostasis and Gene Expression in BCR-ABL-Negative MPN

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Introduction: The classical BCR-ABL-negative myeloproliferative neoplasms (MPN) are characterized by increased proliferation of hematopoietic precursors in the bone marrow resulting. Despite the description of several mutations, these do not completely explain the pathophysiology and clinical heterogeneity of MPN. Epigenetic modifications, like histone acetylation, play key roles in the pathogenesis of several hematological malignancies, and treatment of such disorders with histone deacetylase inhibitors showed efficacy in several hematological malignancies. HDAC inhibition has demonstrated some efficacy in MPN patients. In order to analyze the effects of HDAC inhibitors in MPN, we analyzed the impact of Vorinostat on the cellular biology of MPN. *Material and Methods*: MPN bone marrow samples were collected at diagnosis, mononuclear cells were then isolated by gradient separation and used for culture experiments and lysed for RNA extraction. MPN primary cells and MPN derived cell lines were incubated with Vorinostat and at different time points the cells were lysed for gene expression analysis and cellular differentiation, apoptosis and Reactive Oxygen Species (ROS) levels was analyzed by Flow Cytometry. *Results and Discussion*: Vorinostat induces apoptosis in MPN cells, and incubation of primary MPN bone marrow samples with Vorinostat induced apoptosis, blocked differentiation and also diminished ROS levels. These effects were most marked in the monocytic lineage, a population which expresses the highest levels of ROS. Vorinostat also reduced the levels of GPA and CD61, markers of erythroid and megakaryocytic differentiation, respectively. At the molecular level we identified 9 genes (BIRC3, TNFRSF9, DLL4, IL1B, CDKN1A, FOSL1, CREL, SERPINB9 and EGR1) whose expression increased for at least 4 fold and 2 genes (HIP1 and DTX1) whose expression decreased by at least 0.5 fold in MPN patients relative to normal bone marrow samples. Interestingly, incubation of Vorinostat in MPN cell lines increases the expression of such genes suggesting that can be used to monitor Vorinostat response in MPN patients. Moreover, Vorinostat incubation in MPN cell lines increased the expression of genes associated with apoptosis and growth arrest while decreasing the expression of genes associated with proliferation, growth arrest and JAK-STAT signaling pathway. Our results show that Vorinostat incubation impairs MPN cellular differentiation and reduces ROS and cellular viability, possibly through the down-regulation of genes associated with cellular proliferation, particularly the JAK-STAT target genes, and up-regulation of genes important for apoptosis and growth arrest. Our results point towards the potential role of Vorinostat (and possibly other HDAC inhibitors) in the treatment of MPN. This potential would require clinical trials to investigate its efficacy.

I/we (In case of co-authors) have an interest in relation with one or more organisations that could be perceived as a possible conflict of interest in the context of the subject of this abstract. The relationship(s) is (are) summarised below:Dr Almeida receives consulting fees from Celgene and Novartis and is on the board of speakers for Bristol-Meyer Squibb, Shire and Amgen. The remaining authors of this abstract have no competing financial interests in relation to the work described here.

F2. Bone Marrow protects BCR-ABL-Negative Myeloproliferative Neoplasms from Ruxolitinib- and Vorinostat-Induced Apoptosis

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Introduction: The classical BCR-ABL-negative myeloproliferative neoplasms (MPN) are characterized by increased proliferation of hematopoietic precursors in the bone marrow. However, despite the understanding of MPN biology, there is still no curative treatment for MPN. The discovery of JAK-STAT constitutive activation in MPN patients led to the development of clinical studies targeting MPN with Ruxolitinib, a JAK1/2 inhibitor, which reduced splenomegaly and constitutional symptoms MPN patients, but failed to eradicate the malignant clone. In alternative, histone deacetylase inhibitors, like Vorinostat have shown success in the treatment of hematological malignancies but their efficacy in MPN is limited. Given that Ruxolitinib and the Vorinostat fail to eradicate the neoplastic clone in MPN patients, we tested whether the bone marrow could confer resistance by preventing their cytotoxic effects on MPN cells. *Material and Methods*: MPN derived cell lines (SET-2 and HEL) were cultured alone or in the presence of HS-5 cells (bone marrow stromal cell line) and incubated with Ruxolitinib and Vorinostat. At different time points, cellular viability, gene expression and also activation of specific signaling pathways was analyzed. *Results and Discussion*: Treatment of SET-2 and HEL cells with Vorinostat and Ruxolitinib promoted apoptosis and decreased proliferation. Importantly, apoptosis was significantly abrogated when MPN cells were cultured in the presence of HS-5 cells or HS-5 conditioned medium. These effects correlated with the altered expression of genes associated with inflammatory processes, apoptosis and proliferation (CDKN1A, IER3, TNFRSF9, IL1B, XIAP, CCND1), and with the activation of signaling pathways important for cellular homeostasis, like PI3K-Akt/PKB-mTOR, MAPK-JNK, JAK-STAT and NF-κB as shown by increased phosphorylation of its downstream targets. Overall, we show that bone marrow protects MPN cells from the cytotoxic effects of two clinically effective pharmacological agents. This protective effect is likely achieved, through the up-regulation of genes that inhibit apoptosis and also through the activation of pro-survival signaling pathways such as PI3K-PKB-mTOR. We did not observe any effects of the bone marrow on proliferation or differentiation, suggesting that the main effect of stromal cells is to prevent apoptosis of the neoplastic cells. Here, we identify a possible cell non-autonomous mechanism by which Ruxolitinib and Vorinostat fail to eradicate the neoplastic clone in MPN patients. We believe that by identifying the molecular mechanism induced by the bone marrow we could define novel therapeutic strategies that potentiate the effects of Ruxolitinib and Vorinostat in MPN.

F3. Evaluation of tumor cells' biomechanical properties for assessing the chemotherapy efficacy of anticancer peptides

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Introduction There have been important achievements regarding cancer chemotherapy, however new therapeutic approaches are needed to increase selectivity and efficacy and decrease resistance levels. Therapies based on biologically active peptides such as antimicrobial peptides (AMPs) are an interesting source of new potential anticancer molecules. Indeed, in addition to combining decreased resistance development and citotoxicity, AMPs have proved to be anticancer peptides (ACPs) with tumoricidal activity against human cancer cells. The human neutrophil peptide-1 (HNP-1) is an endogenous AMP that has been implicated in different cellular phenomena such as tumor proliferation. The presence of HNP-1 in the serum/plasma of

oncologic patients turns this peptide into a potential tumor biomarker. The present work details the effects of HNP-1 on solid and hematological tumor cells while highlighting the importance of monitoring cells' biophysical and nanomechanical properties for assessing peptide's efficacy and selectivity. Understanding the mode of action of ACPs such as HNP-1 will certainly potentiate the development of new drugs for cancer treatment. *Material and Methods* The experimental approach of this work relied on the combination of spectroscopic and atomic force microscopy (AFM) techniques for probing solid and non-solid tumor cells' viability and biomechanical properties before and after peptide contact. The effects of HNP-1 on human prostate adenocarcinoma and human acute lymphoblastic leukemia cells' biophysical and nanomechanical properties were followed by flow cytometry, surface charge measurements and AFM (imaging and single cell force spectroscopy). *Results and Discussion* Flow cytometry, surface charge density and AFM studies reveal HNP-1 preferential activity toward solid tumors. The particular use of AFM in oncology allows the study of certain parameters such as the cell membrane structure, morphology and stiffness and consequently pinpoints the cellular structures damaged by the chemotherapeutic agent. These damages ultimately dictate the ability of the tumor cells to migrate and invade different organs after treatment. Cell death occurs without full neutralization of the cancer cell membrane, a distinctive characteristic for ACPs' mode of action. Our results also point to a translocation of the peptide to the interior of the cell and a posterior nuclear DNA and cytoskeleton damage. Finally, the tumor cell collapses due to an apoptotic process. *Acknowledgements:* This work was supported by a grant from Laço. D.G. and J.F. acknowledge FCT-MEC for fellowships SFRH/BPD/73500/2010 and SFRH/BD/70423/2010, respectively.

No conflict of interest.

F4. Azurin, a therapeutic protein that interferes with oncogenic signaling and blocks tumor progression

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Azurin is a small protein from the bacterium Pseudomonas aeruginosa. In the last years, this protein has been studied as a new anti-cancer therapeutic agent. Recently, we have shown that azurin is capable to block invasion of breast cancer cell lines in models where it depends on the overexpression of P-cadherin (Bernardes et al. 2013). This transmembrane protein was found to play an important role for a subset of breast cancers, being a poor prognosis factor associated with increased migration and invasiveness, and is also as a marker for breast cancer stem cells (Ribeiro et al. 2010; Vieira et al. 2012). Also, we observed that azurin impairs a signaling pathway associated to P-cadherin overexpression, mediated by FAK/Src phosphorylation (Bernardes et al. 2013). We have further performed a genome-wide expression analysis of azurin-treated cells, revealing that azurin up-regulated endocytic processes, concomitantly with the decrease in the expression of cell surface receptors and associated signaling, and decreased adhesion to the Extracellular Matrix (ECM). These observations were confirmed by the decrease of integrin subunit receptors (α6, β1 and β4 integrin subunits) and a decrease in the ability to form mammospheres, an important parameter of cancer resistance to therapies (Bernardes et al., 2014). We hypothesize that azurin, exerting its effects through lipid raft regions, alters the communication of cancer cells with their surrounding microenvironment with important consequences at several downstream signaling pathways. Therefore, by disrupting lipid rafts which harbor a variety of signaling components such as integrin subunits or growth factor receptors, this new therapy may target important subset of cancer cells, such as cancer stem cells, alone or in combination with other therapies, improving clinical outcome. Additional research to prove this hypothesis is being now pursued in our lab.

No conflict of interest.

F5. Cell death and oxidative stress profile in human hepatocellular carcinoma cell lines after treatment with amniotic membrane

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Introduction: Hepatocellular carcinoma (HCC) is a primary liver cancer. HCC is a leading cause of cancer death worldwide, mainly due to its ability to withstand conventional therapies. For the last years, the regenerative properties of human amniotic membrane (hAM) were explored in the treatment of liver diseases. Several studies indicated hAM as a possible option in the treatment of liver diseases, particularly in the treatment of hepatic cirrhosis and fibrosis. Nevertheless, until now, there are still no studies exploiting the application of hAM in the treatment of HCC. So, the aim of this study is to evaluate the effect of hAM proteins extracts (hAMPE) treatment in cell death and oxidative stress in three human HCC cell lines. *Material and Methods*: hAM were obtained from cesarean section according to the guidelines of Ethical Committee of Coimbra Hospital and University Centre (Coimbra, Portugal). hAM were washed with phosphate buffered solution and subjected to mechanical actions in order to extract proteins (hAMPE), which were quantified using Nanodrop®. To study the effect of hAMPE in HCC, studies were performed in three human cancer cell lines: HuH7 (mP53), HepG2 (wP53) and Hep3B2.1-7 (nP53). Cells were incubated with 1µg/µL of hAMPE for 72h. After, cellular morphology was analyzed through May-Grunwald-Giemsa staining. Oxidative stress and mitochondrial membrane potential was evaluated through a fluorescence based-assay. *Results and Discussion*: Through cellular morphology analysis, hAMPE seems to be able to induce cell death in all HCC cell lines. In HuH7 cell line, hydrogen peroxide increased after the treatment with hAMPE, being this response accompanied by a reduction of superoxide radical. In this cell line, the mitochondrial potential does not seem to be affected by the treatment. Moreover, in the cell line HepG2 and Hep3B2.1-7 there seems to be an increase of superoxide radical and a decrease in hydrogen peroxide. Mitochondrial dysfunction does not occur in HepG2 cell line, unlike what occurs in Hep3B2.1-7 cells. Through these results, it can be concluded that hAMPE may be useful in the treatment of HCC. However, the action mechanisms of this treatment may depend on the specific tumor profile.

No conflict of interest.

F6. Dll4Fc concurrent systemic therapy improves tumor drug delivery with considerable gain of therapeutic efficacy

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Introduction- Anti-Dll4 therapy was unveiled as a paradox to the world of oncology. It works as a pro-angiogenic therapy but leads to tumoral neo-vasculature that is too malformed to be able to transport blood, leaving the tumor hypoxic and growth limited. Anti-angiogenic therapies have been proposed for the treatment of prostate cancer but clinical trial results have not been encouraging so far. Nevertheless, very little is still known on how to optimize these novel therapies in this setting. Also, it is already known that tumors treated with the currently approved anti-angiogenic drugs can develop resistance to such treatments. Another current issue with anti-angiogenic therapies lies with their effect on tumor drug delivery, with different reports stating either an improvement or impairment on this crucial aspect of current therapeutic modalities. This means that several alternative angiogenesis-based therapies have to come up, to complete existing treatments and improve outcome. *Materials and Methods-* In this work we used the TRAMP mouse prostate cancer model

to assess Dll4Fc, an inhibitor of Dll4-Notch1 binding, therapy effectiveness at two different therapeutic timepoints, when used alone or concurrently with metronomic chemotherapy. The mice were sacrificed at 18 and 24 weeks of age, corresponding to an early or late disease detection protocol. The prostates were collected and evaluated regarding several parameters such as: weight, histopathological classification of the tumor, vascular phenotype, apoptosis, proliferation and tumor drug accumulation. *Results and Discussion*- Results revealed that Dll4Fc therapy was able to reduce prostate cancer growth in all stages of development. Despite this we found that Dll4Fc therapy was more efficacious in advanced tumors, when neoangiogenesis is more highly activated. Although tumor grading did not change from Dll4Fc therapy administration, apoptosis was increased and proliferation was reduced from Dll4Fc therapy. Finally, concurrent administration with doxorubicin improved the therapeutic outcome by increasing doxorubicin tumor accumulation.

No conflict of interest.

F7. The cell response to traditional chemotherapeutic agents - relevance of BER pathway

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Introduction: The different response to chemotherapeutic treatment is subject to wide interindividual variability, which is expressed not only as differences in severity and type of toxicity, but also as differences in effectiveness. Genes associated with DNA repair and drug response can be useful to understand the mechanisms of drug resistance, identify promising therapeutic targets and improve personalized treatment. Being BER the most accurate pathway repairing damaged bases induced by oxidizing agents, our main question is: are the lesions induced by chemotherapeutic drugs and repaired by BER pathway affected by polymorphic genes compromising the therapeutic efficacy? *Material and Methods*: For this study were selected HeLa SilenciX cell lines from Tebu-Bio, silenced for PARP1 and XRCC1 genes and the control one. Cells were grown to subconfluence and were treated with several concentrations of Paclitaxel (PAX), Doxorubicin (DOX) and 5-Fluorouracil (5-FU) for 48 hours. The effect of drug exposure on cell lines was carried-out by flow-cytometric analysis for apoptosis detection by APC-annexin V. Results: Our results showed that the concentrations under study influence the cellular response decreasing cell viability through the increase of apoptotic cellular states. XRCC1 HeLa SilenciX exposed to PAX showed an increased dispersion in early and later apoptotic states compared with HeLa control cell-line. The cells exposed to DOX showed formation of clusters sub-populations specially in later apoptotic state, which is much more evident in HeLa silenced for XRCC1 gene compared with the control cell line, existing more cell death in higher concentrations. Concerning the exposure to 5-FU, major differences were not observed between silenced XRCC1 and control cell-lines. PARP1 HeLa SilenciX showed a dispersion cloud more pronounced compared with the control, after exposure to PAX, increasing the cellular dispersion in later apoptotic state. The percentage of death cells was higher in cell line silenced for PARP1. The exposure to DOX revealed that HeLa silenced for PARP1 gene exhibit an increment of cells in later apoptotic quadrille and more death cells. 5-FU exposure showed an increment of cells in apoptotic states. Discussion: Our results revealed that the HeLa cell lines silenced for XRCC1 and PARP1 genes are more sensitive to chemotherapeutic drugs, suggesting that variations in those genes can alter the efficacy of those agents.

