therapy or for active surveillance as conservative management approach.

**Material and methods** Therefore, we aimed to evaluate the potential of novel or known prognostic biomarkers in high-risk and low-risk prostate cancer tissues, and adjacent normal tissues by RNA sequencing and qPCR techniques. We also investigated tumour heterogeneity by including different foci from primary prostate cancer. A set of prognostic biomarker candidates was identified upon RNA sequencing of radical prostatectomy tumours of patients (n=25) with or without biochemical relapse (>5 year follow-up), as well as adjacent benign tissues. The candidate genes were retrospectively validated by qPCR method in the same and in an independent patient cohort (n=59). Expression variance of genes was investigated in different tumour foci of four primary tumours.

**Results and discussions** Overall, 16 prognostic biomarker candidates were selected from RNA sequencing data analysis according to differential expression between high-risk cancer, low-risk cancer and benign tissues. In total 10/16 candidates were technically sound upon qPCR of in the same cohort. We could clearly show that qPCR is a very robust and sensitive method to verify RNA sequencing data. Additionally, known tumour markers like AMACR and ERG showed expected signatures associated with the clinical phenotype and FISH-based gene fusion status. Data from different tumour loci indicated high expression variances across tumour sections. Independent validation of candidate genes could not confirm significant differential expression between the patient risk groups.

**Conclusion** Tumour heterogeneity might impede the detection and validation of diagnostic and prognostic biomarkers in primary prostate cancer tissues.

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**PO-325 NOVEL RECURRENT HIGH-LEVEL AMPLIFICATIONS IN MICROSATELLITE STABLE COLORECTAL CANCER**

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10.1136/esmoopen-2018-EACR25.355

**Introduction** Colorectal cancer (CRC) is a molecularly diverse disease with few targeted treatment options. Focal and high-amplitude DNA copy number aberrations are potential tumour drivers, and their identification may contribute to improved therapeutic outcomes in small patient subgroups, as recently illustrated by targeting the over-expression of HER2 protein resulting from high-level ERBB2 amplification in KRAS wild-type metastatic CRC. However, few recurrent amplifications have been detected in CRC.

**Material and methods** We analysed focal high-amplifications in 203 microsatellite stable (MSS) primary colorectal tumours using genome-wide high-resolution Affymetrix SNP6.0 arrays. The ASCAT algorithm was used to derive discrete allele specific copy number estimates.

**Results and discussions** The overall copy number profiles confirmed the frequent gains and losses previously described in MSS CRC studies. Extreme focal amplifications (defined as >15 copies and <50 genes in peak) were found in 77 regions in tumours from 22 unique patients, distributed across 16 different chromosome arms. Recurrent amplifications included ERBB2, which was amplified to 97, 27 and 22 additional copies in tumours from three individual patients (amplification frequency 1.5%). The transcription factor TOX3 (16q) was also recurrently amplified in 1.5% of the patients, while MYC (8q), CCND2 (12 p) and a region on 10q22.3-q23.1, where ANXA11 was nominated as a likely target by GISTIC analysis, were recurrent in 1% of the patients. Regions with extreme and focal amplifications were also investigated for lower-amplitude aberrations (5–15 additional DNA copies), revealing a 3% amplification frequency of TOX3 in our cohort.

**Conclusion** We have identified several recurrent amplifications in cancer-critical genes in CRC MSS tumours, including the transcription factor TOX3, suggesting novel drug targets for preclinical studies.

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**PO-326 IMPACT OF MIR-205–5 P AND MIR-425–5 P ON WNT AND AR SIGNALLING PATHWAYS IN CASTRATION RESISTANT PROSTATE CANCER TRANSITION**

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10.1136/esmoopen-2018-EACR25.356

**Introduction** Prostate cancer (PCa) is the second leading cause of cancer mortality in western countries. Prostate tumours initially respond well to androgen-deprivation therapy (ADT). Unfortunately, the majority of tumours evolve, after androgen deprivation, from a hormone-sensitive to a castration-resistant prostate cancer (CRPC). For that reason new agents, targeting the androgen receptor (AR) pathway (abiraterone, enzalutamide among others), have been approved in the last decade. Unfortunately, the emergence of resistance to these treatments is common and CRPC remains highly lethal. Therefore, new approaches and better knowledge of the molecular mechanisms leading to CRPC is still needed. Mechanisms related to CRPC transition include increased expression of AR and activating mutations in this receptor. As in other tumours, there are other signalling pathways that could interfere with AR activation such as Wnt signalling pathway, which has been suggested to play an important role in CRPC. On the other hand, emerging evidences indicate that certain miRNAs are involved in the appearance of treatment resistances in several diseases. The aim of this project was to study miRNA and mRNA expression profiles to identify deregulated miRNAs and genes involved in the Wnt signalling pathway in CRPC.
Material and methods A set of 20 PCa tumour samples, 10 radical prostatectomy (RP) specimens from hormone-naïve patients vs 10 transurethral resection of prostate (TURP) samples from castration resistant patients, were analysed. Total RNA was obtained to study miRNA and mRNA expression using Affymetrix GeneChip miRNA 4.0 and GeneChip Clarion S human arrays, respectively. To identify deregulated miRNAs and their corresponding predicted target mRNA related to AR and Wnt signalling pathways, both miRNA and mRNA expression profiles were integrated by correlation analysis using the TAC software (appliedbiosystems).

