Clinical Cancer Genomics

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Whole genomes and transcriptomes: from clinic to research and back

Abstract book
Oral presentations

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O02: Molecular markers to guide treatment-decision making in metastatic urothelial cancer

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Friday March 21, 10.15-10.30 CET , Session 3: The research perspective: Big data analyses and impact on clinic

PURPOSE: Recent approvals of immune check-point inhibitors (ICI), enfortumab vedotin (EV; nectin-4-directed antibody and microtubule inhibitor conjugate) combined with ICI, and the fibroblast growth factor receptors (FGFR) inhibitor erdafitinib are anticipated to change the therapeutic landscape for metastatic Urothelial Carcinoma (mUC) patients. However, selection criteria to identify the most optimal therapeutic option are lacking, stressing the urgent need for predictive biomarkers to improve patient stratification, optimize treatment of choice, and omit ineffective/combination therapies in patients who are unlikely to benefit. Also, given the large financial burden of these novel therapies on the healthcare system, efficient decision-making is key.

PATIENTS AND METHODS: Using whole-genome DNA- and paired RNA-sequencing data of fresh-frozen metastatic tumor biopsies of 155 mUC patients facilitated by the Hartwig Medical Foundation, we investigated their molecular makeup including tumor microenvironment elements, gene mutations, fusions and amplifications that have been previously correlated with response to ICI, anti-FGFR or EV monotherapy respectively. We stratified mUC patients based on these potential candidate biomarkers, and Kaplan-Meier curves were used to compare response to treatment between the patient groups.

RESULTS: We observed that NECTIN4 amplification, FGFR2/3 mutations, and the RNA-expression-based T-Cell-to-Stroma Enrichment (TSE) positivity score were mutually exclusive ($p \leq 0.001$ in all cases, estimations using the Poisson-Binomial distribution). Previous studies have associated these biomarkers with response to EV, anti-FGFR and ICI, respectively, and may therefore reflect biologically distinct tumors and sensitivity to treatments. For patients with a positive TSE score indicating potential benefit from ICI and who actually received this treatment showed the longest overall survival compared to the other groups who also received the treatment (Figure 1A). Our findings were validated in two independent bladder cohorts: the IMvigor210 study and The Cancer Genome Atlas. Based on these data and the limited evidence of synergistic effect of combined therapies, stratification into subgroups of patients with potential benefit to monotherapy is possible; a) patients with NECTIN4 amplification, who might have most benefit from EV, b) patients with FGFR2/3 mutations/fusions and no NECTIN4 amplification might have most benefit from treatment with an FGFR inhibitor, c) patients with a positive TSE score without NECTIN4 amplification or FGFR2/3 mutations/fusions might benefit most from anti-PD(L)1 ICI, and d) in patients with none of the above molecular markers (~60%), for whom the most optimal therapeutic strategy is unclear and may therefore receive the current standard of care or other therapeutic options (Figure 1B).

CONCLUSION: Our data challenge the current treatment setting of a one-treatment-fits-all and support the rationale for biomarker-guided treatment selection of mUC patients.