No conflict of interest.

F8. Human amniotic membrane-derived proteins: what are the action mechanisms against hepatocellular carcinoma?

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Introduction: Some studies point out the potential anticancer effects of human amniotic membrane (hAM). Although interesting, this research field is quite recent and therefore the cellular mechanisms responsible for hAM anti-cancer properties still poorly understood. The potential of hAM in the treatment of liver diseases has been explored in the past decade and several studies indicate that in a near future hAM may become one of the biomaterials for the treatment of liver diseases. Hepatocellular carcinoma (HCC) has a highest incidence and mortality worldwide, mainly due to the lack of effective treatments in more advanced stages of the disease. So, the aim of this study is to evaluate the effect of hAM protein extracts (hAMPE) in two human HCC cell lines. *Material and Methods*: hAM were obtained from healthy women with informed consent according to the guidelines of Ethical Committee of Coimbra Hospital and University Centre (Coimbra, Portugal). After the receipt, hAM were washed with phosphate buffered solution and subjected to mechanical actions in order to extract proteins, which were quantified using Nanodrop®. To study the effect of hAMPE in HCC, studies were performed in two cell lines: HuH7 (mP53) and HepG2 (wP53). Cells were incubated with 1µg/µL of hAMPE for 72h. After this period, sulforhodamine B and crystal violet assays were performed to assess protein and DNA synthesis, respectively. In order to evaluate DNA damage, comet assay was carried out. Cell cycle was analysed through propidium iodide/Rnase incorporation assay by flow cytometry. P53 and β-catenin expression was analysed through western blot. *Results and Discussion*: Protein content decreased 89.5% and 90.7% after treatment in HuH7 and HepG2 cell lines, respectively. DNA content also decreased after treatment: 54.7% in HuH7 and 68.1% in HepG2 cell line. DNA of HuH7 cell line not seems to be affected by hAMPE. However, there is a 13 times higher damage in treated than in the control HepG2 cells. hAMPE induced a delay in G2/M cell cycle phase in both cell lines. P53 and β-catenin expression decreased in the cell lines under study after treatment with hAMPE. It appears that hAMPE can have multiple cellular targets in HCC cells. It is important to characterize hAMPE in the future and cross this information with the observed cellular mechanisms, thus contributing to the clarification of hAM potential in oncology.

No conflict of interest.

F9. Photodynamic therapy in lung cancer cells

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Introduction: Lung cancer (LC) is leading death cause of cancer worldwide with annual mortality over 1 million. Photodynamic therapy (PDT) relies on administration of a photosensitizer (PS) whose effect is triggered only with light. The PS causes no damage to normal tissues; however, irradiation leads to production of highly reactive oxygen species (ROS) that trigger cell death. PDT was already applied to LC and it preserves lung function, can be repeated and combined with other therapies, being useful in palliation and in the eradicated of stages 0/1 centrally located LC. With this work we show the promising *in vitro* pre-clinical studies of a family of new PS synthetized by our group in lung cancer cell lines. *Materials and Methods*: The PS evaluated were a bromated hydroxyphenyl porphyrin, BBr2HPP, a bromated hydroxyphenyl chlorine, BBr2HPC, and a bromated hydroxyethyloxy porphyrin, BBr2HPP-O (CH2)2OH. The human non-small cell lung cancer cell lines H1299 and A549 were propagated according to standard cell culture conditions. Cells were incubated with several concentrations of the PS and irradiated 24 hours later with a total energy of 10J. The half maximal inhibitory concentration (IC50) was calculated 24 and 48 hours after irradiation by colorimetric test MTT. Further flow cytometry studies were performed in the H1299 cells treated with 50 and 200 nM of BBr2HPC prior irradiation with 10J, in order to evaluate viability and types of cell death, mitochondrial disruption, production of ROS, and the antioxidant defense GSH. *Results and Discussion*: The results obtained with MTT assay show that all the PS are cytotoxic at low concentrations. For the A549 cell line dose-response curves obtained allowed to calculate the IC50s being approximately 50nM for BBr2HPP, 34nM for BBr2HPC and 70nM for BBr2HPP-O (CH2)2OH. For the H1299 cell line dose-response curves obtained allowed to estimate the IC50s being approximately 28nM for BBr2HPP, 34nM for BBr2HPC and 86nM for BBr2HPP-O (CH2)2OH. Preliminary flow cytometry results

showed the reduction of metabolic activity is due to decrease of cell viability being the main pathway of cell death by late apoptosis and necrosis. Disruption of mitochondrial membrane potential was observed. Concerning intracellular ROS production, the treatment with 50nM there is an increase of superoxide anion. Relative to the treatment with 200nM BBr2HPC there is an imbalance where peroxides are reduced and supeoxide anion is augmented. For both concentrations an increase in the GSH defense is observed. The PS are cytotoxic in a nanomolar range of concentrations in the human lung cancer cell lines. BBr2HPC-PDT confirms induction of cell death in a human non-small cell lung cancer cell line through a ROS dependent pathway. This work is supported by Portuguese Foundation for Science and Technology (PTDC/BIM-ONC/0979/2012) and LPCC/Pfizer Research Grant to M. Laranjo.

No conflict of interest.

F10. C1236T (rs1128503) polymorphism in the *ABCB1* gene in chemotherapeutic response and clinical variability in the breast cancer

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Introduction: Response to chemotherapy has been evaluated and was associated with polymorphisms in different genes, which may be associated with metabolism of drugs, independentlyof the drug applied. Among the genes, ABCB1 [ATP-Binding Cassette, Sub-Family B (MDR/TAP), Member 1], which encodes the P-glycoprotein, with important function of efflux drug, has been studied. In the gene, the C1236T polymorphism (rs1128503) has been evaluated and presented intriguing results, including in the breast cancer. In this context, the objective of the present study was to associate the response to chemotherapy in patients with breast cancer and the clinical variability of the disease with the C1236T polymorphism in the ABCB1 gene. *Material and Methods*: The study enrolled 100 female patients with breast cancer. The clinical markers included were: race, use of oral contraceptive, breastfeeding, hormone replacement therapy, smoking, alcoholism, hypertension, diabetes mellitus, histological type, histological and nuclear grades, tumor classification by TNM, clinical stage, radiation therapy, hormone therapy, patient status, age, menarche, menopause, age at first live birth, height, weight, estrogen and progesterone receptors. Statistical analysis: likelihood ratio $\chi2$, Fisher exact, Mann-Whitney and Kruskal-Wallistests. $\alpha=0.05$. *Results and Discussion*: For genotypic analysis, we obtained the values of 39 (39%), 45 (45%) and 16 (16%), respectively for the genotypes, CC, CT and TT. The polymorphism was in Hardy-Weinberg equilibrium (p>0.05). In the present study, we found no association of the polymorphism analyzed with the clinical presentation of patients with breast cancer, except for race (Caucasian versus non-Caucasian) patients (p= 0.048), however there was no positive odds ratio after the data correction. At the same time, the later onset of menopause (p= 0.023) (CC= 49.32±3.87, median= 50, min and max= 40 and 54, CT+TT= 45.85±6.45, median= 46, min and max= 30 and 56) and minor progesterone receptors (p= 0.037) (CC= 43.68±28.42 and median= 40, min and max= 5 to 100; CT+TT= 66.15±24.38, median= 70, min and max= 5 and 95) for the breast cancer patients was observed. However, there was no association with response to chemotherapy used in patients followed in our center (p= 0.598). In conclusion, in this study, the C1236T polymorphism was associated with patient's race, time to onset of menopause and the percentage of progesterone receptors in patients with breast cancer.

No conflict of interest.

F11. Butyrate and Irinotecan: a new approach for colon cancer treatment

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INTRODUCTION: High levels of dietary fiber are related with a lower risk for developing colon cancer (CC). Microbial fermentation of fiber by the gut produces short chain fatty acids, such as butyrate. Butyrate is an important energy source for colonocytes and it plays an important role in maintenance of the colon homeostasis. It was reported that butyrate may be a chemopreventive agent. Irinotecan is used as second-line treatment, but, there remains the doubt about the benefits and risks, namely, the large interindividual variability in pharmacogenetic behavior. The use of natural compounds to turn the resistant cells more sensitive to chemotherapy seems to be a possible solution. The aim of this study is to evaluate the therapeutic potential of the combination of butyrate and irinotecan. *MATERIAL AND METHODS*: C2BBe1, WiDr and LS1034 cells were incubated with increasing sodium butyrate (1-50 mM) and irinotecan (0.1-100 μM) concentrations, separately and in combination. In order to obtain the IC50 (half maximal inhibitory concentration) after 48, 72 and 96 h, cell proliferation was evaluated through MTT assay. Flow cytometry was performed to study the combination effect on cell viability and types of death, BAX and BCL-2 expression and alterations on mitochondrial membrane potential (MMP). *In vivo* studies with Balb/c nu/nu mice were conducted through inoculation of WiDr cells on their back. During several days the body weight and tumor size were monitorized, in order to ascertain the effect of the combination on tumor growth. *RESULTS AND DISCUSSION*: It was observed that as butyrate incubation time increases, cell proliferation decreases, being obtained lower IC50 values. The combination of butyrate and irinotecan significantly decreased cell proliferation compared to monotherapy, in all cell lines, being LS1034 cells the most sensitive to the combination at longer incubation times. In all cell lines, combination of butyrate and irinotecan also decreased cell viability. Preliminary results also revealed an increase of BAX/BCL-2 ratio and a decrease of MMP, most evident on LS1034 cells and that can be correlated with the apoptosis results. The data obtained *in vivo* suggest that butyrate and irinotecan combination synergistically inhibit tumor growth. Our study suggests that butyrate and irinotecan combination has a significant cytotoxic effect on the three CC cell lines and inhibit tumor growth. The use of natural compounds as butyrate in combination with chemotherapeutic agents can be a new solution for CC treatment.

No conflict of interest.

F12. Combination of Vitamin C and Irinotecan as a potential treatment against colon cancer

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Introduction: Colon cancer (CC) is one of the most dangerous forms of cancer. Despite toxicity caused by irinotecan, it is used as second-line treatment for CC. Several studies revealed that ascorbic acid (AA) can be a potential anticancer agent and it can contribute to the development of a promising therapy with reduced doses. AA can act as a pro-oxidant, promoting the formation of reactive oxygen species, such as hydrogen peroxide, which compromise cell viability. The aim of this study was to evaluate, *in vitro* and *in vivo*, the potential synergistic effect of a combination between AA and irinotecan. Methods: LS1034 and WiDr cells were incubated with increasing concentrations of AA and irinotecan, in monotherapy and in combination. Cell proliferation was evaluated through SRB assay after 24, 48, 72 and 96 hours of exposure. The half maximal inhibitory concentrations (IC50) and the combination index were determined. By flow cytometry it was evaluated, in LS1034 cells, the influence of the treatment on cell viability and the induced types of death, the alteration of mitochondrial membrane potential through the fluorochrome JC-1 and the expression of BAX and BCL-2 proteins. For *in vivo* studies, WiDr cells were inoculated on the back of Balb/c nu/nu mice. AA and

irinotecan were intraperitoneal injected separately or in combination. During 14 days, body weight and tumor size were monitorized. *Results and Discussion*: In both cells lines, it was observed that when AA concentration increases, cell proliferation decreases, being obtained lower IC50 values for WiDr cells. The IC50 values of irinotecan when present in combination with AA significantly decreased compared to monotherapy, for all conditions, in two cells line. When a combination with a proportion of 50% of AA IC50 and 50% of irinotecan IC50 was performed, a 34-fold decrease on IC50 value of irinotecan was obtained for LS1034 cells, compared to monotherapy. A synergistic effect was only obtained in LS1034 cells at 48h and 96h. The combination of both drugs caused a decrease on cell viability and, consequently, cell death by apoptosis/necrosis increased. There was also an increase of BAX/BCL-2 ratio a decrease in mitochondrial membrane potential. The results obtained *in vivo* indicate that AA and irinotecan have a synergistic effect, inhibiting tumor growth. Our study suggest that high doses of AA combined with irinotecan allowed to obtain low rates of tumor cells proliferation and a synergistic effect in the multidrug resistant cell line, LS1034. A synergistic effect was also observed in a mice model of CC. AA and irinotecan combination could be a promising solution for CC treatment with reduced side effects.

No conflict of interest.

F13. Combination of carboplatin and piroxicam leads to a synergistic interaction on two human urinary bladder cancer cell lines.

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Introduction Cancer is one of the most feared disease by Man1 and urinary bladder cancer ranks sixth in worldwide cancer incidence.2 Data compiled thus far suggests that the efficacy of conventional anticancer drugs can be enhanced by their use in combination with non-steroidal anti-inflammatory drugs.3 The purpose of this study was to evaluate the effects of carboplatin and piroxicam in isolation, and the combined effects of using both drugs on cell viability, on two human urinary bladder cancer cell lines (T24 and 5637). Furthermore, we also analyzed the type of interaction (synergic, additive or antagonistic) between both drugs. *Materials and Methods* Cells were maintained at a humidified atmosphere with 5% CO_2 at 37ºC. In order to evaluate the effect of carboplatin and piroxicam in cell viability, the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay was performed. Thus, cells were treated with carboplatin (0.05, 0.5 and 1 µM) and piroxicam (167, 333, 500 µM), either in isolation or in combination, over the course of 72 hours. For the study of interaction between carboplatin and piroxicam on cell growth inhibition, a combination index (CI) was performed using the data obtained from MTT assay. Drug combination studies were based on concentration effect curves generated as a plot of the fraction of unaffected cells versus drug concentration, in accordance to the Chou and Talalay method.3 Statistical analysis was performed using the SPSS 17.0 statistical software. The equality of variances was determined by using the Levene F test and the statistical significance of differences between treatments and control groups was compared by Dunnet's Multiple Comparison post-hoc test for the MTT results. *Results and Discussion* In isolation, the drugs decreased cell viability in the two cell lines in a dose-dependent manner, with statistical significant results ($p < 0.05$). The simultaneous treatment with both drugs leads to a greater inhibition of T24 and 5637 cell viability ($p < 0.05$). The CI50 values computed for T24 and 5637 cell lines were 0.65 and 0.17, respectively. Therefore, the combined use of carboplatin and piroxicam was synergistic on the growth inhibition of both cell lines (CI<1). In these experiments, dose reduction index50 (DRI50) of carboplatin and piroxicam were equal to 20 and 1.8 in T24 and 20 and 8.6 in 5637 cells, when the two drugs were used in combination. Our results suggest for the first time the beneficial effects of the carboplatin and piroxicam conjugation on T24 and 5637 urinary bladder cancer cell lines. 1 – Arantes-Rodrigues et al., Anticancer Research 33: 1273-96, 2013 2 – Burger et al., European Urology 63: 234-241, 2013 3 – Chou et al., Advances in Enzyme Regulation 22: 27-55, 1984

F14. Zeolites as drug delivery systems for encapsulation of 5-fluorouracil in an *in vitro* model of colorectal cancer

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Introduction Zeolites are solid inorganic crystalline materials comprised of silicon, aluminum, and oxygen in the three- dimensional structure. Were zeolites have several medical aplications, including the use as drug delivery systems (DDS). However, relatively few studies have explored the potential of zeolites as DDS for cancer applications. *Materials and Methods* The DDS efficacy was evaluated in two human colorectal carcinoma (CRC) cell lines, HCT-15 and RKO. Encapsulation of 5-fluorouracil (5-FU), a traditional drug used in the treatment of several cancers, was carried out with zeolites Faujasite in the sodium form, with different particle sizes (NaY, 700 nm and nanoNaY, 150 nm) and Linde type L in the potassium form (LTL) with a particle size of 80 nm. 5-FU was loaded into zeolites by liquid-phase adsorption. *Results and Discussion* 5-FU was successfully loaded into the zeolite structures with different particle sizes, NaY (700 nm) and nanoNaY (150 nm). Drug loading studies revealed higher encapsulation efficiency for NaY than nanoNaY. The release of the drug from the zeolite structures in buffer solution at pH = 7.4 and 37 °C followed the Weibull model. The effect of the zeolites and DDS on HCT-15 and RKO human colon carcinoma cell viability was evaluated. Zeolites alone presented no toxicity to both cancer cells, while all DDS allowed an important potentiation of the 5-FU effect on cell viability. To assess the interaction between the zeolites and the CRC cells, fluorescence microscopy assays were performed, providing evidence for zeolite-cell internalization. DDS based on zeolites were able to increase the efficiency of 5-FU, a widely used anticancer drug. We believe these systems should be further explored in other cancer models, e.g. *in vivo* models, to confirm the efficiency of the systems. References [1] N. Vilaça, et al. J. Mater. Sci. 46 (2011) 7511-7516. [2] R. Amorim, et al. Phys. Chem. C. 116 (2012) 25642-25650.

