polyps were evaluated and colonic samples were histologically analysed. Immunofluorescent staining of immune cells (neutrophils and T cell populations) was performed to quantify positive cells in three interesting colon areas: mucosa, lymphoid follicle and tumour using an innovative algorithm created with Tissue Studio software. In parallel, quantification of immune cells (lymphoid populations) was performed in mesenteric lymph node (MLNs) by flow cytometry.

**Results and discussions** Using optical imaging, we detected a significant increase of oxidative stress and inflammation in the gut after thirty days of 11 G5 infection. However, using our specific algorithm and CD45 immunostaining, we observed a significant increase of lymphoid follicle size in the gut of mice infected with the 11 G5 strains. Interestingly, follicle size was positively correlated with tumour volume suggesting an association between pro-carcinogenic properties of 11 G5 strain and gut immune response. In addition, we observed an increase of neutrophils on the mucosa and lymphoid follicle of 11 G5 infected mice. These results can be linked to our *in vivo* optical imaging observations. Moreover we observed a trend to decrease of CD3+ T cells on mucosa and tumour for the 11 G5 group, suggesting a possible link between E. coli pks+ and anti-tumour T cells. Finally we noticed a significant decrease of CD4+ CD25+ anti-inflammatory T cells in MLNs of 11 G5 infected mice, negatively correlated with gut bacteria colonisation.

**Conclusion** Here we report a potential immunomodulatory effect on gut microenvironment by pks+E. coli, which could be associated with its carcinogenic effect. Our results suggest that gut lymphoid follicle and immune cells such as neutrophils and T cells could be involved in this process. This work shows a link between immune microenvironment, pathogenic E. coli and tumour development.

**Symposium: Systems Medicine – Making Sense of Big Data**

**PAN-CANCER WHOLE GENOME SEQUENCING REVEALS PATTERNS OF SUBCLONAL MUTATIONS, SIGNATURE CHANGES AND SELECTION**

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**Introduction** During their development, tumour cells accumulate somatic mutations, structural variants and copy number alterations (CNAs). Driver events facilitate clonal expansions and lead to intra-tumour heterogeneity (ITH). While ITH is an important therapeutic challenge, its degree among different cancer types is largely unknown.

**Material and methods** The pan-cancer analysis of whole genomes (PCAWG) enabled us to characterise ITH in an unprecedented set of 2778 tumour samples representing 36 histologically distinct cancer types. We applied six CNA callers and eleven subclonal reconstruction algorithms to integrate their solutions into robust consensus copy number profiles and subclonal reconstructions.

**Results and discussions** Our analysis revealed pervasive ITH in all examined cancer types. We found at least one subclone in 96.7% of the 1801 samples for which we had statistical power to detect subclones. In addition, we find that the average proportions of subclonal point mutations, indels, SVs and CNAs are highly variable across cancer types. These observations suggest distinct evolutionary narratives of each histological cancer type.

Analysis of dN/dS ratios shows clear signs of positive selection within both clonal and subclonal mutations. We also identified subclonal mutations in driver genes that are recurrently hit and we found a significant enrichment of subclonal mutations in genes responsible for chromatin regulation. More than 5% of tumours contain driver mutations in genes for which specific treatment is available only in subclones, indicating the importance of assessing the clonality of targeted mutations for clinical decisions.

Mutational signatures in the analysed samples show changes in activity over the course of tumour development. Characteristic carcinogen signatures, e.g. UV light exposure in melanomas, make less contributions to subclonal than clonal mutations, while APOBEC-induced mutagenesis has increased activity during the subclonal phase.

**Conclusion** The absence of a detectable driver mutation in a majority of subclones suggests that late tumour development is frequently driven by CNAs or genomic rearrangements, or that a significant number of late drivers have yet to be identified. We found that selection is widespread and likely the rule rather than the exception and we identified differential activity of mutational signatures, reflecting successive waves of subclonal expansion.

