adenocarcinoma was quite high (19.5% and 9.6%, respectively). EGFR mutations were more common in women comparing with men, whereas ALK translocation was associated with younger age. High accuracy of ALK-detection by IHC allows using it for screening, however due to low PPV, ALK-positive IHC results should be verified by FISH.

PO-336 THRUPLEX AND PICOPLEX TECHNOLOGIES FOR RARE ALLELES AND COPY NUMBER VARIATION DETECTION FROM CELL-FREE DNA AND SINGLE HUMAN CANCER CELLS

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Introduction Liquid biopsies provide a non-invasive method to acquire the genetic information provided in cell-free DNA (cfDNA) as well as in single circulating tumour cells. Access to this genetic information through next-generation sequencing (NGS) identifies mutations and alterations such as Copy Number Variation (CNV) that play a role in cancer and other diseases.
Material and methods The key to identifying rare mutations is improved sequencing accuracy and the ability to distinguish between biological and PCR duplicates. ThruPLEX Tag-seq was developed with the addition of unique molecular tags (UMTs) to improve sequencing accuracy by accounting for polymerase and sequencing errors and to increase confidence in rare allele identification. Whole Genome Amplification (WGA) for CNV detection was achieved by the thermal cycling quasi-random primed library chemistry of the PicoPLEX DNA-seq single-cell NGS library prep kit.
Results and discussions ThruPLEX Tag-seq libraries were prepared using 10–30 ng of Horizon Discovery's Multiplex I cfDNA Reference Standard Set containing six single nucleotide variants (SNV) for 4 different genes (EGFR, KRAS, NRAS, PIK3CA) present at 0.5%–5% allele frequency. The libraries were enriched with either a 110 kb or 240 kb custom panel or the Agilent ClearSeq Comprehensive Cancer Panel. Enriched libraries were sequenced witan average total read coverage of approximately 5,000X and analysed with and without the UMTs.

For CNV analysis in single cells, a bar-coded PicoPLEX DNA-seq library was synthesised and amplified from 6 single cells from either PBMCs and clonally-expanded PC3 prostate cancer cells. Sequencing was performed on a MiSeq v2 and reads were mapped using BWA-MEM, processed in Picard_-Mark_Duplicates, and further characterised in DNA nexus.classic. All PC3 cells showed reproducible CNV calling, however none of the lymphocyte samples showed any CNVs. Accurate CNV calls for PC3 cells were achieved even at when fastq files were randomly downsampled to 1 50 000 read pairs.
Conclusion Therefore, use of UMTs in the preparation of NGS libraries from cfDNA enhances sequencing accuracy: by distinguishing between biological duplicates and PCR duplicates, increasing read coverage and decreasing background noise, reducing false positives, and in more confident mutation calls. PicoPLEX DNA-seq NGS libraries have a very simple and fast workflow that is suitable reproducible CNV detection in single cells even at low 0.002X average coverage.

PO-337 PRELIMINARY OBSERVATIONS: ARRAY-COMPARATIVE GENOMIC HYBRIDIZATION AS A HIGH-THROUGHPUT APPROACH AGAINST BLADDER CANCER

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Introduction Bladder cancer (BC) starts when urinary bladder cells grow abnormally. It is a solid tumour with high recurrence rates. BC is the eight tumour with the highest mortality and the sixth one with the highest incidence in the worldwide. Since the prognostic tools currently available have limitations and acquired changes in specific genes are thought to be significant in the development of bladder tumours, we needed to improve the research in this field of genetic changes associated with the BC. The aim of this study was the characterisation of the genomic profile of bladder tumours using the array-Comparative Genomic Hybridization (aCGH) technique.
Material and methods Bladder tumour samples were acquired from 28 patients when they were submitted a transurethral resection of bladder tumour (TURBT). The aCGH was done using an Agilent oligonucleotide microarray 4 × 180K. Bladder tissue samples from non-cancer donors are used as controls. Histopathological information from the patients was analysed and clinical data registered.
Results and discussions A few genomic imbalances were verified, using aCGH – a whole genome technique. In these preliminary outcomes, we did not observe a pattern of chromosomal alterations, as, we did not find imbalances in more than 20% of patients. Moreover, the chromosomes with more frequent copy number losses were 1, 6, 10, 13, 20, 21, 22 and X and the chromosomes with more frequent copy number gains were 1, 11, 13, 18 and 21. Additionally, the sizes of aberrations detected for the same chromosome were often variable between patients.
Conclusion This approach allowed us to identify altered chromosomal regions in bladder cancer comparing to normal tissues. In this way, is possible to map fundamental genes related to disease initiation and progression. The correlation between molecular and clinical-pathological data will be fundamental to identify recognised biomarkers with possible diagnostic and prognostic interest.

PO-338 RECURRENT GLIOBLASTOMA: A COMPLEX SCENARIO DOMINATED BY LOSS OF MMR PROTEINS

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Introduction Glioblastoma (GBM) is the most common primary brain tumour in adults and the Stupp protocol represents the standard of care. However, the tumour invariably relapses suggesting marked intra-tumour genetic heterogeneity enabling rapid adaptation to therapy. In-depth characterisation of recurrent GBM (rGBM) might contribute to better understand mechanisms behind tumour progression and enable rGBM treatment with targeted drugs.