F15. Nucleolin-mediated targeting of breast cancer cell sub-populations with different stem-like phenotype

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Introduction: Breast cancer stem cells (CSC) are a cell sub-population with stem-like characteristics, presenting several fundamental deregulated signaling pathways, responsible for tumor growth and relapse, metastization, and active evasion to standard chemotherapy. The acknowledgment that CSC may originate from non-stem cancer cells (non-SCC), turning these into two relevant cell therapeutic targets, provided the necessary accessibility to the CSC niche. In this work, we aim at assessing the specificity of interaction of a nucleolin-mediated delivery strategy towards both CSC and non-SCC using the nucleolin-binding F3 peptide. *Materials and Methods*: Assessment of cellular association of the F3 peptide-targeted nanoparticle (targeting membrane nucleolin) with CSC enriched populations identified in triple negative breast cancer cell line has been performed by flow cytometry. Additionally, assessment of cellular association with mouse embryonic stem cells

(mESC) has also been performed. Cell sorting of putative CSC-enriched and depleted populations was performed in order to evaluate stemness potential using tumorsphere formation assay, tumorigenic potential and mRNA levels of Nanog, Oct4 and nucleolin by qRT-PCR. *Results and Discussion*: Collected data clearly demonstrated that the F3 peptide-targeted nanoparticle associated with both non-SCC, but preferentially, with putative CSC identified in human triple negative breast cancer cell line. Upon sorting, increased tumorigenic and tumorsphere formation capacity by putative CSC was observed, relative to CSC-depleted population, as well as increased mRNA levels of Nanog, Oct4 and nucleolin. In addition, the results suggested that overexpression of cell surface nucleolin per se could be useful for the identification of highly tumorigenic cells. Surprisingly, data also demonstrated that the developed strategy specifically associated with mESC according to pluripotency status. Overall, our results suggested a clear link between nucleolin expression (including cell membrane nucleolin) and the stem cell-like phenotype in breast cancer, namely in the triple negative molecular subtype, emphasizing the potential of nucleolin as a molecular target in both CSC and non-SCC. Acknowledgments Nuno Fonseca is recipient of a fellowship from the Portuguese Foundation for Science and Technology (FCT) (ref.: SFRH/BD/64243/2009). The work was supported by the grants InovC/UC (2013), QREN/FEDER/COMPETE (Ref. 30248/IN0617) and PEst-C/SAU/LA0001/2011. Additional funding was granted by FEDER/COMPETE/FCT PTDC/EBB-EBI/120634/2010 and PDTC/QUI-BIQ/120652/2010 grants and QREN: CENTRO-01-0762-FEDER-00204.

No conflict of interest.

F16. HER family of receptors are potential targets for therapy in cervical cancer: an *in vitro* and *in vivo* study

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Introduction: Despite technological advances, up to 35% of all cervical cancer patients will develop a metastatic disease. Infection of human keratinocytes by oncogenic HPV subtypes is critical for cervical carcinogenesis, however, it is not sufficient for cancer development. In the past two decades we have seen the successful development of Receptor Tyosine Kinases (RTK) signaling targeted drugs, expanding treatment options for cancer patients. However, actually there is no FDA approved molecular targeted therapies in cervical cancer mainly because the preclinical studies are scarce. Thus, the major aim of this work was to screen for effective molecular targeted therapies in cervical cancer cell lines and to find out potential predictive factors for therapy response, with focus in the HER family of receptors. *Materials and Methods*: In a panel of 4 cervical cancer cell lines (HeLa, SiHa, CaSki and C-33A) we screened for the activation of 42 different RTK by using an RTK-phospho array. Next, the tumorigenic effect of a panel of 6 different RTK inhibitors (cediranib, sunitinib, erloinib, lapatinib, imatinib and AST1306) was evaluated in the 4 cell lines using *in vitro* and *in vivo* approaches. Finally, we characterize the expression of HER family receptors (EGFR, HER2, HER3 and HER4) by immunohistochemistry in a series of 231 adernocarcinoma cervical cancer tissues. The correlations with clinicopathological data of patients was done by statistical analysis. *Results and Discussion*: By screening for the activation of 42 different RTK in human cervical cancer cell lines we observed that exclusively HER family of receptors, such as EGFR, HER2 and HER4 were activated. In accordance, we found by cytotoxic *in vitro* assays that only HER inhibitors (such as lapatinib and AST1036) were effective in the studied cell lines. Those results were validated by the *in vivo* Chick Chorioallantoic Membrane (CAM) Assay. Finally, in human cervical cancer tissues we found EGFR, HER2 , HER3 and HER 4 positivity in both cytoplasm and membrane of the cells being HER3 the most expressed (74,7% of cases) and EGFR (18.8% of cases) the less frequent. Importantly, we observed a statistically significant association ($p < 0.05$) between HER2 overexpression and poor prognosis of patients. In conclusion, we performed the first comparative study of HER family receptors expression in a large series of cervical carcinoma cases. We found that the expression HER2 could constitute an independent prognostic marker for this patients. Importantly, we demonstrate for the first time that exclusively HER family receptors are activated in cervical cancer cell lines, being HER targeted therapies effective in the blockage of cervical cancer cells aggressiveness. Thus, with this work we propose HER family inhibitors as potential anti-cancer agents in cervical cancer patients.

F17. Targeting cell surface nucleolin in metastatic and breast cancer

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Introduction: Breast cancer (BC) is the most common malignant disease among women. Despite the developments and great progress achieved in BC treatment, metastatic disease remains an incurable condition and is responsible for cancer-associated mortality. Therefore, new rationally designed therapeutic approaches, targeting both primary and metastatic disease, are required. Herein, we aim to validate cell surface nucleolin as therapeutic target for previously developed F3 peptide-targeted lipid-based nanoparticle. Material and Method: 4T1 and E0771 mouse cell lines were used as metastatic breast cancer (MBC) models. To investigate the relevance of nucleolin in this cell lines, cellular association studies were performed by flow cytometry; cells were incubated with fluorescently-labeled liposomes, either non-targeted (SLpH), or targeted by a non-specific (SLpHNS) or F3 (SLpHF3) peptide, for 1, 4 and 7 h at 37°C, and analyzed for cell-associated fluorescence. Aiming at assessing cytotoxicity, cells were incubated, as previously described, with targeted or non-targeted formulations encapsulating doxorubicin (DXR), for 1 h at 37°C. Cell proliferation was evaluated by resazurin assay, and IC50 values were determined from dose-response curves. To validate 4T1 mouse model of MBC and access clinical potential of the nucleolin-targeting strategy, immunohistochemistry of mouse and human breast tumors were performed in collaboration with Portuguese Institute of Oncology FG, EPE, Coimbra. *Results and Discussion*: A 12 to 35-fold increase in cellular association of F3 peptide-targeted liposomes by 4T1 and E0771 cells relative to non-targeted or targeted by a non-specific peptide, suggests a ligand-specific interaction mediated by surface nucleolin. Superior association led to improved intracellular delivery of encapsulated DXR by SLpHF3 liposomes, resulting in 8.8 to 17-fold increase of drug cytotoxicity relative to the other tested formulations. Immunohistochemistry of the generated orthotopic tumors were positively stained for cell surface nucleolin, reinforcing the potential of this protein as a therapeutic target. Additionally, clinical relevance of targeting nucleolin as a therapeutic approach for MBC treatment was emphasized by its expression on human breast tumors and their corresponding lymph node metastases, including in triple negative patient-derived samples analyzed so far. *Conclusion:* The results show that liposomes targeting cell-surface nucleolin are a therapeutic strategy with great therapeutic potential against metastatic and triple negative breast cancer treatment. Further preclinical evaluation will be conducted using the 4T1 mouse model of metastatic disease.

F18. Gold Nanoparticles for Targeted Delivery of Radiogallium to EGFR-overexpressing Tumors

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Introduction The rapidly advancing field of cancer nanotechnology has generated several innovative radionuclide delivery systems to improve and enhance their targeted transport to the tumor sites. In particular,

gold nanoparticles (AuNPs) have very attractive properties for medical application such as, biocompatibility, easy functionalization with molecular vectors and good biological half-life, with great potential for the development of (nano)radiopharmaceuticals. Herein, we will report on the synthesis, characterization and biological evaluation of AuNPs decorated with chelators for gallium complexation and with a bioactive peptide (GE11 derivative) for targeted radionuclide delivery to epidermal growth factor receptor (EGFr) overexpressing cancer cells. *Materials and Methods* AuNP-DTDTPA nanoparticles were synthesized based by reduction of HAuCl4 with NaBH4 in the presence of a dithiolated DTPA derivative (DTDTPA) as a stabilizer molecule. Loading of the bioactive peptide into these AuNPs was done afterwards by reaction with a GE11 peptide derivative (TA-GE11-DOTA). Radiolabeling with 67Ga was performed using two distinct routes: i) direct labeling of the GE11 peptide-containing AuNPs (Post-DTAu-GE11-DOTA); ii) pre-labeling approach, in which the GE11 peptide derivative was first labeled with 67Ga and then conjugated to AuNP-DTDTPA (Pre-DTAu-GE11-DOTA). Stability studies in physiological media and in the presence of apo-transferrin were performed for the 67Ga-labeled AuNPs, as well as cell uptake studies using EGFr-overexpressing cancer cells (A431). *Results and Discussion* Small core (2-3 nm) AuNPs stabilized with DTDTPA and decorated with TA-GE11-DOTA were successfully synthesized. Both direct and pre-labeling approaches provided 67Ga-labeled AuNPs decorated with a GE11 peptide derivative. Post-DTAu-GE11-DOTA displayed a lower capability to maintain suitable 67Ga coordination in physiological media and in the presence of apo-transferrin, compared with Pre-DTAu-GE11-DOTA. Both radiolabeled nanoconstructs display high internalization in A431 cells, with the highest internalization being observed for Post-DTAu-GE11-DOTA. However, blocking studies with EGF showed no significant difference in internalization for Post-DTAu-GE11-DOTA, while in the case of Pre-DTAu-GE11-DOTA about 30% decrease was observed. Aknowledgements The authors would like to thank Fundação para a Ciência e Tecnologia (FCT) (EXCL/QEQ-MED/0233/2012) for the financial support.

No conflict of interest.

F19. Better outcomes for brain tumor patients using a novel hybrid compound from a triazene-histone deacetylase inhibitor combination

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INTRODUCTION: Brain and nervous system tumors are noteworthy neoplasms. In spite of the number of patients afflicted by them be lower than that from other malignancies, their morbidity and mortality are still extremely significant. Nevertheless, treatment effectiveness, especially regarding the most aggressive grade – glioblastoma, is even distant from what we can desire. *MATERIAL AND METHODS*: Our research team has recently synthetized an innovative antineoplastic hybrid compound (HYBCOM), which by binding two different molecules – temozolomide (TMZ) and valproic acid (VPA) may show increased pharmacological potency toward the use of such molecules alone. The studies we have conducted indicate a better chemotherapeutic effect with HYBCOM as compared to TMZ alone, the currently best antineoplastic for these type of tumors. We performed different assays using the mouse glioma GL261 cell line, treated with a single incubation of TMZ (250µM) or HYBCOM (100µM) during 72h. At the end of the incubation period, and to evaluate cell function/dysfunction we determined cellular viability (MTS test), proliferation (Ki67immunolabeling), cell death (Guava Nexin® Annexin V flow cytometry), cytoskeleton dynamics (F-actin immunolabeling), cell migration (scratch assay) and the expression of the multidrug resistance-associated protein 1 (Mrp1, Western Blot). *RESULTS AND DISCUSSION*: Our results clearly suggest that HYBCOM possess a superior antineoplastic effect when compared to TMZ. Indeed, we observed an enhanced loss of cellular viability (minus 30%, $p < 0.01$) and a marked decrease in the proliferation rate (less 59%, $p < 0.05$). Effect on cell death was similarly observed in either TMZ or HYBCOM assays. Moreover, HYBCOM significantly switched the GL261 cells from a more polar to a non-polar morphological shape (more 54% vs. TMZ, $p < 0.01$), which may be associated with the loss of migratory properties of the GL261 cells, as suggested by the scratch assay. First results also indicate that only HYBCOM is able to decrease the drug resistant phenotype of glioma cells close to control levels. Based on

these encouraging data, we believe that this innovative molecule will constitute a promising therapeutic approach, still demanding further clarification for the mechanisms behind such success. We expect that future preclinical and clinical applications targeting patients may corroborate the use of HYBCOM as a valuable new chemotherapeutic approach for brain tumors.

No conflict of interest.

G1. Immune System Response after Radiotherapy in Non-Small Cell Lung Cancer Patients

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Introduction: Lung cancer (LC) is a disease with a poor prognosis once diagnosed, making it a very aggressive cancer with high mortality. LC can be classified into two types: Small Cell Lung Cancer (SCLC) and Non-Small cell lung cancer (NSCLC). This last one includes adenocarcinoma, squamous-cell lung carcinoma, and large-cell lung carcinoma, each with its subtypes. Chemotherapy and radiation therapy (RT) are standard therapeutic modalities for patients with cancer, including LC. It is well known that RT recruits biological effectors that may have systemic effects as result of treatment. Non-cancerous cells surrounding the tumor (fibroblasts, pro-inflammatory leukocytes and vasculature cells) may play a pivotal role in determining the progression of cancer, as well as its metastasis. Recruitment of cells from the immune system as well as the type of immune response may influence the therapeutic outcome. The purpose of this study was to evaluate T Cells subsets and Tregs as well as TNF-αin NSCLC (Adenocarcinoma) patients after external beam RT. *Materials and Methods*: A group of 10 NSCLC patients was studiedimmediately before radiotherapy(T0), half-treatment (T1) and 30 days after the end of treatment (T2), blood samples were collected to EDTAK3 and dry tube. Blood samples were analyzed by flow cytometry for CD3, CD4, CD8, CD19, CD56, CD25, CD127, FoxP3, CD31 and CD45RA expression. TNF- α levels were evaluated using an immunoassay (ELISA). *Results and Discussion*: The observed significant changes in Tregs, going together with non-significant alterations of NK and DN T cells as well as in TNF- αlevel after RT, may suggest an attempt to decrease/regulate inflammation, in this group of patients, explaining the obtained results. The significant difference for T cells and specifically within Tregs, together with no significant changes in NK and DN T cells as well as in TNF- α levels, this last one increasing from T1 and decrease to T2 may suggests a possible polarization of a Th2 immune response, as well as an attempt to reduce and/or regulate inflammation in this group of patients, thus explaining the results we obtained.