Results and discussions When hormone-naïve samples vs. castration-resistant samples were compared, 62 miRNAs and 1023 mRNAs were significantly deregulated. Further identification of potential target genes found a strong correlation between hsa-miR-205–5p, AR and Wnt5A, and between hsa-miR425-5p and GSK3β. In addition, expression of hsa-miR-425–5p correlated with survival (2-tailed Student’s t test, p-value<0.05) in patients with PCa.

Conclusion Integrated analysis of miRNA and mRNA expression profiles is a useful tool to identify novel therapeutic targets for CRPC. The role of miRNAs involved in CRPC deserves further investigation.

Introduction The aim of this project was to perform genome-wide loss-of-function CRISPR-Cas9 screens across hundreds of genetically-annotated human cancer cell lines to identify synthetic-lethal interaction between cancer genomes and targeted gene deletion. This poster details the pipeline and processes used to successfully screen a large collection of diverse human cancer cell lines.

Material and methods A whole genome CRISPR-Cas9 screening pipeline was set up in the Cellular Generation and Phenotyping cell culture core facility at the Wellcome Sanger Institute. The pipeline consists of the following three stages:

1. Antibiotic titration of parental cancer cell lines to determine the correct kill concentration for selection markers.
2. Production and banking of stable Cas9-expressing lines via lentiviral transduction. Cas9 activity was determined using a BFP-GFP-gGFP reporter vector. Lines which had Cas9 activity of greater than 75% were used for downstream screening.
3. 33 million Cas9-expressing cells were transduced in triplicate with a lentivirally delivered genome-wide guide RNA library at an efficiency of 15%-50% as determined by BFP reporter.

Successfully transduced cells were selected with puromycin on day 3 and expanded, maintaining library representation at x500 coverage. Following two weeks screening, cells were harvested and pelleted for DNA extraction and sequencing to identify essential genes.

Multiple process improvements increased pipeline efficiency, increased Cas9 activity and improved accuracy of scale up of guide RNA library transductions.

Results and discussions The screening pipeline is highly robust and consistent with an overall success rate of >80% of lines successfully Cas9 transduced and screened. The screen has identified hundreds of essential genes across our cancer types, including many core essential genes.

Conclusion Cancer cell lines can be successfully screened at scale to generate robust essentiality data.

Introduction Understanding the mechanisms of mutagenesis in cancer is important for the prevention and treatment of the disease. While it is well known that environmental carcinogens such as tobacco smoke substantially increase disease incidence, other studies have suggested that the number of replicative cycles a cell undergoes is the biggest risk factor for cancer. To reconcile these divergent observations, it is critical to understand of and how DNA replication influences distinct mechanisms of mutagenesis.

Material and methods Here, we developed a methodology to identify replication-strand and timing asymmetry of mutational signatures, representing different mutagenic mechanisms.

Results and discussions Applying this computational framework to whole-genome sequencing data from over 3000 patients, spanning 19 cancer types, reveals a significant impact of DNA replication in 22 out of 29 mutational signatures. This includes some well-known examples, such as the APOBEC class of enzymes that preferentially attack cytosine residues on the lagging strand. Notably, however, we find that most exogenous mutagens, such as UV light or tobacco smoke, also exhibit significant replication strand and timing asymmetry. Even more surprisingly, mutations of epigenetically methylated cytosine residues show clear replication strand asymmetry in cancers deficient of different error correction mechanisms. Our findings demonstrate that DNA replication and the associated repair have a bigger influence on the fixation of DNA damage into somatic mutations than previously acknowledged. In some cases, such as for Signature 17 or methylated cytosine, our results point towards a direct causal role of the replicative machinery in the formation of mutations. In summary, our discoveries shed new light onto the link between tissue regeneration, mutagenesis and cancer.