**GENE FUSIONS IN 1,015 HUMAN CANCER CELL LINES: INTEGRATING LARGE-SCALE GENOMIC DATA, HIGH-THROUGHPUT DRUG AND CRISPR/CAS9 SCREENS TO ASSESS FUNCTIONAL RELEVANCE AND THERAPEUTIC POTENTIAL**

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**Introduction** Translating our understanding of genetic alterations in cancer into clinical care remains a major challenge. The discovery of gene fusions such as *EML4-ALK* in lung cancer and *BCR-ABL1* in chronic myeloid leukaemia have already led to changes in clinical care. Advances in next-generation sequencing have accelerated the rate at which novel gene fusions are discovered, but important questions remain about their roles in promoting oncogenic phenotypes and their relevance in drug response. Here, we combine RNA sequencing, CRISPR/Cas9 screens and high-throughput drug sensitivity data in a panel of 1000 human cancer cell lines to examine
the occurrence and functional relevance of gene fusions in cancer.

**Material and methods** We performed RNA-sequencing on 1015 human cancer cell lines, representing 42 cancer types. We called fusions using three algorithms: TopHat Fusion, DeFuse and RNA-STAR fusion. Further, we integrate high-throughput drug screening data across >350 compounds, single-nucleotide variants, copy number variation, gene expression data and genome-wide CRISPR/Cas9 dropout screening data to systematically search for gene fusions with functional relevance.

**Results and discussions** We find 8546 distinct gene fusion events across our panel of cell lines. These include well understood gene fusions (e.g., ALK-fusions, BCR-ABL1 and EWSR1-FLI1), as well as novel fusions that involve known cancer driver genes. We are able to recapitulate previously identified gene fusion-drug response associations using an unguided statistical analysis. Furthermore, we developed a systematic unguided approach of using CRISPR/Cas9 gene essentiality data to identify essential gene fusions. This approach recapitulates known essential gene fusions and provides evidence for the oncogenic relevance for previously poorly understood gene fusions.

**Conclusion** In this study, we provide an annotation of gene fusions in 1015 human cancer cell lines. Our systematic analysis of the functional role of gene fusions captures the oncogenic and therapeutic relevance of known gene fusions, and highlights potential therapeutic opportunities of previously uncharacterised gene fusions.

### Posters in the Spotlight Sessions

**SPOT-001** **MOLECULAR AND BIOPHYSICAL DETERMINANTS OF CHROMOSOMAL TRANSLOCATIONS: A MULTIOMICS APPROACH**

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**Introduction** Chromosomal translocations are potential cancer-initiating lesions. Despite their pathogenic risk, the fact that chromosomal translocations are rare events has hampered the analysis of the molecular mechanism underlying these genetic aberrations. Over the last decades numerous retro- and prospective studies suggested a number of parameters and conditions that associate with the onset of chromosomal translocations. To pinpoint the mechanisms(s) underlying chromosomal translocations, a clear distinction between potential causal and correlative risk factors is required.

**Material and methods** To meet this goal, we applied innovative integrative analyses of multifactorial genome-wide data. To determine the relevance of a single parameter in a multi-parameter NGS-based big data, requests an effective multiomics integration method. We established a novel paired integrative (PAINT) analysis that enables to determine the relevance of a single parameter in multi-parameter NGS-based data analyses.

**Results and discussions** Our data indicate that the chromosomal translocation risk is causally unrelated to promoter proximal activities, transcriptional activity, or off-targeting activity of the activation-induced cytidine deaminase (AID). Rather, an open chromatin configuration, which is not promoter specific, explained the elevated translocation risk of accessible genes and promoter regions. Furthermore, the fact that gene size directly correlates with the translocation risk in mice and human cancers further demonstrated the general irrelevance of promoter specific activities. Interestingly, a subset of translocations observed in cancer patients likely initiates from DSBs induced by an access independent process.

**Conclusion** Both, our reassessments and analyses led to much more nuanced conclusions. Pomoter activities, promoter-proximal activities and transcription do not determine the translocation risk. The proximity of a gene to a DNA double strand break, its overall accessibility and its size are critical risk determinants for a gene to become involved in chromosomal translocation. We emphasise, that these conclusions were independently validated by our studies on the gene sizes of translocated genes as archived in the translocation databases of human cancers and the experimental HTGTS setting.