No conflict of interest.

G2. Nanoparticle-based cancer vaccine to deliver tumor associated antigens and for immunomodulation

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Introduction: Cancer vaccines have been used as an alternative treatment for breast cancer and have already shown promising results. However, only a small number induced an effective tumor regression that can be explained by the release of potent immunosuppressor molecules by tumor cells, such as TGF-β1. The elimination of the tumor itself and the tumor microenvironment seems to be an ideal therapeutic strategy against this disease. Thus, this project aims to characterize the antitumor efficiency and related immune responses induced by a nanoparticle (NP)-based cancer vaccine designed to deliver incorporated antigen and/or small interfering RNA (siRNA) to target dendritic cells (DCs) and for immunomodulation, by silencing TGF-β1 genes. *Materials and Methods*: Antigen-chitosan complexes encapsulated in poly(lactide acid) (PLA) NPs have been formulated by a double emulsion solvent evaporation method using α-lactalbumin as TAA. These NPs were coated with polyvinyl alcohol (PVA) or with block co-polymer Pluronic to improve stability under physiological conditions. In order to potentiate tumor targeting, NP surface was modified by hyaluronic acid (HA), a targeting moiety that specifically recognizes CD44 receptor, overexpressed on several tumor cells. NP size, surface charge (ZP) and morphology were analyzed by Dynamic Light Scattering, Laser Doppler Electrophoresis and Atomic Force Microscopy (AFM), respectively. Entrapment efficiency (EE) and loading capacity (LC) were quantified by MicroBCA® and HPLC. Finally, antigen integrity and cell viability were determined by SDS-PAGE and MTT assay, respectively. *Results and Discussion*: Overall, NPs presented a mean diameter close to 165 nm with low polydispersity index (PdI) values (≤0.160), ZP close to neutrality, and high EE (>80%) and LC (>20 µg/mg) values. PLA NPs showed no cytotoxicity on targeted cells after 24h of incubation, even at high NP concentration (500 µg/mL). Three different chitosan (Cs) derivatives were used for TAA complexation. However, no significant differences were observed between the physicochemical properties of those three different nanoparticulate systems. Similarly, no significant differences were detected in NP's size, surface charge and cytotoxicity when formulated with PVA and Pluronic as external phase surfactant. Moreover, non-targeted and targeted NPs also presented similar properties. Therefore, it is possible to state that the formulation method followed for PLA-based NP preparation is highly reproducible and this nanoparticulate system constitutes a promising platform for the delivery of TAA and immunomodulators to different cells within tumor microenvironment.

No conflict of interest.

G3. Novel nanoparticulate platform for breast cancer immunotherapy

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Introduction: Therapeutic cancer vaccines are novel immunotherapeutic approaches used to overcome host immunosupression induced by tumor cells. A polymeric platform based on antigen-loaded poly(lactide-co-glycolide) acid (PLGA)-based nanoparticles (NPs) is being developed to deliver the antigen to dendritic cells (DCs) and improve T cell efficiency within tumor microenvironment. *Materials and Methods*: PEGylated-PLGA-based NPs (PLGA-PEG) were formulated by double-emulsion solvent evaporation technique, using alpha-lactalbumine (LALBA) as an antigen. Other polymers were used to best attain the most efficient parameters for cancer immunotherapeutic treatment. NPs size was determined by Dynamic Light Scattering (DLS) and Atomic Force Microscopy (AFM), while surface charge was obtained by Laser Doppler Velocimetry, respectively. Encapsulation efficiency (EE) and loading capacity (LC) were determined by HPLC. Antigen structural integrity was confirmed through SDS-PAGE gel electrophoresis. To confirm NPs safety, DCs were incubated with increasing concentrations of PLGA-PEG-based NPs using MTT and AlamarBlue® assays. *Results and Discussion*: PLGA-PEG NPs presented an average size of 131 nm, with a polydispersity index (PdI) of 0.16 and a zeta potencial (ZP) of –4.78 mV. Encapsulation efficiency (EE) and loading capacity (LC) were 60 % and 19 µg/mg, respectively. Higher mean diameter of PLGA-PEG NPs was observed when chitosan (CS) (PG_CS) was included in NP formulation (178 nm, PdI 0.173), and their ZP values were close to neutrality (-1.59 mV), which is desired for a therapeutic cancer vaccine to overcome its premature capture by macrophages. EE and LC of PG_CS were 92 % and 23 µg/mg, respectively. PG_CS NPs modified by Pluronic F127 (PG_CS_PL) presented higher mean diameter values (181 nm with a PdI 0.146), and ZP (ZP -0.54 mV) and similar EE and LC (90 % and 23 µg/mg). NPs safety was quantified using MTT and AlamarBlue® assays. DCs were incubated 24 h with increasing concentrations (100-1000 µg/mL) of three different batches of PLGA-PEG-based NPs: PG_CS, PG_CS_PL and PG_PL. Formulated NPs did not decrease cell viability. Having in consideration the results herein described,

PLGA-PEG-based NP constitutes a promising platform for the delivery of antigens to DCs, key players in tumor immunology. *No conflict of interest.*

G4. Tyrosine Kinase inhibitor therapy induces alterations of CD137 and CD137L expression in chronic myeloid leukemia patients

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Introduction: CD137 (4-1BB, TNFRSF9) is a member of the TNFR superfamily that provides expansion and survival signals to T cells. Its ligand, CD137L, in addition to its ability to costimulate T cells, signals back into antigen presenting cells, promoting their activation and differentiation. Recently, CD137 has been proposed as a therapeutic target to improve and sustain anticancer immune response. Expression of CD137 or CD137L was described in T leukemia and B lymphoma activated cell lines, respectively, and soluble CD137L has been found in sera of leukemia patients. However, the role of these costimulatory molecules in hematologic malignancies is still unknown. The aim of the present study was to evaluate CD137/CD137L role in Chronic Myeloid Leukemia (CML) patients treated with tyrosine kinase inhibitors (TKI). *Material and Methods*: In this study, 67 CML patients treated with imatinib mesylate and 36 blood donors were investigated. Soluble CD137 was significantly increased in sera and supernatants of *in vitro* stimulated PBMCs from CML patients. Results: CD137 and CD137L were found in T (CD4 and CD8), B and NK cells from CML patients. CD137 was mainly expressed in CD8+ T cells and CD137L was significantly detected in CD4+ T cells, but was also found in B and NK cells. Expression of CD137 and CD137L positively correlated with TKI dose. These results were accompanied by the increased production of IFN . Gene expression of IFN , granzyme B and perforin was found to be upregulated in TKI-treated patients, compared to TKI-naïve patients and healthy controls. Analysis of miRNAs predicted as targeting TNFRSF9 and TNFSF9 revealed a significant increase of hsa-mir-886-3p. The analysis of miRNAs associated with CD137 signaling pathway and the induction of IFN via the ADAP-CBM signaling module (Lck, Fyn, ADAP, CARMA/CARD11, Bcl-10, MALT1, c-Jun and IFN) showed no correlation in TKI treated CML patients. Discussion: Taking together, our results suggest that CD137/CD137L signaling should be further investigated in CML patients undergoing TKI therapy to improve and sustain anticancer immune response. *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.

No conflict of interest.

G5. Dynamic expansion and functional tuning of natural killer cells in chronic myeloid leukemia.

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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. The aim of the present study was to analyze NK cells in CML patients and the effect of therapy and dose-dependent mechanisms on essential features of NK cells. *Material and 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. *Results and Discussion*: Relative frequency of NK cells was found reduced at CML diagnosis and recovered after treatment. CML therapy induces an increase of CD62L+CD56bright 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 CD56dimCD16+ 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. Dasatinib induced the expression of KIR2DS1 (activating receptor) on NK cells, improving NK cell ability to kill cancer cells. In conclusion, 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.

No conflict of interest.

H1. Metabolic Radiotherapy in cholangiocarcinoma: an option in the future?

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Introduction: Cholangiocarcinoma (CC) has a poor prognosis and limited therapeutic options. Thus, it becomes imperative to investigate new therapeutic options for this highly aggressive type of tumour. Recently, it was shown that CC has increased expression of natrium-iodide symporter (NIS), a molecule that mediates the iodine uptake. It is already known that NIS is a key component in the successful metabolic radiotherapy, using iodine-131 (131I), in the treatment of thyroid tumours. These data opening the possibility of a new therapeutic approach for CC. Thus, the aim of this study was to evaluate the therapeutic efficacy of metabolic radiotherapy with 131I in a human CC cell line. *Material and Methods*: The human CC cell line used was TFK-1. Influx studies were performed in order to determine the 131I uptake profile by the cell line. Subsequently, the cells were subjected to 1, 5, 10 and 20 Gy of 131I, in order to evaluate and characterize the effects of metabolic radiotherapy. The effect on cell survival was assessed by clonogenic assay. Then, it was used flow cytometry in order to evaluate the type of induced cell death, as well as BAX, BCL-2 and cytochrome C expression, mitochondrial membrane potential, cell cycle changes, intracellular peroxides, superoxide anion, and glutathione production. Superoxide dismutase activity was also evaluated. To determine the possible damages in the DNA it was performed the comet assay. *Results and Discussion*: Through uptake studies it was observed that the 131I uptake peak of TFK-1 cells occur in the first minutes, keeping constant for the remaining time. It was observed that treatment with 131I induced a decrease in cell viability dependent on the dose. The predominant type of cell death was apoptosis, followed by an increase in the BAX/BCL-2 ratio. In agreement with these results, there was also the release of cytochrome C, mitochondrial membrane depolarization and the occurrence of pre-G0 peak during the cell cycle. Interestingly, it wasn't possible to detect differences in the production of intracellular peroxides, superoxide anion, glutathione, and superoxide dismutase. It was also found that 131I induce DNA breaks in TFK-1 cells. Metabolic radiotherapy with 131I causes a decrease in TFK-1 cells survival, inducing cell death mainly by apoptosis, at least in part through mitochondrial pathway. Thus, the 131I could a promising option for the treatment of CC.

No conflict of interest.

H2. Ionizing Radiation Effects in Lung Cancer – An *in vitro* study

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Introduction: Lung cancer (LC) is one of the leading causes of cancer-related death worldwide. In general, LC can be split into two main types: Non-Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC). The NSCLC type includes such as adenocarcinoma, squamous-cell lung carcinoma, and large-cell lung carcinoma, each with subtypes. Although the number of studies discussing LC is vast, treatments efficacy is still suboptimal due to the wide range of factors that affect the patient's outcome. Our propose is to evaluate the effects of X radiation (X-rays) in three LC cell lines after irradiation, two of NSCLC (H1299 and A549 cells) and one of SCLC (H69 cells). *Materials and Methods*: The assays were performed after the cell lines were exposed to 4MeV X-rays in the Varian 600C Linear Accelerator 12D10. Studies included control cells and cells irradiated with several doses of radiation from 0.5 to 60 Gy. Cell survival was studied by clonogenic assay after 12 days of incubation. Measurement of X-rays impact was achieved by using flow cytometry (FC) after an incubation period of 48 hours. Cell death was evaluated using double staining with annexinV /propidiumiodide. By using FITC-conjugated monoclonal antibodies BAX and BCL-2 were quantified and subsequently a BAX/BCL-2 ratio was calculated. Oxidative stress was determined by FC through superoxide anion, peroxides and reduced glutathione levels using the following probes, dihydroethidium, 2',7'-dichlorofluorescin diacetate and 1-(4-chloromercuriophenylazo)-2-naphthol, respectively. Mitochondria membrane potential was measured by FC using the fluorescent probe 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl benzimidazolcarbocyanine. Cell cycle was performed with PI/RNase assay. *Results and Discussion*: We observed that X-rays induces a decrease in cell proliferation and viability in a dose, time and cell line dependent manner, inducing cell death preferentially by apoptosis. These anti-proliferative and cytotoxic effects are in agreement with the observed cell cycle arrest. However, our results show that A549 and H1299 cells are more sensitive to cell death induced by radiation, being the H69 cells more resistant. These results may be related with differences in the P53 expression or stress oxidative response.However, the sensibility and/or resistance to radiation may be dependent on molecular LC characteristics which could influence the response to radiotherapy and consequently treatment success.

No conflict of interest.

H3. Treatment response of radiosensitive and radioresistant colorectal cancer cells

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Introduction: Chemoradiation as neoadjuvant treatment for locally advanced rectal cancer is one of the most common approaches, since it allows downstaging and improves local control. To understand how radioresistant cells behave after treatment is extremely important in order to improve treatment modalities. In this study we aimed to compare cell viability, DNA damage, oxidative stress and GSH expression of sensitive parental colorectal cancer cells to its radioresistant derivatives, after treatment with 5-fluorouracil (5-FU), radiation alone and combined therapy. *Material and Methods*: Parental WiDr cell line and the radioresistant derivative WiDr/10r, previously obtained by our group, were used to study radioresistance. Cells were submitted to 20 µM of 5-FU for chemotherapy and irradiated with 0, 2 and 10 Gy for radiation alone, in a Varian Clinac 600 linear accelerator with a 4MV photon beam. Cells treated with combined therapy were exposed to 20 µM of 5-FU three hours prior irradiation and then irradiated with the same doses. Cell viability and oxidative stress were assessed by flow cytometry 96 hours after treatment and DNA damage was measured by comet assay immediately after treatment. *Results and Discussion*: WiDr cells showed a significant decrease of cell viability after exposure to 10 Gy and to 5-FU + 10 Gy, comparing to their derivative radioresistant cells WiDr/10x (respectively 49,1±5,0% vs 66,0±8,1%, p=0,008; and 50,9±2,4% vs 63,8±4,2%, p=0,003) and a significant increase of necrosis, regarding 10 Gy (30,9±8,5% vs 19,3±4,2%, p=0,001), 5-FU

(22,5±5,2% vs 17,8±2,7%, p=0,009) and 5-FU + 10 Gy (27,0±6,6% vs 16,2±2,3%, p=0,015). Although there were no differences in superoxide anion concentration, WiDr cell line showed a significant decrease in formation of peroxides when compared to WiDr/10x after treatment with 5-FU + 10 Gy (160,0±10,0% and 200,0±10,0% respectively, p=0,006). WiDr cell line exhibited lower mitochondrial membrane potential than WiDr/10x cell line, i.e., superior monomers/aggregates ratio, after treatment with 10 Gy and 5-FU (540,0±80,0% vs 360,0±50,0%, p=0,012; and 230,0±30,0% vs 180,0±30,0%, p=0,015, respectively). DNA damage was superior in WiDr/10x cell line than in WiDr cell line, since the tail moment parameter was significantly higher in the first one for all treatment conditions (p<0,001). These results showed that radioresistant WiDr/10r cell line is less affected by higher doses of radiation, regarding cell viability and oxidative stress. However, radioresistant cell line acquired more DNA damage than radiosensitive cell line, which means that cells can carry DNA damage and still be viable and resistant to treatment.

No conflict of interest.