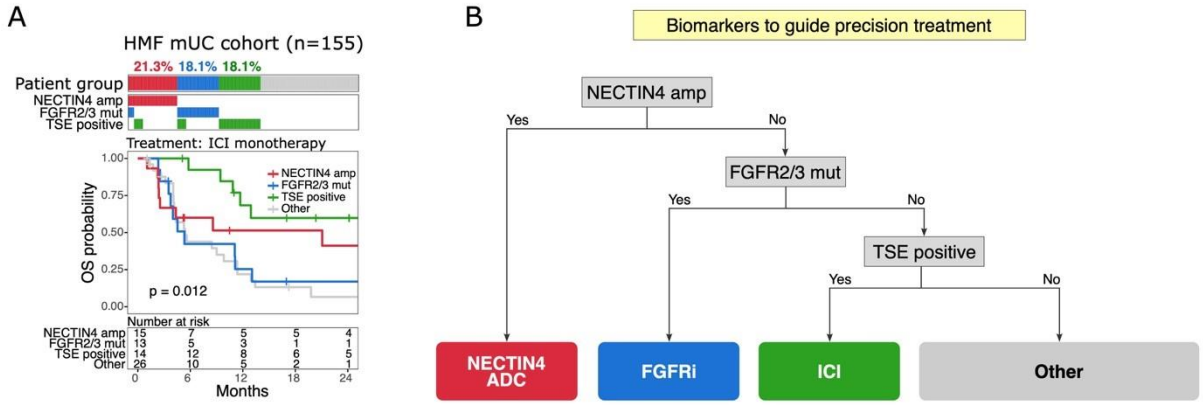

Figure 1. Biomarkers to guide targeted therapy in metastatic urothelial carcinoma (mUC). A) Patient populations were stratified based on NECTIN4 amplification, FGFR mutations/fusions (no NECTIN4 amplification) and positive TSE score (no FGFR mutations and no NECTIN4 amplification). Survival outcomes of these group of patients with available follow up who were treated with immune check-point inhibitor (ICI; n=68) are compared. B) Flowchart for biomarker-guided treatment in patients with mUC.
HMF = Hartwig Medical Foundation, ADC = antibody drug conjugate, FGFRi = FGFR inhibitor.

O05: Liquid biopsy in cancer: towards whole genome-based analysis of ctDNA in clinical practice

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Friday March 21, 14.45-15.00 CET, Session 4: The future perspective: data-driven cancer care

Liquid biopsy routine diagnostics have so far predominantly focused on the detection of actionable somatic hotspot variants in circulating tumor DNA (ctDNA) to guide treatment decisions. Upcoming ctDNA analysis go beyond the detection of single hotspot variants by utilizing whole genome sequencing, for example in the context of minimal residual disease (MRD) detection, treatment monitoring and early cancer detection. Such genome wide approaches have great potential to detect ctDNA independent of the presence of somatic hotspot variants with high sensitivity and to provide additional insights into the tumor biology of individual patients, such as epigenetic signatures and gene expression status of tumor cells. Therefore, personalized whole genome based ctDNA analyses are likely to complement current standard approaches in clinical practice in the foreseeable future.

Material and Methods
200 longitudinal plasma samples from 55 patients with colorectal cancer (CRC) and 50 control samples were subjected to whole genome sequencing for ctDNA analysis using our custom bioinformatic analysis pipeline LIFE-CNA, for LIquid biopsy Fragmentation, Epigenetic signature, and Copy Number Alteration analysis. Furthermore, plasma samples from patients diagnosed with diverse tumor types, including 2 breast cancer, 9 prostate cancer, and 2 Lynch Syndrome (LS)-associated cancer cases, as well as 7 intraductal papillary mucinous neoplasm (IPMN) cases presenting with a risk to develop pancreatic cancer, were analyzed with LIFE-CNA.

Results
We present our ongoing work on whole genome ctDNA analysis (LIFE-CNA), based on distinct cutoffs for the detection of ctDNA based on global and regional fragmentation patterns, transcriptionally active chromatin, and somatic copy number alterations
(CNVs) that were established for our CRC cohort. All breast and prostate cancer cases exhibited significant alterations in both fragmentation profiles and CNVs. In addition, 6/7 IPMN cases showed an overlap with a distinct epigenetic signature. In contrast, both LS cases showed no significant variations.

Discussion & Outlook
Our proof-of-principle study underscores the utility of employing multiple metrics in ctDNA analysis across tumor types and highlights LIFE-CNA's adaptability to detect various cancer types in a minimally invasive manner. However, cohort expansion is crucial to confirm the sensitivity and specificity of this approach across tumor entities; considering functional differences between tumor entities could further improve the analytical performance. Important challenges in the routine diagnostic implementation of WGS methods in ctDNA analysis remain 1) the large cohort sizes needed for analytical and clinical validation of individual analysis pipelines, 2) the current lack of reference materials for quality control and 3) the need to react quickly to new developments in a fast-moving scientific field for the benefit of individual patients.

O01: Whole genome sequencing improves tissue of origin diagnosis and treatment options for cancer of unknown primary

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Thursday March 20, 16.25-16.40 CET, Session 2: The clinical perspective: implementation and impact

