LONGITUDINAL TRANSCRIPTOMICS REVEALS HETEROGENEOUS DYNAMICS THROUGH THE COURSE OF DISEASE AND THERAPY IN BREAST CANCER

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Introduction Tumours are continuously evolving through their course of progression and treatment, a major process contributing to resistance to therapy. Increasingly, it becomes clear that unravelling the dynamics of gene expression over time, during stages of cancer progression and therapy is fundamental to interpretation of tumour evolution and resistance mechanisms. We postulate that in order to understand the emergence of resistance it is important to immensely profile matched biopsies from individual patients accompanied with their long-term clinical follow-up.

Material and methods We collected triplets of archived samples from 33 individual patients that underwent neo-adjuvant (pre-operative) treatment. Matched biopsies of tumour pre-treatment, post-treatment and adjacent normal epithelium were used. Full transcriptome analysis was performed by mRNA sequencing, after optimising this method for archived samples. Comprehensive clinical and pathological information was collected. A dedicated longitudinal pattern analysis method was developed to follow dynamic expression fluctuations of individual patients. Pathifier was used to calculate pathway deregulation scores.

Results and discussions Principle component analysis showed clustering of the samples according to their type. Dynamic fluctuations across the 3 time-points were classified into 8 theoretical patterns, each representing a different scenario through the tumour progression and treatment stages. Genes were divided into two main types: 1. Sharing a common temporal expression pattern across most patients. These genes were associated with tumour progression pathways. 2. Genes that were divided into two or three dominant patterns and this division showed correlation with pathological response score. The dynamic pattern classification enabled to pinpoint genes associated with response that otherwise were difficult to identify using single-time point or two-time points datasets. Furthermore, the dynamics of pathway deregulation scores enabled to detect pathways that were correlated with response to therapy.

Conclusion The longitudinal approach of serial sampling and analysis reveals heterogeneous dynamic behaviour across patients through the course of disease. This individual dynamics has higher sensitivity than single-time point measurements in detecting clinically relevant genes that are associated with resistance to therapy and with tumour progression.

GENOME-WIDE ANALYSIS OF SITE-SPECIFIC HOTTOPSPOTS IN CANCER

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Introduction According to models of known mutational processes, site-specific hotspots of even just a few mutations become unlikely in large cancer genomic datasets (four mutations in our case). These hotspots may affect cancer development or be a consequence of localised mutational processes. Here, we identify and characterise protein-coding and non-coding site-specific hotspots.

Material and methods We use whole genome sequencing data from 2583 cancer patients across 37 cancer types from Pan-Cancer Analysis of Whole Genomes (PCAWG) under ICGC/TCGA. We identify SNV and indel hotspots genome-wide, annotate them with their genomic features, and investigate expression-correlation and cancer allele fractions.

Results and discussions We find 566,760 SNV and 1,698,39 indel hotspots, which are genomic positions with two or more SNVs/indels across patients. A small fraction of the hotspots are in protein-coding regions (0.7% for both sets; 3.9x enrichment of local mutation rate in genomic region for SNVs; 1.7x for indels) and regulatory elements of protein-coding genes (0.9%/1.3x for SNVs; 1.8%/1.04x for indels). Only a small fraction of the protein-coding hotspots fall in the known drivers from Cancer Gene Census (0.9% for SNVs; 0.8% for indels).

Among the top-20 SNV hotspots are 13 positions in known driver sites in protein-coding genes, a known driver site in the TERT promoter, two positions in the PLEKHS1 promoter and a position in a GPR126 intron now known to likely be caused by APOBEC editing, and four non-coding sites possibly caused by different mutational processes.

In contrast, none of the top-20 indel hotspots overlap protein-coding genes or regulatory elements. All 20 are deletion-hotspots, and they are located at least 14 kb away from the transcription start site of the nearest protein-coding gene.

One third of the SNV hotspots are almost exclusive to a single cancer type. Cancers with high mutational burden and cancer-type specific mutational processes top the list. E.g. colorectal cancer hotspots, likely caused by patients with microsatellite-instability, and melanoma hotspots, likely caused by UV-induced DNA damages.

Moreover, analyses of cancer allele fractions and expression correlation in stratified promoter sets indicate a weak signal of positive selection on a few hotspots in promoters of oncogenes.

Conclusion We see no clear driver signal from other non-coding hotspots than two already known positions in the TERT promoter. Mutational processes appear to be the dominating contributor to non-coding hotspots.