H4. 111In-estradiol based complexes for breast cancer targeting

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Introduction Imaging estrogen receptor (ER) status is considered a useful strategy in the treatment planning and ultimate outcome of breast cancer patients since many tumour cells express high ER concentrations as compared to normal cells [1]. Additionally, it can help in the early prediction of the tumour responsiveness to hormonal therapy and in the treatment follow-up. Hence, the search for novel imaging agents to specifically target ER in tumours is an important but very demanding task that may benefit the selection of patients for individual therapy [2]. Herein, we describe the synthesis and biological evaluation of several 111In-estradiol based complexes to access their feasibility for imaging ER positive tumors. *Materials and Methods* New estradiol derivatives bearing different spacer chains were conjugated to three bifunctional chelating agents (BFC): DTPA, DOTA, DOTAGA. The BFC-estradiol conjugates were then coordinated with In. The relative binding affinity (RBA) of the new compounds to human ER was evaluated by competitive binding assay. Radiochemical purity and *in vitro* stability of the 111In complexes, obtained by reaction with 111InCl3, were evaluated by HPLC. Radiochemical stability was assessed in PBS and human blood serum and by exchange with apo-transferrin and DTPA solutions. Cellular uptake kinetics was assessed in suitable human breast cancer cell lines, MCF-7 (ER+) and MDA-MB-231 (ER-). Biodistribution and *in vivo* stability studies were also performed in immature female rats. *Results and Discussion* New estradiol-BFC conjugates have been successfully synthesized. The estradiol-DOTAGA chelators presented high or moderate affinity and selectivity to ER. 111In-estradiol complexes were obtained in high radiochemical yield and purity at low ligand concentrations. HPLC analysis indicated that most of the 111In complexes are kinetically stable at 37º C in the presence of the iron-transport protein apo-transferrin and human blood serum. Moreover, radioactive complexes do not undergo relevant transchelation in presence of DTPA and are stable in human blood serum. Cellular studies indicate moderate uptake of the 111In complexes in MCF-7 which decreases in the presence of estradiol. In MDA-MB-231 cells, the uptake is even lower than in the MCF-7 cells probably due to the low lipophilicity of the complexes (-0.99 to 0.43). Biodistribution studies indicated high *in vivo* stability and rapid clearance from main organs. ER binding affinity and cellular uptake studies suggest that the uptake of DOTAGA-estradiol complexes may occur via an ER-mediated process. Acknowledgments: S. Cunha and F. Vultos thank FCT for PhD grants (SFRH/BD/43432/2008; SFRH/BD/84509/2012). Work was supported by PTDC/QUI-QUI/111891/2009 and EXCL/QEQ-MED/0233/2012. References [1] Bai Z, Gust R, [2009], Arch Pharm Chem Life Sci 342:133-149 [2] Linden HM, [2013], Semin Nucl Med 43:324-329

No conflict of interest.

H5. Block copolymer micelles for therapy of bone metastases

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Introduction: Cancer is one of the leading causes of death worldwide. Several malignant cancers, e.g., breast and prostate, have high tendency to metastasize to bone. The development and progression of bone metastases have a high impact in the quality of life of patients, causing drastic morbidity as bone pain and pathologic fractures. Bisphosphonates (BPs) are compounds with high affinity for the bone mineral matrix, binding strongly to hydroxyapatite and accumulate in areas of high bone metabolism such as metastases. Consequently, BPs are being extensively explored for therapy/diagnostic of bone diseases.[1]Block copolymers micelles (BCMs) have attracted significant attention in themedical field due to their advantages as drug delivery agents. BCMs are able to accommodate antitumor drugs (e.g., docetaxel, DTX) in their hydrophobic core increasing the solubility of hydrophobic drugs.[2] Moreover, the nanosize of the particles can increase drug accumulation in tumors by the enhanced permeability and retention effect (EPR). Our main goal is to explore BCMs for the simultaneous delivery of radiation/BPs/chemotherapy to bone metastatic lesions. So far, we have synthesized and characterized a novel type of non-loaded and DTX-loaded micelles decorated with pyrazolyl-containing ligands to coordinate 99mTc for a preclinical evaluation. *Materials and Methods*: The copolymers Me-PEG-b-PCL, NH2-PEG-b-PCL and Pz-PEG-b-PCL were synthesized and characterized by 1H NMR and FTIR spectroscopy.The BCMs were synthesized by the thin-film hydration method loaded or not with DTX.[3] Hydrodynamic diameter (Dh) and critical micelle concentration (CMC) were determined by DLS, morphology by TEM and DTX loading content by HPLC. The functionalized micelles were radiolabeled with fac-[99mTc(CO)3]+ and purified by 10 kDa centrifuge filters. In vitro stability was evaluated at 37ºC in PBS and in cell culture medium by GPC. *Results and Discussion*: The block copolymers were successfully synthesized. The non-loaded and DTX-loaded BCMs studied by DLS revealed an effective Dh of 64±27 and 50±17nm, and zeta potential of -13±3 and -9±2 mV, respectively. By TEM the shape of the obtained BCMs was spherical. The estimated CMC for Pz-CONH-PEG-b-PCL was 65 mg/L and the DTX content was 1.4%. The BCMs were radiolabelled with 99mTc in high yield and high radiochemical purity. The 99mTc-BCMs were stable in physiological and cellular culture media. Cellular uptake studies in the MDAMB231 metastatic breast cancer cells showed a maximal uptake of 3 e 2% at 3 h incubation for non-loaded and loaded BCMs,respectively. Acknowledgments: This work is supported by FCT EXCL/QEQ-MED/0233/2012 project. E. Ribeiro thanks FCT for a grant. References:[1]L Costa et.al., Nat Clin Pract Oncol, 6: 163–74, 2009. [2]C Allen et.al., Colloids Surf B Biointerfaces, 16, 3–27,1999.[3]Y Zhang, et.al., Colloids Surf B Biointerfaces, 44, 104–9, 2005.

No conflict of interest.

H6. Novel radiotracers for molecular imaging of EGFR positive tumors

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Introduction Cancer is recognized as a major leading cause of death worldwide. Traditional anticancer treatments such as chemotherapy and radiotherapy are often hindered by toxicity, lack of specificity, and efficacy. Such disadvantage resulted in an emergent interest in developing novel cancer-specific agents to increase survival and improve quality of life. Epidermal growth factor receptor (EGFR) is overexpressed in a wide variety of solid tumours and has become an attractive target for cancer therapy [1]. Hence, the non-invasive evaluation of EGFR status by molecular imaging modalities, such as PET or SPECT, may help in the selection of patients who are expected to benefit from anti-EGFR targeted therapy and to monitor their response to personalized cancer management. For this reason, remarkable efforts are being devoted to find suitable radiotracers for *in vivo* imaging of EGFR [2]. In the present work, we describe the synthesis and biological evaluation of several radiolabeled tyrosine kinase inhibitors (TKi) and small radiopeptides targeting EGFR. The potential of these radiotracers for molecular imaging of EGFR positive tumors, will be discussed. *Materials and Methods* Peptides were synthesized by Fmoc-based microwave solid-phase peptide synthesis using orthogonal protecting groups and were purified by HPLC and characterized by ESI-MS. The TKi and peptide derivatives, functionalized with DOTA or NOTA chelators, were labelled with 67Ga by reaction with 67GaCl3. The radiochemical purity and *in vitro* stability of the 67Ga complexes were evaluated by HPLC. The inactive gallium complexes were prepared to characterize the radioactive congeners and to evaluate their

ability to inhibit autophosphorylation and tumour cells proliferation. *Results and Discussion* In the present work, TKi and small peptides targeting EGFR have been synthesized, conjugated to DOTA and NOTA-like chelators through spacer chains of variable length and were successfully labeled with 67Ga. The radioactive complexes were obtained in high radiochemical yield and purity even at low ligand concentrations. *In vitro* stability studies indicated that most of the 67Ga complexes were stable in PBS pH7.4 and human blood serum, at 37º C. The inactive gallium complexes were prepared and used to characterize the radioactive congeners and to evaluate their ability to inhibit autophosphorylation and tumor cells proliferation. *In vitro* studies have indicated that the coordination of the TKi to the metal leads to compounds that still inhibit EGFR autophosphorylation and A431 cell growth. Biodistribution studies in tumor bearing mice are currently underway. Acknowledgments: A. Gonçalves thank FCT for BI grant (BL43/2014_IST-ID). Work was supported by EXPL/QEQ-MED/1950/2013 and EXCL/QEQ-MED/0233/2012. References [1] J. J. Laskin, [2004], Cancer Treatment Reviews,1-17 [2] C. Fernandes, [2008], Dalton Trans, 3215–3225.

No conflict of interest.

I1. Differential allelic expression is a powerful approach to prioritize GWAS candidate loci for functional studies

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Introduction: Breast cancer is the most common cancer affecting women in the developed world. However, the current knowledge of breast cancer genetic risk can only explain one-third of cases. Furthermore, genome-wide association studies (GWAS) have failed to identify low-frequency intermediate risk loci and do not contribute directly to the understanding of the underlying mechanism. Previous results demonstrated that cis-regulatory variation is involved in risk for several cancer, including breast cancer. We propose an innovative approach to prioritise GWAS candidate risk loci for further functional characterization and validation: to cross GWAS results with information on differential allelic expression (DAE), a powerful method to identify cis-regulatory variants. *Material and Methods*: We conducted a preliminary genome-wide study of allelic expression in sixty-four normal breast samples. DAE quantification, including genotyping and allelic expression quantification, was performed using commercial microarrays. Genome-wide breast tissue DAE data was crossed with published breast cancer GWAS top hits according to chromosome location. Loci with DAE and GWAS associated SNPs in linkage disequilibrium (LD) were further analyzed for evidence of cis-regulatory potential. The top candidate regulatory SNPs were selected for in-vitro and in-vivo functional analysis. *Results and Discussion*: We found that 21% of the array coding SNPs displayed DAE (tagging genes under cis-regulatory control), with approximately 3% displaying highly significant fold differences between alleles (p<1.0E-5). The crossing of published breast cancer GWAS data with our DAE map allowed the identification of over 200 loci that contain risk-associated SNPs and DAE SNPs. In 13 of these loci the risk-associated SNP and the DAE SNP were in strong LD with each other, supporting a cis-regulatory role for the risk-associated SNP in breast cancer susceptibility. Candidate cis-regulatory variants in the top three loci are being currently functionally studied to assess their potential to alter the binding affinity of transcription factors (TFs). In this study we report a large overlap between GWAS and DAE data, confirming that cis-regulatory variants are indeed major players in breast cancer susceptibility. We propose a novel and efficient method to prioritise candidate GWAS loci, for further functional analysis, which will contribute to a better understanding of the biology underlying breast cancer risk.

No conflict of interest.

I2. Can polymorphisms in the *MTHFR* gene (C677T AND A1298C) alter the risk and severity for sporadic breast cancer in brazilian women

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Introduction: Breast cancer (BC) is the most commonly diagnosed form of cancer. It is the main cause of cancer death in women in many parts of the world. BC is a complex disease, varying in risk factors, natural history, histological and molecular patterns and treatment response. Recent studies have associated the rs1801133 (677C → T) and rs1801131 (1298A → C) polymorphisms in the Methylenetetrahydrofolate Reductase gene (MTHFR) to cancer development in different organs, including the breast. Therefore, we investigated the association between the frequency of sporadic BC, including clinical variables, and the C677T and A1298C polymorphisms in the MTHFR gene. *Material and Methods*: DNA samples from 251 women diagnosed with sporadic BC of the Laboratory of Molecular Genetics of Cancer, State University of Campinas (Brazil) and 227 women over the age of 50 years and no history of BC in the family (control) were enrolled. For the genotyping, the Restriction Fragments Length Polymorphism PCR technique was used. The results were analyzed by SPSS vs 21.0 software. The 2 test was employed to investigate the distribution of the polymorphisms, and to the difference in the percentage of these, we calculated the odds ratio. In the case of the numerical distribution, the Mann-Whitney and Kruskal-Wallis tests were used. *Results and Discussion*: No significant difference between groups was observed in the occurrence of sporadic BC for both polymorphisms [C677T (p = 0.300), A1298C (p = 0.419)]. The association between genotypes and clinical variables, we found that the CC genotype (C677T polymorphism) had lower prevalence in Caucasian patients (OR = 0.330, 95%CI = 0.128 to 0.787); the same genotype was associated with lower risk for metastasis (OR = 0.192, 95%CI = 0.20 to 0.957). For the A1298C polymorphism, the CC genotype was associated with protection from stage 0 compared to the others (OR = 0.048, 95%CI = 0.252 to 0.764). For the other clinical variables, there was no significant difference. We conclude that the rs1801133 (677C → T), and rs1801131 (1298A → C) polymorphisms do not appear to alter the risk for developing sporadic BC, however, may be associated with its severity.

No conflict of interest.

I3. Cis-regulatory locus at 12q24 is associated with breast cancer risk

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Introduction: Genome-wide association studies (GWAS) for breast cancer identified over 70 risk-associated loci. The major challenges now include: mapping the casual variants of risk-loci, understand their biological link to disease aetiology. It is predicted that most unidentified susceptibility variants for breast cancer have a regulatory function. We performed a genome-wide mapping of cis-regulatory variation in breast tissue, and cross-compared it with GWAS data. Over 200 candidate loci were identified that contain variants with strong cis-regulatory potential and an association in GWAS. We are functionally characterising the 12q24 locus, for which there is a GWAS hit and 3 DAE SNPs, in the AACS gene, one being the same as the GWAS hit. *Material and Methods*: DAE was quantified using allele-specific real time PCR in breast tissue samples (n=18) from healthy controls, and control and patient's blood samples (n= 12 and n=18, respectively). Each region was then analysed for evidence of regulatory elements using ENCODE data. In-silico analysis proceeded to identify candidate rSNPs that were likely to alter transcription factor binding affinity (TFs), in an allele specific manner. Electrophoretic mobility shift assays (EMSA) are currently being carried out to assess *in vitro* these predictions. *Results and Discussion*: Easton et al (2007) reported an association with breast cancer risk at locus 12q24, tagged by rs7307700 (Odds ratio per minor allele [95% Confidence Interval] = 1.02 [0.99-1.05]; p-value 0.002). We have detected 3 SNPs in the same region showing DAE. LD analysis of rs7307700 and our DAE targets, revealed strong correlation between them. We performed further LD and haplotyping analysis, as well as DAE mapping, which generated a list of 13 candidate causal variants, which overlapped confirmed genomic regulatory elements. TRANSFAC analysis of these candidates predicted allele-specific differences in TF binding, which we are currently testing in-vitro (EMSA experiments). We will follow on with chromatin immunoprecipitation in the region to further confirm TF binding in-vivo. We also found that AACS is controlled by cis-regulation in the two tissue types, and that there is an association between DAE and breast cancer risk (case-control study). Using a multidisciplinary approach, we are identifying cis-variants driving susceptibility at the 12q24 risk locus for breast cancer. We also have results suggesting that DAE in AACS could be a marker for susceptibility to breast cancer. This study shows that integrating cis-regulation and GWAS data, is an efficient method for prioritising and functionally analysing risk-loci for breast cancer. Further understanding of the

biology underlying the risk associated to this locus will allow improvements in individual risk prediction and development of new preventive therapy targets.

No conflict of interest.