Cancer of unknown primary (CUP) is a metastatic cancer without a primary site diagnosis after standardized investigations. Genomic testing can inform both treatment targets and identify the primary tissue of origin (TOO). While whole-genome sequencing (WGS) can increase the diagnostic yield over cancer panel testing a systematic head-to-head comparison of these methods has not been reported in CUP patients. Through a retrospective analysis of CUP patients recruited to the Australian Solving Unknown Primary cancER (SUPER) study we directly compared the diagnostic yield of whole-genome and transcriptome sequencing (WGTS) to comprehensive cancer panel testing. WGTS was successfully applied to 73 tumours from 72 patients with 59 (81%) involving use of formalin-fixed paraffin embedded (FFPE) tissues. Panel testing (386-523 genes) was applied in 71 of these cases. WGTS detected all reportable DNA features identified by panel as well as additional mutations of diagnostic or therapeutic relevance in 76% of cases. Curated WGTS features as well as the Hartwig Medical Foundation CUP prediction algorithm (CUPPA) informed TOO in 71% of cases that could not be diagnosed by clinicopathology review alone. WGTS indicated either cancer type dependent standard of care and/or clinical trial treatments in 79% of all patients, compared to only 62% by panel testing. Finally, we applied WGS to blood plasma cell-free DNA where high circulating tumour DNA fraction (>7%) was detected and there was sufficient DNA for library preparation, representing ~25% of all CUP patients screened. High-likelihood CUPPA predictions were made in 9/22 (41%) of these cell-free DNA cases WGS tested, and CUPPA predictions were either concordant with pathology review or within a likely diagnostic differential. Importantly, four cell-free DNA cases with high-likelihood predictions were from patients where tissue-based WGS was not feasible due to sample availability. WGTS is therefore superior to panel testing, broadens treatment options, and is feasible using routine FFPE clinical samples. Application of WGS to cell-free DNA can also enable algorithmic TOO prediction in a subset of CUP patients.

O07: (Ultra-) fast deep-learned classification algorithms for diagnosing pediatric CNS and solid tumors

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Friday March 21, 16.10-16.25 CET, Session 4: The future perspective: data-driven cancer care

Introduction
Cancer is the leading disease-related death in children and young adults. The 5-year survival rate for these tumors varies significantly based on tumor type and location, ranging from over 90% for Wilms tumors to less than 40% for malignant rhabdoid tumors. The first line of treatment often involves surgical resection to reduce tumor burden, relieve symptoms, and obtain tissue for (molecular) diagnostic testing, which is crucial for guiding further treatment.

Many tumor entities now require molecular profiling for a definitive diagnosis, which can take days to weeks to finalize. We therefore previously developed and published a deep-learned algorithm, i.e. Sturgeon, that allows for ultra-fast central nervous system (CNS) tumor classification during surgery using shallow whole genome nanopore sequencing (Vermeulen, et al. Nature 2023 622:842-849). This method allows for intra-operative decision making based on a molecular diagnosis.

In addition, using the same methodology we developed a deep-learned algorithm that allows for the classification of pediatric solid tumors, i.e. Tucan. This algorithm not only allows for the classification of 80 pediatric solid tumor types (including over 40 types of sarcoma) by nanopore sequencing, but also generates a copy number profile, including focal amplifications, within hours. This allows for immediate start of treatment for the 10-20% of the pediatric patients for which immediate action is warranted.

Methods
Both Sturgeon and Tucan are trained on methylation array data. In brief, the methylation array data is first binarized and subsequently sampled in order to simulate nanopore sequencing data. We then train a fully connected multi-layer neural network using a cross validation approach in which 50% of the samples are used for training, 25% are used for fine-tuning and the remaining 25% are used for testing.

Results
We have now validated Sturgeon both retrospectively and prospectively and found that Sturgeon provides the correct classification in over 95% of samples within 2.5 hours of acquisition of the material from the patient. Sturgeon is now implemented in standard clinical care and used routinely to provide a molecular diagnosis during surgery. For Tucan performance is similar with over 95% of samples receiving the correct classification. Combined, Tucan and Sturgeon can provide a same day molecular diagnosis for over 150 different (pediatric) tumortypes.

Conclusion
We conclude that machine-learned diagnostics based on methylation profiling using shallow whole genome nanopore sequencing is reliable and can impact treatment-decision making. Either by same day diagnosis for starting of chemotherapy treatment or intra-operative diagnosis for surgical decision making.

O03: The temporal evolution of cancer hallmarks

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Friday March 21, 11.50-12.05 CET, Session 3: The research perspective: Big data analyses and impact on clinic

Cancer hallmarks describe key physiological characteristics that distinguish cancers from normal tissues. The temporal order in which these hallmarks appear during cancer pathogenesis is of interest from both evolutionary and clinical perspectives but has not been investigated before. Here, we order hallmarks based on the allele frequency and selective advantage of mutations in cancer hallmark genes across >10K untreated primary tumors and >8K healthy tissues. Using this novel approach, we identified a common evolutionary trajectory for 27 of 32 cancer types with genomic instability appearing first and immune evasion appearing last. We demonstrated widespread positive selection in cancer and strong negative selection in normal tissues for all hallmarks. Notable exceptions to the hallmark ordering in tumors were melanomas (uveal and skin) suggesting that strong environmental factors could disrupt common evolutionary paths. Clustering of hallmark trajectories across patients revealed 2 clusters defined by early or late genomic instability, with differential prognosis. We finally validated our results in about 3K primary tumors from the PCAWG consortium. Our study is the first to identify the temporal order of cancer hallmarks during tumorigenesis and demonstrate a prognostic value that could be exploited for early detection and risk stratification across multiple cancer types.

