### 503 In vivo metastatic profile of breast cancer cell lines expressing hormonal receptors versus triple negative

M. Carvalho[1], A.M. Abrantes[2], M. Laranjo[2], F. Falcão[3], S. Cabrita[4], M.F. Botelho[2], C.F. Oliveira[5], I. Torgal[6]. [1]*Gynaecology Service University Hospitals of Coimbra Biophysics and biomathematics Institute IBILI CIMAGO, Faculty of Medicine of Coimbra University, Coimbra, Portugal*, [2]*Biophysics and biomathematics Institute IBILI CIMAGO, Faculty of Medicine of Coimbra University, Coimbra, Portugal*, [3]*Gynaecology Service University Hospitals of Coimbra, University Hospitals of Coimbra, Coimbra, Portugal*, [4]*Experimental Pathology Institute, Faculty of Medicine of Coimbra University, Coimbra, Portugal*, [5]*CIMAGO, Faculty of Medicine of Coimbra University, Coimbra, Portugal*, [6]*Gynaecology Service University Hospitals of Coimbra, Faculty of Medicine of Coimbra University, Coimbra, Portugal*

**Background:** Triple negative (TN) tumours are a group of breast cancers with severe clinical behavior. The early metastization is one of the factors that contribute to the aggressiveness. Some clinical studies suggest a differential metastatic spread and a preference for hematogeneous dissemination. Despite these, it is not established a metastatic profile characteristic of each breast cancer type.
The aim of this study is the characterization of metastatic spread *in vivo* of breast cancer cell lines that express hormonal receptors (HR) comparing with TN after injection in the tail vein. The objective is to determine a specific metastization profile, particularly considering the lung and liver.
**Material and Methods:** It was performed injection in the tail vein of female mice Balb/c nude with 4−6 weeks of age with $1.5 \times 10^6$ cells of each breast cancer cell line (MCF7 and HCC1806). Eight weeks after cells injection, the animals were sacrificed and the lungs, liver, kidneys, brain and all sites with suspicious lesion were collected for histological analysis. For morfometric studies were used a histological image analysis focusing on regions of interest (ROI), in order to obtain lesion areas in pixels.
**Results:** The necropsy revealed a macroscopic pelvic tumour and a bone metastasis in mice injected with MCF7. Considering lung analysis, in all animals injected were found metastatic foci on histological study. On one hand, the number of lung foci was not significantly different considering MCF7 and HCC1806 injection. On the other hand, the mean area of lung metastasis in MCF7 cases were significantly higher than in HCC1806 ($p = 0.023$). The histological study of liver showed 47% of metastasis. The number of liver foci was higher in the group injected with HCC1806 than MCF7, reaching statistical significance ($p = 0.006$). The mean area of liver metastasis was not different in the groups considered (p = n.s.). The logistic regression revealed a potentiating model for liver metastasis with HCC1806 (odds ratio = 16; $p = 0.03$). The number and area of lung metastasis foci were not predictive for liver dissemination.
**Conclusion:** The mice injected with HR positive breast cancer cells in the tail vein were associated with huge lung metastatic areas. Liver metastization foci were more relevant in TN than HR positive cell lines. TN cells seem to potentiate liver metastasis. The lung metastization does not influence the presence of hepatic metastasis foci after injection in tail vein.

### 504 WAVE-3 knock-down results in reduced invasion and motility in prostate cancer cells via reduced phosphorylation of paxillin

M. Moazzam[1], L. Yee[2], H. Kynaston[1], W. Jiang[2]. [1]*University Hospital Wales, Urology, Cardiff Wales, United Kingdom*, [2]*University Hospital Wales, Surgery, Cardiff Wales, United Kingdom*

**Introduction:** WAVE-3 is a member of Wiskott-Aldrich syndrome proteins family, regulating cellular migration through actin related protein complex. WAVE-3 activity is controlled by Rho GTPases. Paxillin serves as a nexus for the control of the Rho family of GTPases for their essential role in regulation of actin cytoarchitecture and adhesion dynamics. This study investigates the possible role of paxillin in the changes induced by WAVE-3 knock-down in prostate cancer cells.
**Methods:** Expression of WAVE-3 was knocked down through a transgene consisting of hammerhead ribozyme and antisense specific to WAVE-3, cloned in to a PEF6 expression factor and transfected in to PC-3 cell line through electroporation. After confirming knock-down, *in vitro* assays were used to assess cell growth, adhesion, motility and invasion. Expression and phosphorylation status in different cell lines was assessed by PCR, immunoblotting and immunofluorescence cytology.
**Results:** Stably transfected $PC3^{WAVE\text{-}3\ KD}$ cells exhibited reduced expression of WAVE-3 at both mRNA and protein levels. PC-3 $^{\Delta WAVE3\ KD}$ cell line showed reduced invasion ($P < 0.01$) and motility ($P < 0.01$). The active phophorylated form of paxillin was significantly reduced ($P < 0.01$) in PC-3 $^{\Delta WAVE3\ KD}$ cells on western blotting as compared to $PC\text{-}3^{WT}$ & $PC\text{-}3^{pEF}$ cells and failed to show any significant increase in phosphorylation following growth factor stimulation with HGF.
**Conclusion:** Optimal levels of phosphorylated paxillin are reduced following elimination of WAVE-3 in prostate cancer cells and contribute to a reduction in the invasive phenotype of prostate cancer cells.