J1. Attempt of an early diagnosis by the concentration of lactate dehydrogenase (LDH) using ionic-liquid-based systems

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Introduction Prostate cancer represents, nowadays, the third most common cause of death among men, and currently there is no effective treatment when the tumor is diagnosed at an advanced stage. Given this incapacity, an early diagnosis is essential to increase the treatment success rate. Nevertheless, most of the methods employed for diagnosis display several disadvantages, namely an extensive sample processing, the need for identification and characterization of specific antibodies and highly specialized technical personnel. In this sense, and in order to develop an efficient method for the detection of biomarkers associated with prostate cancer in human fluids at an early stage, and to overcome the limitations of the traditional analytical equipment, the extraction and concentration of one of the biomarkers associated to prostate cancer (lactate dehydrogenase (LDH)) was here investigated. *Materials and Methods* In this work, a series of liquid-liquid systems, in particular aqueous biphasic systems (ABS), majorly composed of water, and combined with phosphonium-based ionic liquids and citrate-based buffered solutions have been investigated. The respective ternary phase diagrams, as well as the tie-lines and tie-line lengths, were determined at 25 ºC, in order to ascertain on the phase-forming components composition required for two phases formation as well as on the concentration factors that can be achieved. After, a careful separation of both phases was performed and the amount of LDH in each phase was quantified by SE-HPLC (Size Exclusion High-Performance Liquid Chromatography). *Results and Discussion* The results obtained revealed extraction efficiencies of 100% for the ionic-liquid-rich phase, attained in a single operational step, and the possibility to concentrate up to 20 times the LDH present in an initial aqueous phase. According to the obtained results, ABS composed of ionic liquids and salts represent a new platform for the concentration of cancer biomarkers from human fluids and further studies are ongoing aiming at reaching higher concentration factors. *Acknowledgements:* This work was financed by national funding from FCT - Fundação para a Ciência e a Tecnologia through the project Pest-C/CTM/LA0011/2013. The authors also acknowledge the financial support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Capes for the PhD grant (2740-13-3) of M.M. Pereira. M. G. Freire acknowledges the European Research Council (ERC) for the Starting Grant ERC-2013-StG-337753.

No conflict of interest.

J2. Concentration of Prostate Specific Antigen (PSA) using Good-Buffers-based Ionic Liquids

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Introduction After the Prostate Specific Antigen (PSA) discovery in human fluids it has become one of the most important tumor biomarkers in cancer detection [1]. In general, a PSA value > 4.0 µg/L has been defined as abnormal and it is frequently used as a cut-off value indicative of prostate cancer [2]. Nevertheless, this value is below the detection limit of most analytical equipment, and therefore early diagnoses are difficult to carry.

Currently, Enzyme-Linked Immunosorbent Assays are the most common used in the identification and quantification of cancer biomarkers. This technique require the use of immunoassay-qualified antibodies, being thus an highly-cost and laborious methodology [3]. Therefore, in order to develop an efficient method to extract, purify and concentrate PSA from human fluids, aiming at overcoming the detection limitations of traditional analytical equipment, in this work, aqueous biphasic systems (ABS) composed of Good Buffers Ionic Liquids (GB-ILs) were investigated. *Materials and Methods* Phase diagrams and tie-lines (TLs) The binodal curves of each ABS composed of GB-IL + potassium citrate (K3C6H5O7) + water was determined through the cloud point titration method at 25 (± 1) ºC and atmospheric pressured [4].The tie-lines (TLs) of each phase diagram, at the mixtures compositions for which the extraction/concentration of PSA was carried out, were also determined. Extraction efficiencies and concentration factors of PSA The ternary mixtures compositions used in the partitioning experiments of PSA were gravimetrically prepared at fixed mixture compositions. Each mixture was vigorously stirred, centrifuged for 10 min, and left to equilibrate for at least 10 min at 25 (± 1) °C to achieve the complete PSA partitioning between the two phases. After, a separation of the phases was performed and the amount of PSA in each phase was quantified by SE-HPLC. *Results and Discussion* GB-ILs were shown to be able to form aqueous biphasic systems when combined with aqueous solutions of potassium citrate. Outstanding extraction efficiencies of 100% were obtained, without any losses on the protein content. Concentration factors up to 20 times were already achieved and further work aiming at obtaining higher concentration factors is ongoing. References 1. Pinsky, P. F., Andriole, G., Crawford, E. D., Chia, D., Kramer, B. S., Grubb, R., Greenlee, R., and Gohagan, J. K., Prostate-specific antigen velocity and prostate cancer gleason grade and stage. Cancer, 2007. 109(8): p. 1689-95. 2. Heidenreich, A., Bellmunt, J., Bolla, M., Joniau, S., Mason, M., Matveev, V., Mottet, N., Schmid, H.-P., van der Kwast, T., Wiegel, T., and Zattoni, F., EAU guidelines on prostate cancer. Part 1: screening, diagnosis, and treatment of clinically localised disease. European urology, 2011. 59(1): p. 61-71. 3. Acevedo, B., Perera, Y., Ruiz, M., Rojas, G., Benítez, J., Ayala, M., and Gavilondo, J., Development and validation of a quantitative ELISA for the measurement of PSA concentration. Clinica chimica acta; international journal of clinical chemistry, 2002. 317(1-2): p. 55-63. 4. Taha, M., e Silva, F. A., Quental, M. V., Ventura, S. P. M., Freire, M. G., and Coutinho, J. A. P., Good's buffers as a basis for developing self-buffering and biocompatible ionic liquids for biological research. Green Chemistry, 2014. 16(6): p. 3149-3159. *Acknowledgements:* This work was financed by national funding from FCT - Fundação para a Ciência e a Tecnologia through the project Pest-C/CTM/LA0011/2013. The authors also acknowledge the financial support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Capes for the PhD grant (2740-13-3) of M.M. Pereira. M. G. Freire acknowledges the European Research Council (ERC) for the Starting Grant ERC-2013-StG-337753.

No conflict of interest.

J3. Sol-gel biomimetic material designed to target CEA cancer biomarker

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Introduction Carcinoembryonic antigen (CEA) is an important tumor marker responsible for clinical diagnosis of over 95% of all colon tumors, 50% of breast tumors, as well as tumors of the lung cancer or ovarian carcinoma [1]. The detection of CEA levels in biological samples plays an important role in the pre-diagnosis evaluation and in the follow-up examination during therapy stage [2]. The most common tool for the analysis of CEA in hospitals and clinical laboratories relies on ELISA-based procedures using antibodies as capturing probe. The overall principal offers the selectivity and sensitivity out coming from the use of antibodies, but it could be further improved by assembling the biosensors over a receptor platform and establishing a label-free measure by electrical impedance spectroscopy (EIS). Thus, the present work proposes the development of an immunosensor for CEA. *Materials and Methods* Electrochemical signals were measured in a Methrom Autolab potentiostat/galvanostat (Autolab PGSTAT302N) interfaced to a computer and controlled by NOVA 1.9 software. The chemical modification of the surface of the conductive glass was characterized by Raman spectroscopy with confocal microscopy (Thermo Scientific). The immunosensor was assembled by modifying conductive glass (with ITO) with an amino silane compound (APTES), activating the antibody via carbodiimide chemistry (EDAC/NHS) and binding the antibody to the amine surface over the ITO glass. The performance of the imunosensor was evaluated by electrochemical techniques, namely electrochemical impedance spectroscopy (EIS) and square wave voltammetry (SVW). *Results and Discussion* The immunosensor made with

an optimized composition displayed linear behavior against CEA concentration by EIS and SWV techniques. The corresponding linear ranges were 0.502-1.5 and 0.252-1.5ng/mL, with detection limits of 0.417 and 0.043 ng/mL, respectively. Overall, the obtained device may be potential method to apply for screening CEA in point-of-care due to the simplicity of fabrication, short time response, low cost and good sensitivity when compared to other analytical techniques, such as ELISA assays. [1] Kemmegne-Mbouguen, J. et al., 2014, Int. J. Electrochem. Sci., 9, 478 – 492. [2] Liua, M. et al., 2010, Talanta, 81, 1625–1629. Keywords: Cancer biomarker, CEA, antibody, biosensors, sol-gel. *Acknowledgements:* This work had the financial support of 3P's Starting Grant/ERC (GA 311086).

J4. Biomimetic sensor for breast tumour antigen detection

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Biomimetic sensor for breast tumour antigen detection Intoduction Monitoring protein biomarkers in circulating fluids is currently an important approach towards non-invasive procedures in cancer screening. Clinical application of biomarker screening has been unfeasible until now in a wide range of the population or in point-of-care testing (where speed of response is a prime consideration). It requires highly sophisticated equipment and/or relies on the use of biological unstable material. Herein, an alternative device is proposed, including a biomimetic film as synthetic receptor for Breast Tumour Antigen, the first protein acting as cancer biomarker in breast cancer disease. *Material and Methods* The electrochemical measurements were conducted with a potentiostat/galvanostat from Metrohm Autolab and a PGSTAT302N, equipped with a FRA module and controlled by Nova software. Raman measurements were performed using a Thermo Scientific DXR Raman microscope system with a 100 mW 532 nm excitation laser. The biomimetic film was obtained by electro-polymerizing o-phenylenediamine around the Breast Tumour Antigen target that was adsorbed on a gold (Au) support and incubated in charged monomers. The gold (Au) working area was included in a screen printed electrode (SPE), in order to allow the production of disposable devices and enable point-of-care application. After terminating the polymerization, the protein structures that remained in the outer surface were enzymatically removed, leaving behind vacant sites to which the same kind of protein could favourably rebind. RAMAN analyses were performed to follow the surface modification of the Au-SPE. The ability of the biomaterial to rebind the protein biomarker was measured by electrochemical techniques, namely impedance spectroscopy (EIS) and square wave voltammetry (SWV). *Results and Discussion* The Au-SPE/oPDA devices displayed linear responses to Breast Tumour Antigen both in EIS and SWV assays, down to 0.5 U/mL and 10.0 U/mL, respectively, with detection limits of 40.0 and 0.005 U/mL. Further tests are progressing and point out that the here described sensor seems a promising tool for screening Breast Tumour Antigen in point-of-care, due the simplicity of fabrication, reusability, low time response, low cost, good sensitivity and selectivity. Acknowledgements The authors acknowledge the financial support of European Research Council though the Starting Grant, ERC-StG-3P's/2012, GA 311086 (to MGF Sales).

No conflict of interest.

J5. A biomimetic sensor for monitoring oxidative stress biomarker in point-of-care

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(*Introduction*) Free radicals and other reactive species are constantly generated *in vivo* and can cause oxidative damage to biomolecules, a process that seems to play an important role at the origin of cancer. 8-Hydroxy-2'-deoxyguanosine (8-OHdG) is a major product of DNA hydroxylation and is considered a biomarker of damage caused by oxidative stress (OS). Thus, early diagnosis of OS biomarkers may be used as a fundamental tool in

cancer prevention and in more efficient therapeutic strategies. For this purpose, a biomimetic sensor for 8-OHdG detection and quantification by Electrochemical Impedance Spectroscopy (EIS) is proposed herein. (*Materials and Methods*) The biomimetic sensor was obtained by modifying a clean gold (Au) electrode with a OH-terminal thiol compound, followed by direct electropolymerization of phenol in the presence of 8-OHdG. The biomimetic/Au acted as working electrode, while glassy carbon and Ag/AgCl were used as counter and reference electrodes, respectively. Electropolymerization of phenol was performed by Cyclic Voltammetry (CV) over the potential range 0.2 to 0.9 V in pH 7.0 PBS buffer, enabling the formation of a non-conductive layer. Non-imprinted materials (NIM) were also performed by removing the template from the procedure and, then, the ability of the polymer to interact non-specifically with the template was measured. (*Results and Discussion*) Preliminary results showed the development of a direct and label-free biomimetic sensor with good performance, stability and sensibility. In particular, only MIP material was able to rebind to the target molecule and produce a linear response against EIS on the range 0.010 to 10ng/ml. Overall, the biosensor described herein is simple, precise and may allow routine use for biological samples on-site. (Acknowledgments) GVM acknowledges FCT the PhD Grant ref. SFRH/BD/94159/2013.

No conflict of interest.

J6. A new biomimetic sensor for detecting carnitine, a potential biomarker in ovarian cancer

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Introduction Carnitine (CRT) displays an important role in cellular metabolism and energy production. It has actions that include the metabolites associated with glycolysis and β-oxidation of fatty acids. The change of its levels in biological fluids has been associated to the presence of ovarian cancer, making CRT a potential biomarker of the disease. Sensitive CRT determination (in low levels) becomes therefore important, for which a low cost and sensitive device would be appreciated. A biomimetic polymer is proposed herein for this purpose, produced by bulk electropolymerization around a hydrophobic paper substrate that was made conductive by casting a graphite-based ink. *Materials and Methods* The electrode substrate was prepared by modifying cellulose paper, first with solid wax and after with carbon ink. The hydrophobicity of the paper was tested by contact angle and the ink properties evaluated by Thermogravimetry, Raman Spectroscopy and FTIR. Two different biomimetic materials were electropolymerized over the carbon conductive support: 3,4-ethylenedioxythiophene (EDOT) and dodecilbenzenesulfonic acid sodium salt (NaDBS). The polymeric film depositions were obtained by chronoamperometry at 0.9 V vs Ag/AgCl during 240 s. The obtained sensors were characterized by Electrochemical Impedance Spectroscopy (EIS), in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer at pH 7.0. Results The EDOT and NaDBS-based biomimetic sensors were calibrated by EIS using CRT standard solutions. The results showed linear responses over a wide concentration range, with average slopes of 303 and 450 Ω×L/mol, and detection limits of 1×10^{-9} and 1×10^{-8} mol/L, respectively. Both sensors exhibited good selectivity for CRT in diluted urine samples. Their application to the analysis of spiked urine samples revealed relative errors < 16% and the possibility of reusing the sensor after each calibration. In addition, the conductive ink proved good thermal stability and reusability. Overall, the biomimetic sensors described herein seem a successful approach for the determination of CRT in urine. Acknowledgement: The authors acknowledge the financial support of the European Research Council, through Starting Grant 3P's/ERC (GA 311086).

No conflict of interest.

J7. Electrochemical sensors in breast cancer

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Introduction The detection of tumor markers can have a major contribution to the management of breast cancer. A simple blood test that could detect cancers in their earliest stages could prevent the deaths of millions of people and reduce the suffering of patients, and their families, and the cost to society. Cancer antigen 15-3 (CA 15-3) and the extracellular domain of the human epidermal growth factor receptor 2 (HER2-ECD) are among the established circulating biomarkers for breast cancer [1]. Electrochemical sensors (ES) have the potential to be alternatives for the detection of these cancer markers because of their high selectivity and sensitivity and the possibility of their inclusion in point-of-care devices. Another advantage is the possibility of developing multiplexed detection systems based on ES. In our group several ES (immunosensors and molecularly imprinted polymer (MIP) sensors) were developed for the analysis of CA15-3 and HER2-ECD. *Materials and Methods* For the development of the immunosensors (individual and multiplexed detection of CA15-3 and HER2-ECD) a sandwich immunoassay was employed and the analytical signal, based on the stripping of enzymatically deposited silver, was detected by linear-sweep voltammetry. Screen-printed carbon electrodes (SPCEs), modified with gold nanoparticles (nAu), were the transducers for these sensors. The nAu were deposited on the SPCE by electrochemical reduction of ionic gold from a solution. Furthermore, a MIP sensor was developed for HER2-ECD analysis. In this case a gold electrode was used. The MIP was formed by surface imprinting and electrochemical impedance spectroscopy and cyclic voltammetry were used for detection purposes. *Results and Discussion* The following conditions of the immunoassays were optimized: capture and detection antibody concentration, surface blocking and reaction times. The best limits of detection were 37.5 U/mL and 4.4 ng/mL for CA15-3 and HER2-ECD, respectively. Regarding the MIP sensors; the most adequate polymer was chosen and the electropolymerization, template removal, and incubation conditions were optimized. The lowest HER2-ECD concentration that was analyzed was 50 ng/mL. The obtained results indicate that the developed ES could be promising tools in breast cancer diagnostics and follow-up. However, further studies should be conducted using patients' blood samples and the results of these assays should be validated with the established analysis procedures for these cancer biomarkers. Acknowledgments This work received financial support from the European Union (FEDER funds through COMPETE) and National Funds (FCT, Fundação para a Ciência e a Tecnologia) through projects PTDC/SAU-ENB/114786/2009 and Pest-C/EQB/LA0006/2013. References [1] C. Paoletti, D.F. Hayes, Molecular Testing in Breast Cancer, Annual Review of Medicine 65 (2014) 95-110.