ALK INHIBITOR, CERITINIB, ABROGATES ACTIVATION OF THE NOVEL ALK- I1171T MUTATION IN NEUROBLASTOMA

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Introduction Mutations in the kinase domain of Anaplastic Lymphoma Kinase (ALK) are undoubtedly implicated in the pathogenesis of the childhood cancer, neuroblastoma. However,
clinical response of ALK positive neuroblastoma patients to the first generation ALK inhibitor has been rather disappointing.

Here we report the appearance of a novel ALK mutation in neuroblastoma together with other chromosomal aberrations that mediate neuroblastoma initiation and progression.

**Material and methods** Genomic tumour DNA from biopsy samples was extracted and exome sequencing was performed through paired-end sequencing on Illumina platforms.

The novel ALK-I1171T mutation was biochemically analysed by western blot and neurite-outgrowth assay in PC12 cells, and foci formation assay in NIH3T3 cells.

**Results and discussions** Analyses of genomic tumour DNA from biopsy samples initially showed an 11q deletion, 17q gain with a mutation of the ALK gene at protein position 1171, which mediate an amino acid change from isoleucine to threonine. We show that mutation of I1171 to threonine generates a potent gain-of-function mutant, as observed in two independent systems. Firstly, in PC12 cell lines expressing ALK-I1171T display ligand independent activation of ALK, neurite outgrowth and further downstream signalling activation. Secondly, ALK-I1171T mediate foci formation in a NIH3T3 transformation analysis. Finally, pharmacological inhibition of ALK-I1171T employing ceritinib, an FDA approved ALK inhibitor show 14-fold better ability to abrogate ALK-I1171T compared with crizotinib.

**Conclusion** This study suggests that ceritinib presents a viable therapeutic option for ALK-positive neuroblastoma.

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**Abstracts**

**PO-315**

**THE MUTATIONAL AND TRANSCRIPTOME LANDSCAPE OF INFANT B-CELL ACUTE LYMPHOBlastic LEUKAEMIA: THE INTERFANT TREATMENT PROTOCOL EXPERIENCE**

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**Introduction** Infant B-cell precursor acute lymphoblastic leukaemia (iBCP-ALL) has dismal prognosis, especially with MLL-gene rearrangements (MLLr) which are hallmark clonal leukemicogenic drivers. Molecular pathogenesis of MLLr-iBCP-ALL remain somehow enigmatic and in vivo recreation of MLLr-iBCP-ALL is challenging.

**Material and methods** We performed whole-genome, exome, targetted and RNA-sequencing on an Interfant study discovery cohort of 50 iBCP-ALLs (27MLL-AF4+, including relapses, 5MLL-AF9+ and 10non-MLL). An independent validation cohort of 82iBCP-ALLs (43MLL-AF4+, 11MLL-AF9+, and 28non-MLL) was used for targeted DNA-sequencing/qRT-PCR.

**Results and discussions** iBCP-ALL shows an extremely low frequency of somatic mutations, irrespective of the presence subtype of MLLr, with the predominant leukemic clone carrying a mean of 2.5 non-silent mutations. Recurrent mutations were exclusively found in KRAS and NRAS, which were more frequent in the MLL-AF4+ than in MLL-AF9+/non-MLL iBCP-ALL due to common NRAS mutations found in MLL-AF4+infants (32% vs 6%; p<0.01). These mutations were subclonal and frequently lost at relapse, despite a larger number of non-silent but non-recurrent mutations (19.5 mutations/patient). RNA-seq/qRT-PCR validation revealed that there are deregulated protein coding genes related to three important pathways such as cell cycle regulation, DNA integrity check point and DSB DNA repair. Also, deregulated LncRNAs were found that could provide further mechanisms of tumorigenesis. Furthermore, different isoforms of the reciprocal fusion AF4-MLL were expressed only in 55% of the t(4;11)+patients, and HOXA cluster genes are uniquely expressed in AF4-MLL-expressing t(4;11)+patients. AF4-MLL/HOXA-expressing patients displayed higher 2 year event-free survival than patients lacking AF4-MLL expression (65% vs 34%, p=0.15). Opposite to paediatric/adult BCP-ALLs, BCR repertoire analysis revealed only minor, non-expanded B-cell clones in t(4;11) +iBCP ALL.

**Conclusion** iBCP-ALL shows a silent mutational landscape regardless the MLL status. The expression of AF4-MLL associates to a better prognosis and specific upregulation of HOXA cluster genes. A pre-BCR early progenitor/stem cell may represent the cell-of-origin for both the t(4;11) and RAS mutations.