somatic alteration, including 30 regions with gains (NF1, CCND1, MYC, TP53, DCC, MAP2K4, AURKA, IGF1R, PIK3CA, GAB2, NXXK2-1, BCL2L2, C8orf4, BCL2L1, ZNF217, EGFR, NCOA3, SKP2, FADD, ORAOV1, EEF1A2, REL, TERT, AKT3, PRKCJ, MAPK7, DCUN1D1, BIRC2, YAP1, MYCN) and 14 losses (FHT, GPCS, IRS2, BRC2, RB1, PGDFRA, CDK6, SHH, APC, FOXO1, MET, MTF2, KDR, KIT).

Conclusion The characterisation of a new cervical cancer cell line, HCB-514, will constitute an important in vitro tool for further biological and therapeutic studies of cervical cancer.

PO-344 OSTEOSBLASTOMA IS CHARACTERISED BY RECURRENT REARRANGEMENTS OF FOS AND FOSB

Introduction Osteoblastoma, and the related entity osteoid osteoma, are the most common benign bone-forming tumours. Large, inexcusable, and recurrent tumours can cause considerable morbidity. On occasion, there can be diagnostic uncertainty with osteosarcoma, a malignant tumour that requires multimodal therapy. We sought to define the somatic changes that underpin osteoblastoma.

Material and methods We analysed the whole genomes of 5 osteoblastomas and 1 osteoid osteoma and catalogued all somatic variants. RNA-seq was used to corroborate DNA changes and call gene fusions. FOS fusions were validated with Sanger sequencing. We used FISH and IHC to validate the finding of FOS/FOSB rearrangements in 55 osteoblastomas, 17 angiosarcomas and 183 osteosarcomas. We analysed 55 osteosarcoma and 2652 pan-cancer whole genomes for similar rearrangements.

Results and discussions There was a paucity of somatic alterations in osteoblastoma, with a median mutation burden of 319 substitutions (range 123–700) and 28 indels (range 14–50) per genome. Copy number analyses demonstrated diploid tumours with few aberrations. Only a small number of mutations affected the coding sequence of genes, none of which were plausible driver events.

Analysis of structural variants revealed breakpoints in the AP-1 transcription factor FOS, in 5/6 cases, and its parologue FOSB in the sixth case. All were validated with RNA-seq reads and FOS fusions were validated with Sanger sequencing. FOSB fusion brought expression under the control of the PPP1R10 promoter. FOS fusion were all between exon 4 and intronic or intergenic regions. FOS fusions resulted in the introduction of a stop codon within 30 bp of the breakpoint.

In a validation cohort of 55 tumours, FISH identified breakapart signals in 1 and 48 tumours respectively (89%). Osteoblastoma cellularity is low, hampering FISH sensitivity. IHC for the preserved N-terminus of FOS revealed marked immunoreactivity in all FOS rearranged cases, including the 3/6 FOS FISH negative cases with available material. Only 1/183 osteosarcoma cases had comparable FOS immunoreactivity. No osteosarcoma or pan-cancer whole genome harboured similar rearrangements.

PO-343 TARGETED RESEQUENCING IDENTIFIES NOVEL AND ULTRA-RA HIGH-IMPACT VARIANTS IN BREAST CANCER SUSCEPTIBILITY GENES IN AN IRISH POPULATION

Introduction Breast cancer (BC) remains the most common female malignancy worldwide (incidence of 89.7/100,000 women). Pathogenic variants in BRCA1 and BRCA2 account for 3% of all BC cases. A further 25% of BCs demonstrate familial clustering that may be accounted for by variants of reduced penetrance in other BC susceptibility genes. Next-generation sequencing facilitates massively parallel sequencing of multiple genes in a cost-effective manner. Multiple expanded gene panels exist, including high- and moderate risk BC susceptibility genes, as well as loci with weak/putative association with disease. Expanded gene panels may increase diagnostic yield, but also increase identification of variants of uncertain significance.

We aimed to investigate the frequency of high impact variants in known or putative BC susceptibility genes in an Irish population using a custom-designed multi-gene panel.

Material and methods Targeted resequencing of 168 gDNA samples (91 patients with BC; 77 unaffected ethically-matched controls) was performed on an Illumina NextSeq using a Roche Nimblegen custom 282-gene panel capture. GATK best practices, 2016 were implemented for data analysis. PLink1.9 was utilised for confirmation and removal of first-/second-degree relationships within our cohort. 1000Genomes samples (91 patients with BC; 77 unaffected ethnically-matched controls) was performed on an Illumina NextSeq.

Results and discussions 69 high-impact loss-of-function (LoF) variants were identified in 54 genes (including 51 not typically tested in diagnostic setting). 50 patients and 40 healthy controls were heterozygous for ≥ one LoF variant. Frequency data for 34 variants was absent from population databases, while 23 variants were reported as ultra-rare (minor allele frequency ≤ 1.5 × 10⁻5). Two LoF variants were identified in BRCA1; 1 in ATM and 1 CHEK2 (c.1100delC). Considering all variants, enrichment in cases v- controls was not significant (p=0.761). 9 LoF variants were observed in genes that are recurrently somatically mutated in BC.

Conclusion After controlling for ethnicity and relatedness, NGS identified a high-impact variant in 56% of cases and 58% of controls. However, variants in only 3 out of 54 genes in which variants were identified currently impact clinical management.

Our results confirm that NGS increases diagnostic yield, but of variants in genes with moderate-weak association with BC. Further analyses are required to determine contribution and clinical utility of testing these variants in patients with BC.

