assessors (1 surgery, 1 pathology). Survival was analyzed using Kaplan–Meier curves and the Log-rank test.

Results: There is increased tumour epithelial cytoplasmic staining for NR4A2, and an altered nuclear-cytoplasmic ratio with more cytoplasmic NR4A2 in tumour compared to matched normal tissue (p = 0.001). High tumour cytoplasmic NR4A2 is associated with a significantly worse overall and disease specific survival in colorectal cancer. (5-year DSS 64% high cytoplasmic NR4A2 versus 80% low cytoplasmic NR4A2, log-rank test p=0.049, 5-year OS p=0.003)

Conclusions: There is marked cytoplasmic mislocalisation of NR4A2 in colon cancer. High tumour cytoplasmic NR4A2 is associated with an adverse prognosis.

0921: LOCALISED INHIBITION OF MICRORNA-15 AND -16 RESULTS IN AN IMPROVED ANGIOGENIC RECOVERY FOLLOWING LIMB ISCHAEMIA

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Introduction: MicroRNAs (miRNA; miR) are small non-coding RNAs which negatively regulate mRNA translation. miR-15 & miR-16 are upregulated in response to myocardial ischaemia, whilst inhibition protects against cardiac ischaemic injury in a mouse model. We investigated the effect of adenovirus-mediated local miR-15/16 inhibition in a mouse model of hindlimb ischaemia.

Method: We generated an adenovirus capable of overexpressing a miRNA inhibitor (Ad.Decoy-15/16), containing multiple, tandem complementary binding sites for miR-15 and miR-16, and thus allowing specific and efficient depression of miR-15/16 expression. Unilateral hindlimb ischaemia was surgically induced in anaesthetised CD1 mice (n=12 per group) and Ad.Decoy-15/16 or an Ad.Null control (10⁹ virus particles) were delivered to the ipsilateral adductor muscle.

Results: Blood flow to the ischaemic limb was significantly improved by Ad.Decoy-15/16 at 7 (p<0.04), 14 (p<0.01) and 21 (p<0.03) days post-surgery, with an average increase of ~50% compared to control mice. Subsequent histopathological analyses at day 21 demonstrated an increase in both capillary density (by ~30%, p<0.041) and arteriolar density (by ~40%, p<0.03) in the ischaemic adductor muscles of Ad.Decoy-15/16-treated mice compared with control.

Conclusions: These data indicate a pro-angiogenic effect of localised miR-15/16 inhibition in the setting of peripheral limb ischaemia in mice.

0942: THE ROLE OF MICRORNA-30C-2* AS AN ANTIANGIOGENIC MEDIATOR

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Introduction: MicroRNAs (miRs) negatively regulate the expression of targeted mRNAs. Here, we aim to elucidate the contribution of miR-30c2* in endothelial cell (EC) dysfunction in diabetes.

Methods: miR30c2* functional studies were performed in human umbilical vein endothelial cells (HUVECs) under conditions of high glucose (HG, mimics diabetes) and low growth factors (LGF, mimics ischaemia). Expressional analyses were performed in mice, with and without diabetes, using whole limb muscle and the ECs derived from these tissues.

Results: MicroRNA-30c-2* expression was upregulated under LGF, HG and a LGF/HG combination (p<0.01). In addition, miR-30c-2* was three-fold upregulated in muscular ECs of diabetic mice (p<0.01). miR-30c-2* overexpression induced HUVEC apoptosis and impaired angiogenesis. Conversely, miR-30c-2* inhibition prevented LGF- and LGF/HG-induced apoptosis and impaired angiogenesis.

The cell cycle regulator minichromosome maintenance complex component 7 (MCM7) is a putative miR-30c-2* target. MCM-7 mRNA levels were decreased in p75NTR-HUVECs (p<0.05 vs. Null) and in HUVECs cultured in HG/LGF (p<0.05). miR-30c-2* overexpression reduced MCM7 mRNA (p<0.01).

Conclusions: In conclusion, the p75NTR-induced miRNA miR-30c-2* represses EC survival and angiogenesis, and may prove to be a novel therapeutic target for diabetes-induced endothelial damage in limbs.

0967: CHARACTERIZATION OF INITIATING PHYSIOLOGICAL EVENTS OF MAMMARY GLAND INVOLUTION: EVIDENCE FOR CATHEPSIN B INVOLVEMENT

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Introduction: The mechanism of one of the most extensive physiological cell death in mammals, postlactational regression (involution) of the mammary gland, was recently characterized as a non-classical, lysosomal-mediated and caspase-independent pathway of cell death. Expression of cysteine proteinases cathepsins B and L was shown to be highly increased via Stat3 pathway during this process. The present study aimed to investigate the intracellular trafficking and potential involvement of cathepsins B and L in the physiological events leading to involution mediated by lysosomal membrane permeability.