No conflict of interest.

J8. From cardiology to oncology - new imaging probes for detection of cancer multidrug resistance

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Introduction Resistance to chemotherapeutic agents is a major obstacle in the successful treatment of cancer patients. Therefore, non invasive detection of multidrug resistance (MDR) to chemotherapeutic agents is highly advantageous to define a successful therapeutic regimen. Cationic radiotracers originally developed as myocardial perfusion agents, such as 99mTc-Sestamibi, tend to localize in tumour cells due to the increased negative mitochondrial potentials and have been used for both cancer early detection and non-invasive monitoring of MDR by SPECT. Recently, we developed a new 99mTc complex- 99mTc-TMEOP with potential for myocardial imaging. It presented high initial and persistent heart uptake associated to rapid blood and liver clearance. The goal of this work is to assess the usefulness of 99mTc-TMEOP for functional assessment of MDR. *Material and Methods* The *in vitro* uptake and efflux kinetics of 99mTc-TMEOP was evaluated in human cancer cell lines, H69 (lung small cell carcinoma) and MCF-7 (breast cancer) and the corresponding drug-resistant H69 Lx4 and MCF/MDR-1, which overexpress PgP, the most widely studied transporter associated to MDR. We have further evaluated *in vivo* the role of PgP in the efflux of 99mTc-TMEOP in rats, and its pharmacokinetics and tumour uptake in MCF-7 and MCF/MDR-1 xenografted tumour models. *Results and Discussion* The uptake kinetics of 99mTc-TMEOP is comparable with 99mTc-Sestamibi, being significantly reduced in the cells over-expressing PgP and increased in the presence of verapamil, a modulator of PgP. The biodistribution data of 99mTc-TMEOP in rats show that the effect of cyclosporine A, a PgP inhibitor, induces a significant decrease in the washout rate from liver, kidneys and lungs, organs with a high PgP expression. In nude mice bearing MDR-negative and MDR-positive tumour xenografts, the biodistribution of 99mTc-TMEOP was similar in noncancerous organs. However, the tumour uptake was almost 2 times higher in the MCF-7 xenografts

compared with the MCF/MDR-1 tumours. The *in vivo* MDR phenotype of the tumours was confirmed by detection of protein expression levels by Western blot. The overall results of the intro and *in vivo* evaluation of 99mTc-TMEOP indicate that it could act as substrate of the PgP. In summary, 99mTc-TMEOP is a promising candidate for tumor imaging and functional assessment of MDR mediated drug resistance. The financial support of Covidien, Petten, The Netherlands is acknowledged. Guilhermina Cantinho and Rafael Fragoso (Nuclear Medicine Institute, Faculty of Medicine, Lisboa, Portugal) are acknowledged for the imaging studies.

No conflict of interest.

J9. Development of a biosensor for miRNA in Breast Cancer

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Introduction: MicroRNA (miRNA) has emerged as a possible new kind of biomarkers of early diagnosis in cancer. It plays an essential role in biological processes such as development, cell proliferation, apoptosis, stress response, and tumorigenesis. Finding new and simple methods the carry out this determination in biological fluids and in point-of-care is therefore an emerging issue. Biosensors are currently an alternative to conventional techniques in clinical analysis, providing fast results, being portable and allowing direct sample reading. In brief, these devices convert the interaction of a biorecognition element with a target analyte into a measurable signal. In the present study, the biorecognition element is a complementary single-stranded oligonucleotide of the target miRNA. Electrical signals are an outcome of the hybridization event between target and complementary strands, thereby acting as an electrochemical biosensor. The combined use of electrochemical biosensors for detecting MiR-155 is presented herein, applied in breast cancer. This miRNA is markedly overexpressed in breast cancer tissues and is one of the most potent miRNA suppressors of apoptosis in breast cancer cells. To our knowledge, there are no other works in this context reported in the literature. *Materials and Methods*: The electrochemical biosensor is a commercial screen-printed electrode (SPE), in which the working area is made of gold. The biorecognition element is a thiol-terminated single-stranded oligonucleotide that is complementary to the targeted species. Its immobilization is granted by covalent attachment of the –SH group of the oligonucleotide on the receptor surface. The modification of the gold-surface is followed by Raman Spectroscopy and FTIR techniques. The electrical signals generated are checked several electrochemical approaches, including cyclic voltammetry, square-wave voltammetry, and electrochemical impedance spectroscopy. Results: The hybridization event hinders the electrical flow generated by a standard probe of Fe(II)/Fe(III) standing on the sensing area, which generates an impedance change. The co-immobilization of linear and short carbon chain species with different functional groups is tested for improving the correct orientation of the oligonucleotides on the surface and non-specific binding. The electrical response of the biosensor is calibrated against standard miRNA solutions, for subsequent application in spiked serum samples. Acknowledgement: The authors acknowledge the financial support of the European Research Council, through Starting Grant 3P's/ERC (GA 311086).

No conflict of interest.

K1. Paradoxical and contradictory effects of imatinib in two cell line models of hormone-refractory prostate cancer

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Introduction: The last stages of aggressive prostate cancer are associated with loss of androgen responsiveness, a condition described as hormone-refractory prostate cancer (HRPC). This causes the failure of classical androgen ablation therapies and markedly restricts the therapeutic options available for this usually lethal form of disease. Therefore, the development of effective therapies for this stage of disease is of the uttermost importance. Imatinib mesylate is a chemoterapheutic drug that inhibits the tyrosine kinase activity of c-KIT receptors among others, which has been successfully used to treat leukemias and gastrointestinal stromal tumors. However, its application for treatment of HRPC has not been totally effective with preclinical models and clinical experimentation producing not always concordant results. In the present study we analyzed the cytotoxic effects of Imatinib in two cell line models of HRPC. Methods: DU145 and PC3 cells were incubated with 20 µM Imatinib for 48 and 72 hours. The MTS assay was used to assess cell viability in response to Imatinib and the colorimetric measurement of the enzymatic activity of caspase-3 was included as an end-point of apoptosis. The expression of cell-cycle and apoptosis regulators was determined by real-time PCR and Western Blot. *Results and Discussion*: Imatinib decreased the viability of DU145 cells at 48 and 72 hours. Although the viability of PC3 cells decreased upon 6 hours of treatment, thereafter cell viability significantly increased in relation to control. Accordingly, the enzymatic activity of caspase-3 was increased in DU145 cells whereas diminished activity of caspase-3 was observed in PC3 cells treated with Imatinib. Moreover, DU145 cells displayed reduced expression of anti-apoptotic protein Bcl-2 and increased levels of the executioners of apoptosis caspase-8 and caspase-9. No differences were observed on the expression levels of these apoptosis related proteins in PC3 cells. Also, the mRNA levels of angiogenic factor VEGF were decreased in DU145 cells in response to Imatinib but the opposite effect was seen in PC3 cells. To start explaining the differential response of DU145 and PC3 cells to Imatinib, we characterized the expression of c-KIT receptor in these cell lines. The expression of the active membrane-bound c-KIT is decreased in PC3 cells relatively to DU145. In addition, PC3 cells presented increased expression of cytoplasmic truncated isoforms of c-KIT. The present results indicated that Imatinib was effective inducing apoptosis of DU145 cells likely through the inactivation of c-KIT. On the other hand, the paradoxical effects of Imatinib in PC3 cells may be associated with the presence of cytoplasmic isoforms of c-KIT for which no definitive role has been established. The present findings contributed to understand the discrepancies in the efficacy of Imatinib as therapeutic option in HRPC.

No conflict of interest.

K2. GLUT1 inhibition as a therapeutic approach in hepatocellular carcinoma

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Introduction: Glucose transporter 1 (GLUT1) is overexpressed and confers poor prognosis in a wide range of solid tumors including hepatocellular carcinoma (HCC). Studies have shown that suppression of GLUT1 expression by siRNA (small interfering RNA) significantly impaired HCC cells *in vitro*, suggesting GLUT1 as an innovative therapeutic target for this type of tumor. This work aims to study the role of GLUT1 in the tumorigenicity and chemosensitivity of HCC. *Material and Methods*: In this work a genetic inhibition of GLUT1 using shRNA (small hairpin RNA) was carried out. Thus, using a vector containing three specific lentiviral plasmids a human HCC cell line, HuH7, was transfected in order to deplete GLUT1 expression. In order to prove the transfection efficiency, the GLUT1 expression was evaluated in transfected cells and in the parental cell line by western blot. Subsequently, using the MTT test the ratio between the metabolic activity of the parental cells and transfected cells was calculated. Using the same test, the effect of doxorubicin, 5-FU and sorafenib on metabolic activity was evaluated. The drug concentrations used correspond to the IC50 value previously obtained with the same drugs in the parental cell line. Using the same drug concentrations its effect on cell viability and cell death was also evaluated through the double staining with annexin V and propidium iodide by flow cytometry. *Results and Discussion*: Trough the western blot results, it was found that the GLUT1 expression by the transfected cell line is considerably lower than the expression by the parental cell line. The MTT test revealed that metabolic activity is smaller in transfected cells than in parental cells. The treatment with doxorubicin, sorafenib and 5-FU induced an inhibition of metabolic activity higher than 50%, accompanied by cell death by through apoptosis.It was found that inhibition of GLUT1 induced a decrease in metabolic activity. Moreover, it was also noted that the transfected cell line is less chemoresistant than the parental cell line. Apparently, GLUT1 inhibition may provide a new therapeutic approach for this highly aggressive type of tumor.

No conflict of interest.

K3. Cholangiocarcinoma therapy and diagnosis, the role of Sorafenib and 18F-Fluorocholine

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Introduction: Cholangiocarcinoma (CC) is the second most frequent primary liver tumor, and surgery is currently the only curative therapy. In most cases the diagnosis occurs late and patients are conducted to chemotherapy with conventional drugs which in majority of the cases is only palliative. More recently, new drugs for targeted therapies have been developed, including sorafenib. In cancer diagnosis some improvements have also been observed, including the development of new radiopharmaceuticals to use in PET, such as 18F-Fluorocholine. Thus, the aim of this study was to test the efficacy of various conventional chemotherapy agents, and sorafenib in a human CC cell line, as well as to verify the uptake profiles of 18F-FDG and 18F-Fluorocholine by the cell line studied. *Material and Methods*: TFK1 cell line was used. Cells were incubated with increasing concentrations of cisplatin, doxorubicin, 5-FU and sorafenib during 24, 48, 72 and 96 hours. After, the effect on metabolic activity was evaluated using the MTT assay, in order to calculate the half maximal inhibitory concentration (IC50). The type of cell death and the percentage of live cells were assessed by flow cytometry using double staining with annexin-V and propidium iodide. In parallel, uptake studies using 18F-FDG and 18F-Fluorocholine were performed. *Results and Discussion*: The drug with less potential for metabolic activity inhibition was 5-FU. Sorafenib was the drug that showed the best results for shorter incubation times, being also the drug with which it has obtained the best fit to the kinetic function, which corresponds to a better pharmacokinetic effect. All drugs induced cell death, preferentially, by apoptosis, except sorafenib that induced cell death by necrosis. In terms of diagnosis, the radiopharmaceutical with higher uptake by the cell line used was 18F-Fluorocholine, about 10 times more than 18F-FDG.In general, the CC cell line shown to be highly resistant to the drugs used, what is concordant with the disappointing results that chemotherapy usually presents in patients with CC. However, the results obtained with sorafenib are promising, demonstrating that targeted therapies, and particularly the use of tyrosine kinase inhibitors may be a therapeutic options for the CC. Regarding to diagnosis, 18F-Fluorocholine uptake was higher than 18F-FDG

uptake, demonstrated that cell proliferation indicator may be more useful for CC diagnosis than the metabolic activity indicator. *No conflict of interest.*

K4. Thymic microenvironment in the malignant transformation of thymocytes in TEL-JAK2-induced leucemia

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Introduction T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy of thymic T cell precursors, it affects mainly children and young adults, disseminating throughout the body invading several organs, and can be fatal without early diagnosis and appropriate therapy. T-ALL is known to be associated with different types of genetic alterations, yet, little is known about the role of thymic microenvironmental factors contributing to the development and/or progression of the disease. An important player in the thymic microenvironment, the transcription factor FoxN1, is expressed in thymic epithelial cells (TEC) since the early stages of embryonic development throughout mammalian adult life, and is essential for thymic development and maintenance. Using a transgenic mouse model of T-ALL driven by the TEL-JAK2 fusion protein, we aim to characterize stromal cell alterations in the thymic lymphomas. *Materials and Methods* Thymic lymphomas from EμSRα-TEL-JAK2 transgenic mice (TJ2-Tg) were used to characterize stromal cell alterations in the thymic microenvironment, by RT-qPCR and immunofluorescence of cryosections. To study the role of FoxN1 in T-cell leukemogenesis we have bred TJ2-Tg mice with mice carrying the spontaneous nude mutation of Foxn1. The TJ2-Tg;Foxn1 cohorts were monitored for the development of leukemia and cytometry of T cells. *Results and Discussion* TEL-JAK2-induced thymic lymphomas show a differential expression of stromal markers. In the TEL-JAK2-leukemic samples, the expression of medullary (Keratin5) and cortical (Keratin8) epithelial cell markers is altered, not only at the gene expression level but also in the way that these Keratin5- and Keratin8-expressing cells are distributed in the thymus. Our results indicate that TJ2 transgenic mice carrying only one wild-type allele of Foxn1 (TJ2-Tg;Foxn1+/nu) develop T-cell leukemia with a statistically significant delay as compared with transgenic littermates with two wild-type Foxn1 alleles (TJ2-Tg;Foxn1+/+). However, inactivation of one Foxn1 allele did not affect the tumor load, at a terminal stage of the disease. Cytometric analysis of thymocytes, from both cohorts of TJ2-Tg;Foxn1 animals, at an earlier time-interval, independently of the presence of visible symptoms of the disease, continue to point to a delay in the development of the T-cell leukemia. TEL-JAK2-induced leukemic mice present a different organization and proportion of thymic stromal epithelial cells, revealing alterations in the thymic microenvironment. The importance of the microenvironment, is further revealed by the delayed onset of the TEL-JAK2-induced leukemia in animals lacking one of the wild-type allele of Foxn1.

No conflict of interest.

K5. Contribution of diffusion models in Diffusion–Weighted Magnetic Resonance Imaging (DWI) for improved breast tumor characterization.

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Introduction: Different diffusion models in DWI can be used to obtain information from breast tumor tissues. The Apparent Diffusion Coefficient (ADC) parameter, derived from the Gaussian model of water diffusion, translates how easily water molecules move in tissues; Diffusion Kurtosis Imaging (DKI) assesses the deviation from gaussianity due to tissue barriers, which is quantified by the Mean Kurtosis (MK) parameter. The Intravoxel Incoherent Motion (IVIM) model distinguishes diffusion from perfusion, using the True Diffusion (D), Pseudo-diffusion (D*) and Perfusion Fraction (PF) parameters. All these parameters reflect tissue microarchitecture: lower ADC and D, and high MK values indicate diffusion restriction as expected in tissues with high cellularity; whilst high D* and PF values are expected in highly vascularized tissues. This work

evaluates the combined use of DWI, DKI, and IVIM diffusion models in the characterization of breast tumor lesions. *Materials and Methods*: A total of 39 breast tumor lesions, comprising 4 benign lesions (Fibroadenoma, FA) and 35 malignant lesions (27 Invasive Ductal Carcinomas, IDC; 6 Ductal Carcinoma In Situ, DCIS; 2 Invasive Lobular Carcinoma) were studied after informed consent was obtained. Data was acquired using a 1.5T MRI scanner with a dedicated breast coil and a DWI sequence with 3 orthogonal diffusion gradient directions and 6 b values between 0 and 1000s/mm^2. ADC and IVIM parameters were obtained from fitting data to mono- and biexponential models, respectively. MK parameter was obtained from fitting data to the diffusion kurtosis model. Finally, mean values were compared between benign and malignant lesions, and between histological types. Non-parametric statistics were used (α=0.05). *Results and Discussion*: Significantly higher ADC and D values were observed in benign ((1.43±0.25, 1.34±0.33)×10^{-3}mm^2/s, respectively) when compared to malignant lesions ((0.94±0.22, 0.85±0.16)×10^{-3}mm^2/s, respectively), and the opposite occurred with MK (benign=0.50±0.44, malignant=1.16±0.43). Supporting these results, FA lesions showed higher ADC and D, and MK lower mean values compared to IDC lesions, which are characterized by increased cellularity, restricting diffusion. For IDC lesions, high PF mean values were observed relatively to DCIS lesions, indicating IDC lesions' higher vascularization. Results suggest that a combined analysis using different diffusion models contributes to a better characterization of breast tumor lesions.