Material and methods Matched GBM samples have been collected at diagnosis and recurrence from adult patients (n=57) treated with the Stupp protocol. Expression of mismatch repair (MMR) proteins (MLH1, PMS2, MSH2, MSH6) was evaluated by IHC, followed by exome sequencing of 3 pairs showing loss of MSH6 reactivity as well as of 3 MSH6 positive pairs. In addition, established genetic and epigenetic markers of GBM were investigated along with their correlation with loss of MMR proteins and patients' survival.

Results and discussions According to IHC results, 13 out of 52 rGBM samples (25%) lacked expression of MMR proteins. In particular, 11 among the 13 samples (85%) showed partial or total reduction of MSH6 expression. Conversely, almost all GBM samples at diagnosis (96.4%) stained positive for the 4 MMR markers. Consistent with IHC data, exome sequencing disclosed lack of variants in MMR genes in primary samples whereas rGBM samples lacking MSH6 expression were mutated in the abovementioned genes and shared a c.3438 +1G>A* splicing variant in MSH6 with a potential loss of function effect. Moreover, MSH6 negative relapsed specimens were characterised by 30 to 100-fold more variants compared to the matched primary ones and lacked microsatellite instability. Notably, MMR deficiency was associated with significant telomere shortening. Conversely, the tumour pairs expressing MMR proteins showed an almost comparable number of mutations in primary versus relapsed samples and absence of variants in MMR genes both in the initial tumours and in their recurrent counterpart.

Conclusion Our study shows that IHC staining is a valuable tool to identify a subset of rGBM patients with alterations in MMR genes linked to high mutational burden and, hence, potentially eligible for drugs targeting immune checkpoint inhibitors.

PO-339 IMPLICATIONS OF ORGAN-WISE EXTRACTION OF CANCER MUTATIONAL SIGNATURES USING 2577 WHOLE GENOMES

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Introduction With the advent of next generation sequencing of whole exomes and whole genomes, we can now obtain a comprehensive view of the somatic mutations that are present in tumours. Using latent variable discovery approaches such as non-negative matrix factorisation (NMF), recurrent somatic mutational patterns, called mutational signatures, have been identified, some of which have been attributed to mutational processes, such as sunlight exposure or homologous recombination deficiency. As more cancer whole genomes are available, the identification of new signatures and disambiguation of known signatures become possible. We can now begin to ask new questions, such as whether the same mutational process may induce different, organ-specific, mutational signatures.

Material and methods We used somatic mutations from 2577 tumours from the PCAWG dataset, organised in 21 organs. We then performed 21 independent local organ-specific signature extractions and one global signature extraction with all the samples. Extraction is performed using NMF. The optimal number of signatures for each extraction is determined by the clustering properties of repeated NMF runs. Cluster analysis based on cosine similarity was performed to determine similarity of signatures across organs.

Results and discussions Several of the mutational signatures obtained from organ-specific extractions resemble known COSMIC mutational signatures. COSMIC signature 1 can be found in almost all organs, with the exception of the Liver. Other signatures, such as COSMIC signatures 2, 3, 13, 17 and 18, are independently obtained from multiple organs. All the signatures that are found in multiple organs present organ-specificity. Further investigation is required to determine how much of this specificity is biological and how much is due to a dataset bias.

Conclusion Our work is the first attempt to determine the variability of mutational signatures across different organs. It reveals that some mutational signatures are more robust than others, and that the same mutational process may induce slightly different mutational signatures in different organs, though part of these differences may be attributed to a dataset bias. The use of organ-specific mutational signatures may be critical for the correct assignment of mutations in a tumour to signatures and, in turn, the correct identification of mutational processes that are at work in a tumour.

PO-340 DYSREGULATION OF THE TP53 NETWORK AND PRC2 ACTIVITY ARE INDEPENDENT POOR PROGNOSTIC FACTORS IN PATIENTS WITH MALIGNANT PERIPHERAL NERVE SHEATH TUMOURS

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Introduction Malignant peripheral nerve sheath tumours (MPNSTs) are rare and aggressive tumours with neuroectodermal origin, mainly affecting adolescents and young adults. Genetic aberrations in the *EED* and *SUZ12* genes, encoding core components of the Polycomb repressive complex 2 (PRC2), have been found in up to 80% of MPNSTs. Homozygous losses of PRC2 components have been shown to result in complete loss of trimethylation of lysine 27 (H2K27m3) and this has been found to be an indicator of poor prognosis for MPNST patients. Furthermore, *TP53* is one of few recurrently mutated genes, but the clinical relevance of the TP53 network in MPNSTs remains inconclusive. Here, we have analysed the prognostic impact of dysregulated PRC2 and TP53 activity on the gene expression-level in an aggregated series of MPNSTs.

Material and methods Frozen samples from 60 MPNSTs and 15 neurofibromas from patients treated at specialised sarcoma centres in Norway, Sweden, and Italy were subjected to gene