### 505 Novel potential markers of tumour-iniziating cells in colon cancer

M.A. Puglisi[1], A. Sgambato[2], M. Barba[1], E. Giorda[3], N. Saulnier[1], A. Boninsegna[2], A.C. Piscaglia[1], R. Carsetti[3], A. Cittadini[2], A. Gasbarrini[1]. [1]*Politechnical University "A. Gemelli", Internal Medicine, Rome, Italy*, [2]*Politechnical University "A. Gemelli", General Pathology, Rome, Italy*, [3]*Bambino Gesù Paediatric Hospital, Cytofluorimetry Laboratory, Rome, Italy*

**Background and Aim:** "Cancer stem cells" (CSC) represent a minority of cancer cells responsible for tumour initiation, maintenance and spreading. Several in vitro assays have been used to identify CSC, including Hoechst dye efflux properties which define the side population (SP), or by the expression of cell surface markers, such as CD133. However, each of these methods has potential pitfalls that complicate interpretation of the results. Our research group have previously demonstrated the presence of a significant CD133+ cell fraction in human primitive and metastatic colon cancer (CC). In the present study, using the CaCo-2 cell line, we wanted to confirm that CD133 expression is a valid method for isolating CSC in CC and identify new antigens in order to increase the specificity of this marker.
**Methods:** CD133+ and CD133− cells were isolated from CaCo-2 cell line by FACSorter and the tumour-initiating potential of CD133+ cells was assessed *in vitro*, by soft-agar colony formation assay, and *in vivo*, upon transplantation into nude mice. Furthermore, the gene expression profile of CD133+ versus CD133− CaCo-2 cells was compared by the means of microarray analysis. Then, in the effort to identify a common "tumour stem cell" signature for CC, the most relevant transcripts resulting from gene expression profiling on CD133+ cells was assessed by real-time PCR on SP-fraction isolated from the same cell line.
**Results:** Using the CaCo-2 cell line, we showed that only CD133+ cells have a tumour-initiating potential in vitro and in vivo. Furthermore, microarray analysis of CD133+ versus CD133− CaCo-2 cells revealed a significant overexpression of various transcripts involved in cell proliferation, invasion and stemness in CD133+ cell fraction. Phenotypic analysis displayed that CD133 expression was higher in the SP fraction than no-SP fraction. Comparison of the transcripts by real-time PCR revealed that the genes of Endothelin-1, Smad-7, S100P and NR4A2 are highly expressed in both CD133+ cells and in SP fraction.
**Conclusion:** Overall, we showed that only CD133+ cells exert a tumour-initiating potential *in vitro* and *in vivo*, confirming that CD133 is a good marker for colon CSC. Furthermore, microarray analysis revealed a unique molecular profile of the CD133+ cells. In particular, four genes are highly expressed in both CD133 + cells and in SP fraction. These genes are involved in regulating cell proliferation and metastasis, so they may be excellent markers to increase the specificity of CD133 in identifying CSC in CC.

### 506 Influence of wild-type MLL on glucocorticoid sensitivity and response to DNA-damage in paediatric acute lymphoblastic leukaemia

A.H. Beesley[1], J.L. Rampellini[1], M.L. Palmer[1], J.Y.S. Lee[1], M.J. Firth[2], U.R. Kees[1]. [1]*Telethon Institute for Child Health Research and Centre for Child Health Research University of Western Australia, Leukaemia and Cancer Research, Perth, Australia*, [2]*Telethon Institute for Child Health Research and Centre for Child Health Research University of Western Australia, Biostatistics and Genetic Epidemiology, Perth, Australia*

**Background:** Rearrangement of the mixed-lineage leukemia gene (*MLL*) is found in 80% of infant acute lymphoblastic leukemia (ALL) and is associated with poor prognosis and resistance to glucocorticoids (GCs). We have recently observed that GC resistance in T-ALL cell lines is associated with a proliferative metabolism and reduced expression of *MLL*. In this study we have further explored the relationship between *MLL* status and GC sensitivity.
**Material and Methods:** Studies were performed using a cell line panel comprising nine T-ALL lines derived in our own laboratory from paediatric ALL bone marrow specimens, plus six additional T-ALL cell lines obtained from external sources. Sensitivity of T-ALL cell lines to methylprednisolone (MPRED) and dexamethasone (DEX) was measured using the MTT assay with drugs incubated over four days. For gene expression profiling RNA was extracted from cell lines in exponential growth phase and hybridized to Affymetrix HG-U133A microarrays. Published microarray data used for *in silico* analysis was downloaded from publicly available depositories or authors' websites.
**Results:** Negative correlation of *MLL* expression with GC resistance in 15 T-ALL cell lines was confirmed by quantitative RT-PCR. The absence of *MLL*-rearrangements in the panel of T-ALL cell lines suggested that this relationship represented expression of wild-type *MLL*. Analysis of *MLL* expression patterns revealed a negative relationship with cellular metabolism, proliferation and anti-apoptotic transcriptional networks. *In silico* analysis of published data demonstrated that reduced levels of *MLL* mRNA are associated with relapse and GC resistance in T-ALL patients and adverse clinical outcome in children with *MLL*-rearranged ALL. RNAi knockdown of *MLL* expression in T-ALL cell lines significantly increased resistance to dexamethasone and gamma