No conflict of interest.

K6. An Interferon-γ-delivery system based on chitosan/poly(γ-glutamic acid) polyelectrolyte complexes modulates macrophage phenotype

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Introduction: Macrophages represent a large component of the tumour microenvironment and are described to establish interactions with cancer cells, playing crucial roles in several stages of cancer progression. The functional plasticity of macrophages upon stimulation from the environment makes them susceptible to the influence of cancer cells but also points them as promising therapeutic targets. In this report, we describe a drug delivery system to modulate macrophages from the pro-tumour M2 towards the anti-tumour M1 phenotype, based on the incorporation of a pro-inflammatory cytokine (Interferon-γ) in Chitosan (Ch)/Poly(γ-glutamic acid) (γ-PGA) complexes. Ch is a biocompatible cationic polysaccharide extensively studied and γ-PGA is a poly-amino acid, biodegradable, hydrophilic and negatively charged. These components interact electrostatically, due to opposite charges, resulting in self-assembled structures that can be modulated to carry and deliver active molecules such as drugs, proteins and others. *Materials and Methods*: Ch and γ-PGA were self-assembled into polyelectrolyte multilayer films (PEMs) PEMs buildup and thickness were characterized by quartz crystal microbalance with dissipation. IFN-γ quantification and release kinetics were studied by protein radiolabeling. IL-10-treated macrophages were placed in indirect contact with PEMs and free IFN-γ and its effect on macrophage phenotype was evaluated by actin/tubulin immunostaining, IL-6 and IL-10 quantification (ELISA) and *in vitro* invasion assays with AGS cells. Results: Ch and γ-PGA were self-assembled into polyelectrolyte multilayer films (PEMs) of 371 nm thickness, using the layer-by-layer method. IFN-γ was incorporated within the Ch layers at 100 and 500 ng/mL. Ch/γ-PGA PEMs with IFN-γ were able to modulate the phenotype of IL-10-treated macrophages at the cell cytoskeleton and cytokine profile levels, inducing an increase of IL-6 and a decrease of IL-10 production. Additionally, the pro-invasive role of IL-10-treated macrophages was hindered, as their stimulation of gastric cancer cell invasion decreased from 4 fold to 2 fold, upon modulation by Ch/γ-PGA PEMs with IFN-γ. Discussion: Based on these findings, we hypothesize that the controlled release of IFN-γ at the tumour site would critically increase its therapeutic efficacy in reversing the phenotype of tumour-associated macrophages. This is the first report proposing Ch/γ-PGA PEMs as IFN-γ delivery systems with the aim of modulating macrophage phenotype, and thus counteracting their stimulating role on gastric cancer cell invasion.

No conflict of interest.

K7. Development of a metastatic orthotopic model of prostate cancer in mice

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Introduction: Prostate cancer (PCa) is one of the most common cancers in men and is one of the leading causes of cancer related death worldwide. The development of animal models able to reproduce PCa behaviour to study the mechanisms involved in its progression is essential to improve of the strategies of diagnosis and therapy. The orthotopic transplantation of human cancer cells in rodents, compared with the heterotopic option, leads to the development of PCa in animals with more similar behaviour to human cancer. However, one of the major problems with these models is the lack of metastatic capacity. *Material and Methods*: This study was performed with PC3, a PCa cell line androgen and estrogen independent, obtained from ATCC. For orthotopic inoculation of PCa cell line, Balb/c nu/nu nude male mice (6-8 weeks) were used. After anesthesia, animals underwent surgery in order to inoculate 15x106 cells/animal. Two different types of inoculation were made: in the dorso-lateral portion of the prostate gland (completely orthotopic) and in the seminal vesicles (locally advanced model). It was performed a daily behavioral evaluation of the mice and a weekly body weight measurement. *Results and Discussion*: In the completely orthotopic animals there was no macroscopic tumor formation after 1, 4 or 8 months. However, in locally advanced model, a macroscopic tumor was observed in the seminal vesicles 3 weeks after surgery, as well as secondary liver lesions. The tumor and the metastasis were composed of solid sheets, with no glandular differentiation, and macronucleolus (Gleason Score 5+5=10) and negative for PSA staining. Through this study it is confirmed that the microenvironment is critical for *in vivo* PCa development. In fact, it appears that seminal vesicles microenvironment, which is rich in mitogenic factors, is favorable to the development of this type of cancer. The development of this variant of orthotopic model, which simulates a locally advanced stage, resulted in local disease as well as a metastatic process. This behavior resembles what we know from the human cancer. Further studies are necessary to better characterize this animal model.

No conflict of interest.

K8. A novel scalable strategy for *in vitro* recapitulation of tumour microenvironment and disease progression

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Introduction: Cancer drug discovery is remarkably inefficient with high rates of attrition even at the late stages of development. Aiming at reducing these high attrition rates, the scientific community has been focusing on the development of advanced preclinical models for target validation, in which the complexity and heterogeneity found in human tumours can be recapitulated. Three dimensional (3D) cell models enable cells to maintain cell-cell and cell-ECM interactions, mimicking *in vivo* tissue organization. Moreover, tumour-stroma crosstalk is being increasingly studied as tumour microenvironment influences tumour progression and drug resistance. The current 3D cell models are typically generated in non-scalable culture systems, with poor robustness and use undefined, biologically active matrices. In this work, we present a novel strategy for *in vitro* recapitulation of solid tumours microenvironment. *Materials and Methods*: MCF7 (human ER+ breast cancer) and H1650 (human NSCLC lung adenocarcinoma) cells were selected for the establishment of breast and lung cancer models, respectively, and incorporated with human fibroblasts in alginate microcapsules. The constructs were cultured in stirred-tank bioreactors and continuously monitored throughout 15 days of culture; and

characterized by analysis of cell viability, proliferation and apoptosis along culture time, as well as metabolic profiling, phenotypic characterization and analysis of ECM deposition. *Results and Discussion*: An alginate microencapsulation method for co-culture of breast and lung tumour cells with fibroblasts was implemented in stirred-tank bioreactors. This method developed allowed the formation of stable and inert hydrogel microcapsules, with tumour aggregates presenting epithelial phenotype with partial polarization, surrounded by fibroblasts, recapitulating tumour-stroma organization *in vivo*. Co-cultures of breast and lung cancer cell aggregates with normal fibroblasts led to the deposition of collagen type I fibers inside the capsules, along with altered tumour cell phenotype, with loss of epithelial character and the acquisition of an epithelial-mesenchymal mixed phenotype. In conclusion, we have developed a scalable, robust and versatile strategy for long-term *in vitro* recapitulation of tumour-stroma crosstalk, suitable for investigation of disease progression mechanisms in different cancer types.

No conflict of interest.

K9. Microenvironment and colorectal cancer. Different localization different metabolic profile

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Introduction: Due to temporal and spatial heterogeneity of oxygenation that occurs in solid tumors the adaptation to the variability of its microenvironment is critical in cancer cells. Therefore, tumor hypoxia negatively affects the treatment outcome of radiotherapy and chemotherapy. In this work we aimed to characterize and compare the metabolic profile of three colon cancer cell lines of different localization. *Material and Methods*: Colorectal cancer cell lines (LS1034, WiDr and C2BBe1-ATCC) were characterized concerning glycolytic and oxidative flows using NMR isotopomer analysis upon incubation with [U-13C]glucose. Citrate synthase and complex IV activities were also evaluated. All experiments were performed in normoxia and hypoxia with 5 mM (low) or 25 mM (high) glucose. *Results and Discussion*: 1H NMR analysis showed that hypoxia resulted in an increase in the rate of lactate production with high glucose in all cell lines, being LS1034 the most glycolytic, but for low glucose cells behaved differently - WiDr reduced lactate production while LS1034 and C2BBe1 had an increase. Under normoxia, WiDr is the most glycolytic cell line followed by C2BBe1 and finally LS1034. 13C NMR isotopomer analysis revealed very distinct 13C3-Lac/13C3-Ala ratios, indicative of very different cytosolic redox states, and very distinct 13C3-Lac/13C4-Glu ratios, denoting major differences in metabolic coupling between glycolytic and Krebs cycle fluxes. Concerning the C4Q/C4D45 ratio, there is a decrease on hypoxia condition in WiDr and LS1034 cell lines. In C2BBe1 such ratio remains approximately constant, in accordance with the reduced oxidative character of this cell line. These results are in accordance with those obtained for the complex IV and CS, in which LS1034 revealed the higher activity. *Conclusions:* Results of lactate production, even in aerobic conditions, reveal the occurrence of the Warburg effect. Hypoxia proved to have a distinct effect on the three colorectal cell lines. This microenvironment alteration demonstrates that LS1034, the cell line resistant to chemotherapeutic drugs, has dramatically increased anaerobic metabolism to account for its energy requirements. For the remaining cell lines, WiDr and C2BBe1, as they are less dependent on oxidative metabolism they are less likely to change their metabolic phenotype due to the hypoxic insult.

No conflict of interest.

K10. Tumor microenvironment influencing molecular imaging. - An *in vitro* study in three different colorectal carcinoma cell lines

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Introduction: Tumor hypoxia negatively affects the treatment outcome of radiotherapy and chemotherapy in various cancers, emphasizing the need for noninvasive detection of tumor hypoxia. The application of 18F-FDG PET imaging in oncology is based in the upregulation of glucose transporters and glycolytic enzymes, and tumor hyperglycolysis. On the other hand, 18F-FCHO is metabolized to form phosphatidylcholine, a major membrane phospholipid, because in malignant cells there is an increase on choline and phosphocholine synthesis. These two radiopharmaceuticals can give us different information about the bioenergetics of colorectal cancer. Aim: Determine the pattern of uptake of 18F-FDG and 18F-FCHO in three colorectal carcinoma cell lines in normoxia and hypoxia conditions and correlate these results with the expression of GLUT-1, -3 and p53. *Material and Methods*: Studies were performed in colorectal carcinoma cell lines (WiDr, C2BBe1 and LS1034). Uptake studies with 18F-FDG and 18F-CHO were carried out. After incubation in a cell suspension of 2×106 cells/ml (25µCi/ml), samples were collected, radioactivity of pellets and supernatants was measured and percentage of uptake calculated. Experiments were conducted in normoxia and hypoxia environment. GLUT-1, -3, as well as p53 expression was assessed by flow cytometry and western blot, respectively. Results: p53 protein quantification revealed that C2BBe1 cell line does not express p53 while WiDr and LS1034 do. Related to 18F-FDG uptake, LS1034 and WiDr cell lines increased the uptake in hypoxic conditions in contrast with C2BBe1. In all cell lines, uptake of 18F-FCHO in hypoxic conditions was higher comparing with normoxic conditions. Concerning GLUT-1 and -3 expression we observed that hypoxia (2 and 48 hours) induced an increase of these glucose transporters. *Conclusions:* In solid tumors, as colorectal cancer, the uptake of 18F-FDG and 18F-FCHO is influenced by tumor microenvironment. GLUTs, the main glucose transporters, can be responsible for these results. Genetic background also reveals to have a key role in cells uptake.

No conflict of interest.

K11. Knowing disease: patients first

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Introduction The health field has been witnessing a redefinition of the disciplines that structure it, which results in a rearrangement of the knowledge and skills of their agents. This evidence is expressed in variable degrees and points to a more diligent and informed attitude of patients in the management of their illnesses. Usually, the biomedical perspective strictly aims to reset the state of health but it does not incorporate the multiple dimensions that the illness experience encloses throughout its extent. This paper reports on a multidisciplinary project entitled "Knowing disease: patients first", developed in the Institute of Molecular Pathology and Immunology at the University of Porto (IPATIMUP, Portugal) and funded by Calouste Gulbenkian Foundation. The project was built upon a list of Frequently Asked Questions (FAQs) produced by pathologists of Johns Hopkins University, USA. These FAQs concern reports of the most frequent biopsy cancers and their precursor lesions. *Materials and Methods* This ongoing two-step study explores the multidimensionality enrolled in illness experience through the discourse analysis of oncological patients, using a strategy of action inquiry framed in a contemporary approach of Grounded Theory methodology. The first step corresponds to assessing patient's illness perception through semi-structured interviews. Its script is based on McGill Illness Narrative Interview (MINI), designed to stimulate illness narratives in health research. The qualitative analysis has been performed

with NVivo 10 software. The second step comprises patient's understanding regarding FAQs content by means of an originally designed interview script. The qualitative analysis of this interview was adapted to a quantitative approach (SPSS analysis), according to Grounded Theory methodology flexibility. Socio-biographical profile of the population was also collected in order to contextualize the information derived from patient narratives in their social conditions, symbolic and material resources and respective paths of life. The population is composed of 100 patients of both genres with breast, lung, colon, esophagus and prostate pathologies who accessed specialty appointments of two public hospitals in Porto. *Results and Discussion* This project highlights the benefit and usefulness of incorporation of subjective dimensions of oncologic patients in the objective dimensions of the biomedical approach to illness. Grounded Theory methodology assisted us to develop a model concerning the personal and social construction of oncologic illness, and produces findings with potential usefulness to patients, professionals and researchers. Introducing a new concept of medicine and citizenship, "Knowing disease: patients first" promotes an increasing level of health knowledge of population groups and, subsequently, improves health care provision.

No conflict of interest.

K12. Gastric Synovial Sarcoma: an uncommon location for a common sarcoma

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Introduction: Although very rare at this location, with the largest publication featuring only 10 cases of primary gastric synovial sarcomas (GSS), gastrointestinal synovial sarcomas (GISS) have been increasingly reported, presenting the typical morphological, immunohistochemical and cytogenetic features, but with a peculiar clinical behaviour. Case Discussion: A 41-year-old male was submitted to an emergent gastric surgery due to gastric bleeding, with a diagnosis of gastric leiomyoma and no further treatment was instituted. Fourteen years later the patient was submitted to emergency laparotomy due to massive intrabdominal bleeding after rupture of two gastric lesions diagnosed as a wild-type gastrointestinal stromal tumor (GIST). Subsequently he was referred to our Institution where pathological review of previous lesions showed that both had morphological and immunohistochemical characteristics of monophasic synovial sarcoma, as well as the typical t(X;18).At re-staging, he presented multiple peritoneal lesions on PET-CT and, despite cytotoxic chemotherapy, no response was observed and he died within a few months. *Conclusions:* Our case not only raises the awareness that not all spindle cell tumors of the gastrointestinal tract are GIST's, but also emphasizes the need for all uncharacteristic mesenchymal GI tumours to be reviewed by a multidisciplinary team on a referral oncological center. Correct diagnosis and management of rare cases demand specialized professionals and criterious use of all the available diagnostic tools.

No conflict of interest.

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