Methods: Fluorescent immunohistochemical staining of mammary gland sections from wild type and Stat3 KO mice was performed for cathepsins B, L and α–lactalbumin, alongside co-staining of the lysosome associated membrane protein-2 (LAMP2). Intracellular (co-) localization was analysed by deconvolution fluorescence microscopy.

Results: Both cathepsins B and L, but not α–lactalbumin, showed strong co-localization with LAMP2, indicating lysosomal translocation, in the presence of functional wild-type Stat3. The co-localization with the lysosomal membrane was significantly reduced, most severely in the case of cathepsin B, in Stat3 KO mice compared with wild-type.

Conclusions: The strong Stat3-dependent lysosomal membrane localization of cathepsin B suggests a role for this proteinase in triggering the lysosomal membrane permeability associated with the mammary gland involution.

0988: M1 POLARISED MACROPHAGES DEVELOP AN ENDOTOXIN TOLERANCE-LIKE PHENOMENON IN RESPONSE TO BACTERIAL STIMULATION

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Introduction: To establish a predominant M1and M2 macrophage polarisation pattern in vitro. To examine the inflammatory cytokine response to bacterial stimulation in polarised cells. To determine the phagocytic activity in polarised cells after bacterial stimulation.

Methods: Peritoneal and bone marrow derived macrophages were harvested from C57BL/6 mice. Cells were exposed to polarising stimuli for 18-24 hours. (M1 - LPS and IFN-γ, M2 - IL-4). Polarised cells were further stimulated with heat-killed Staphylococcus aureus and Salmonella Typhi or FITC-labeled E coli. Inflammatory cytokine production and phagocytosis were assessed by ELISA and FACScan analysis.

Results: M1 macrophages were characterised by high levels of TNF-α and IL-12p70 and M2 macrophages by high levels of TGF-β and low levels of IL12p70. M1 polarized macrophages, when exposed to gram-positive bacteria, had lower levels of TNF-α than M2 macrophages. Phagocytosis assays revealed similar results for both macrophage subpopulations.

Conclusions: M1 macrophages are expected to produce higher levels of TNF-α, however we found that M1 macrophages, when stimulated with bacteria, had lower levels of TNF-α compared with their M2 counterparts. This unexpected result indicates a tolerisation effect developed during the M1 polarisation and further work is required to clarify the underlying mechanism(s).

1094: ASSOCIATION BETWEEN LEVELS OF MATRIX METALLOPROTEINASE-3 AND JOINT REPLACEMENT OF THE HIP AND KNEE IN RHEUMATOID ARTHRITIS

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Introduction: Within 20 years 25% of rheumatoid arthritis (RA) patients will undergo total joint replacement. Matrix metalloproteinase-3 (MMP-3) is released during the disease process. Previous studies suggest that MMP-3 levels decrease following joint replacement. The objective of this study was to investigate if serum levels of MMP-3 at baseline are associated with future joint replacement of the hip and knee.

Methods: Clinical data and MMP-3 levels were recorded prospectively for 278 RA patients over a 5-year period. The mean (sd) age of the cohort was 59 (9.9) years with a disease duration of 11(9.2) years and 187 (67%) of the cohort were female.

Results: Significantly higher levels of MMP-3 were found at baseline for those patients who required a joint replacement during the five year follow up period (n=49) than those that did not: median (IQR) MMP-3 level (27,050 (37,146) vs. 19,892 (18,894), p =0.005). No significant difference was found between baseline MMP-3 levels and having a joint replacement prior to the start of the study (n=32): median (IQR) MMP-3 level (25,705 (21,283) vs. 20,031 (20,258), p =0.25).

Conclusions: High levels of MMP-3 are associated with future joint replacement of the hip or knee in RA patients.

1275: THE EFFECTS OF SOCS7 KNOCKDOWN IN BREAST CANCER CELLS: THEIR IN VITRO RESPONSE TO HEPATOCYTE GROWTH FACTOR (HGF)

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Introduction: Phospholipase Cγ-1 (PLCγ-1) is an important Hepatocyte Growth Factor (HGF) receptor (C-MET) downstream mediator, and recent evidence showed that Suppressor of Cytokine Signalling 7(SOCS7) - a member of the SOCS family - interacts with PLCγ-1. Here, we aimed to investigate SOCS7 knockdown effect on breast cancer cellular growth and migratory responses to HGF treatment, and whether this involves HGF-PLCγ-1 pathway using the PLCγ-1 blocker U73122.

Methods: Two breast cancer cell lines (MCF7 and MDA-MB-231) were transfected with anti-SOCS7 ribozymal transgene, creating sublines with SOCS7 knockdown (MCF7$^{\Delta SOCS7}$ and MDA-MB-231$^{\Delta SOCS7}$), verified by RT-PCR. The growth and migration of the cells were evaluated with and without HGF and U73122 pre-treatment using growth assay, scratch-wound and Electrical Cell Impedance Sensing (ECIS) migration assays.