Abstracts
Conclusion FOS or FOSB rearrangements define osteoblastoma and osteoid osteoma. The truncated FOS transcript and protein bears a striking resemblance to the retroviral oncogenic form of v-fos, known to induce osteosarcoma in mice. This is the first report of FOS alteration in a bone-forming tumour.

Functional Genomics

**PO-345** THE ROLE OF THE DEUBIQUITINASE USP11 IN ENDOCRINE-DRIVEN BREAST CANCER

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**Introduction** Approximately 80% of breast cancers overexpress the oestrogen receptor α (ERα) and depend on this key transcriptional regulator for growth. The discovery of novel mechanisms controlling ERα function represent major advances in our understanding of breast cancer progression and potentially offer new therapeutic opportunities. Here, we investigated the role of deubiquitinating enzymes (DUBs), which remove ubiquitin moieties from proteins, in regulating ERα activity in breast cancer.

**Material and methods** To identify DUBs involved in ERα transcriptional activity, we performed an RNAi loss-of-function screen using a library of shRNA vectors targeting all 108 known or putative human DUB genes. We found that suppression of the BRCA-associated DUB, USP11, downregulated ERα transcriptional activity in ZR-75-1 cells. Dual luciferase reporter assays and qRT-PCR were used to determine ERα activity in stable ZR-75-1 USP11 knockdown cell lines. PTMScan technology, which allows for enrichment of ubiquitinated proteins and quantitative profiling by mass spectrometry, was used to reveal the ubiquitinome in these cells and results are pending. RNA sequencing (RNA-seq) technology was carried out in estrogen-independent LCC1 USP11 knockdown cells. USP11 was hypothesised to play a vital role. We carried out RNA-seq technology was performed in estrogen-independent LCC1 USP11 knockdown cell lines. Two experiments of MNT silenced and carried out RNA-seq. This resulted in 158 genes whose expression was altered. Cell cycle, two experiments of MNT silenced and carried out RNA-seq. This resulted in 158 genes whose expression was altered. Cell cycle, DNA replication and DNA repair genes were downregulated upon USP11 silencing in URMT and URMMax34 cells and confirmed by RT-qPCR. Changes in protein levels were analysed by western blot. Co-immunoprecipitation and proximity ligation assays were used to study protein-protein interactions.

**Results and discussions** Knocking-down of USP11 in ZR-75-1 cells decreased ERα transcriptional activity and mRNA expression of ERα target genes. These results were further validated in a HEK293T USP11 CRISPR knockout model with ectopic ERα expression. Interestingly, USP11 expression was upregulated in LCC1 cells, an isogenic, estrogen-independent model derived from MCF-7 cells. Knockdown of USP11 in LCC1 cells decreased the expression of multiple ERα target genes and cell cycle-associated genes, as determined by RNA-seq. IHC staining of a breast cancer tissue microarray (103 ER + patients) and subsequent Kaplan-Meier analysis of this cohort revealed a significant association between high USP11 expression and poor overall (p = 0.030) and breast cancer-specific survival (p = 0.041). In silico analysis of publication available breast cancer gene expression datasets further supported an association between high USP11 expression and poor prognosis.

**Conclusion** These results suggest a role for USP11 in ERα transcriptional activity and reveal a novel mechanism as to how this receptor is regulated in breast cancer.

**PO-346** THE MYC ANTAGONIST MNT BEYOND MAX INTERACTION

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**Introduction** MNT has been described as an antagonist and modulator of MYC, one of the most prevalent oncoproteins in human cancer. Both MYC and MNT are bHLH-LZ transcription factors that heterodimerize with MAX, bind to E-boxes within regulatory regions of target genes, and generally activate (MYC) or repress (MNT) their transcription.

**Material and methods** The cell lines used, URMT and URMMax34, derive from MAX-deficient PC12 (rat pheochromocytoma), and carry a pHeBo-MT (empty vector) and a pHBeo-MT-MAX vector (MAX-inducible with Zn2+), respectively. Knockdown of MNT and MLX were achieved with short hairpin RNA constructs (shMNT and shMLX). Proliferation was assessed by cell counting and clonogenic assays; subG0-G1 population was determined by flow cytometry. RNA-seq was performed from two experiments of MNT silencing in URMT and URMMax34 cells and confirmed by RT-qPCR. Changes in protein levels were analysed by western blot. Co-immunoprecipitation and proximity ligation assays were used to study protein-protein interactions.

**Results and discussions** Knocking-down of MNT in UR61 cells resulted in an important decrease in cell proliferation, together with a decrease in both survivin and cyclin A, which are markers of pro-survival and cell proliferation, respectively. DNA content was measured by flow cytometry, revealing an increase in sub-G0 population in shMNT cells. Thus, MNT is required for optimal proliferation of these cells. This is the first evidence of a MAX-independent function of MNT. Then, we extracted RNA from two experiments of MNT silenced and carried out RNA-seq. This resulted in 158 genes whose expression was altered. Cell cycle, DNA replication and DNA repair genes were downregulated upon MNT silencing. However, there were other up-regulated genes like the cell cycle inhibitor CDKN1C (p57). As we confirmed gene regulation by MNT without MAX, we wondered whether it could be working as an heterodimer with MLX or as an homodimer. Co-immunoprecipitation and proximity-ligation assays showed MNT’s ability to form homodimers and heterodimers with MLX. Finally, we carried out MLX knockdown and determined the genes regulated by MNT-MLX or MNT-MNT complexes.

**Conclusion** In summary, we report novel MAX-independent functions of MNT. In our MAX-deficient model, MNT can be found in homodimers (MNT-MNT) or heterodimers (MNT-MLX) and it supports proliferation and regulates cell cycle and DNA repair genes. This new data about MNT can open new insights into cell biology and tumour development promoted by MYC.