Results: Under basic conditions, both MCF7$^{\Delta SOCS7}$ and MDA-MB-231$^{\Delta SOCS7}$ cells showed higher growth and migration compared to control cells. Additionally SOCS7 knockdown appeared to synergistically enhance their growth and migratory responses to HGF. U73122 pre-treatment was found to abrogate this synergistic effect.

Conclusions: HGF pre-treatment and SOCS7 knockdown have a synergistic effect on the growth and migration of MCF7 and MDA-MB-231 cells. This is lost with pre-treatment with U73122, an alternative PLCγ-1 blocker, indicating a precise anti-PLCγ-1 regulatory role for SOCS7.

Breast surgery

0008: USE OF AUTOLOGOUS FAT GRAFTING FOR RECONSTRUCTION POST-MASTECTOMY AND BREAST CONSERVING SURGERY: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Introduction: There is growing interest in the potential of autologous fat grafting (AFG) for breast reconstruction. However, concerns remains regarding its effectiveness, safety and interference with mammography.

Methods: A protocol was published a priori. All studies investigating AFG for women undergoing reconstruction post surgery for treatment of breast cancer were considered. We assessed six domains; Oncological, clinical, aesthetic/functional, patient reported, process and radiological. Electronic databases and grey literature sources were searched to June 2013.

Results: 31 studies were included in this review (3,521 patients). Fat necrosis is the commonest reported complication at 4.4% (the majority was managed conservatively). Other harms include the anxiety caused by the need for further radiological investigation through interval mammograms (11.5%) and the need for biopsy (2.5%) to exclude malignancy. The

weighted mean recurrence rate was 4.4% at a median of 18.3 months. Random effects Meta-analysis showed no significant difference (p=0.10). We were unable to comment from the data on whether AFG is more successful in combination with other techniques or alone.

Conclusions: The need for long-term follow up is underscored by this review. High quality research is required to demonstrate long-term oncological ramifications and to determine the potential for AFG as a total breast reconstruction.

0244: AN EVALUATION OF THE CORRELATION BETWEEN PRIMARY TUMOUR AND LYMPH NODE RESPONSE FOLLOWING NEOADJUVANT THERAPY IN BREAST CANCER

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Introduction: Neoadjuvant therapy (NAT) offers an opportunity to assess tumour response to systemic agents. However discrepancy may exist between response of primary tumour & involved nodes. This study sought to assess the frequency of discordance in this response post NAT.

Methods: All node positive patients receiving NAT at Cork University Hospital, Republic of Ireland from 2009-2012 were identified. Basic demographics, radiological & pathological features were tabulated & analysed. Nodal response was estimated from standard pathological response to treatment measurements. Statistical analysis was performed.

Results: 66 node positive patients had completed surgery & were eligible for inclusion. Median age was 50 years, all patients underwent axillary clearance and 64% underwent mastectomy. There was an overall positive correlation between tumour and lymph node (LN) response following NAT (Spearman correlation coefficient 0.541, p<0.001). Eleven patients achieved a LN complete pathological response (CPR) with all having a CPR in tumour also. A CPR in the tumour predicted complete nodal response in 73% of cases.

Conclusions: While overall correlation was seen, 27% of primary tumours with CPR had persistently positive LN's. This represents a significant discordance that may be due to biomolecular differences and represents a concern for the potential lack of response of occult systemic metastasis to NAT.

0333: BREAST CANCER, WHAT WOULD YOU CHOOSE? – A SURVEY OF HEALTH PROFESSIONALS

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Introduction: To investigate whether medical professionals, when confronted with a breast cancer diagnosis, would ignore evidence-based medicine in favour of personal preference. Compare the opinion of the breast team with the actual treatment chosen by their patients.

Methods: Anonymous questionnaire with 3 scenarios given to all (39) members of the regional MDT and comparison made with patients' treatment choices.

Results: For a 10 mm, grade 1 cancer 82 % (32/39) of health professionals favoured WLE over mastectomy, compared to 51 % (31/60) of patients (p=0.003). For a 25mm, grade II, invasive cancer 55% (21/38) of medical professionals favoured WLE compared to 49% (28/57) of patients (p=0.67). For 60mm, high grade DCIS, 26% (10/39) of health professionals would chose mastectomy without reconstruction, compared to 75 % (9/12) of patients (p=0.005). Following a mastectomy, 74 % of health professionals would have reconstruction: 28 % immediately, 46 % delayed; whilst of the 25 % of patients with reconstructions all had immediate reconstruction.

Conclusions: A significant majority of health professionals would choose WLE for grade I and II invasive ductal carcinoma compared to their patients' treatment choices. For 60mm DCIS, health professionals were more three times more likely than their patients to choose reconstruction.

0388: THE UTILISATION OF MAGNETIC RESONANCE IMAGING IN THE INVESTIGATION OF INVASIVE LOBULAR CARCINOMA – A RETROSPECTIVE STUDY IN TWO DISTRICT GENERAL HOSPITALS

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