O04: Multiomics in the diagnostics of mesenchymal tumors reveals new genetic aberrations

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Department of Oncology and Pathology, Karolinska Institutet, Stockholm, Sweden.

Friday March 21, 12.05-1.20 CET, Session 3: The research perspective: Big data analyses and impact on clinic

Background:
Mesenchymal tumors, including both benign and malignant neoplasms in soft tissue and bone, are genetically diverse. Whole genome sequencing (WGS) is increasingly used in cancer care. Combined with histopathology, WGS can help the diagnostic process. We aim at mapping clinically potentially relevant events detected by WGS, whole transcriptome sequencing (WTS), and methylation clustering analyses in a large cohort of mesenchymal tumors.

Methods:
Patients are recruited at Sarcoma Center Karolinska, Stockholm, Sweden. Inclusion criteria are suspected mesenchymal tumor and available tumor tissue and blood control. We isolate DNA and RNA from fresh frozen tumor tissue, and DNA from peripheral blood. Sequencing is performed on the Illumina platform. All pathogenic single nucleotide variants, translocations creating fusion genes or truncating tumor suppressors, and copy number aberrations are manually inspected in the light of the histopathological diagnosis. WTS data was analyzed with the RNAfusion pipeline. Methylation status was characterized by Infinium MethylationEPIC v2.0 BeadChip with the same tumor DNA sample as WGS and clustered by the DKFZ (Supervised) and EpiDiP (unsupervised) platform.

Results:
So far, we have analyzed 311 samples. Inclusion is ongoing, with data generated from 387 samples currently. In 12% (37/311), genetic findings have changed the diagnosis. In the other cases, genetic findings support or confirm the histopathological diagnosis in 94% (257/274). Among the most common key genetic findings are TP53 and RB1 loss, MDM2 amplification, complex copy number profiles, and high tumor mutational burden. There are 39 cases with relevant fusion genes detected by WGS and/or WTS. These include eight cases with fusions not detected by the clinical pipeline, and a changed diagnosis based on this study.
Both the supervised and unsupervised clustering analyses supported the correct diagnosis in 57% (170/298) of all cases with available methylation data. For one case, they changed the diagnosis (a supposed myxofibrosarcoma which clustered as an undifferentiated sarcoma). In the rest, the diagnosis class was missing from the supervised data set or the clusters, or there was no support of the correct diagnosis.

Discussion:
The cohort included in this study represents cases referred to our specialized sarcoma center, spanning many different diagnoses. Each subgroup within the study is small, especially for extremely rare diagnoses such as clear cell chondrosarcomas (n=2). To facilitate for researchers and clinicians to find collaborations and increase cohort sizes when dealing with ultra-rare diagnoses, we have created a publicly available portal:
pathologycaseportal.com. We invite the research community and medical institution staff to use this tool, and we hope it will increase our knowledge about rare diagnoses.

O06: Clinical utility of long read sequencing for comprehensive analysis of cancer patient genomes

Rowan Howell, Timothy Freeman, Adam Giess, Melanie Tanguy, Greg Elgar, Kirsty Russell, Alex Younger, Alona Sosinsky, Emma McCargow, Mike Hubank, Christopher Watson, Polly Talley, Zandra Deans and Sue Hill

Friday March 21, 15.45-16.00 CET, Session 4: The future perspective: data-driven cancer care

Long read sequencing (LRS) technologies offer a number of advantages for the clinical characterisation of cancer genomes over the short read sequencing (SRS) technologies currently employed in clinical practice. Genomics England in conjunction with NHSE have initiated a pilot programme to establish the clinical utility of Oxford Nanopore® (ONT) LRS for a subset of cancer patients.

We have developed an approach to generate high depth, paired (50X tumour, 25X normal) whole genome sequences with LRS and a bioinformatics pipeline to call somatic variants. Our pipeline calls Single Nucleotide Variants (SNVs) and small insertions and deletions (indels) with ClairS, Structural Variants (SVs) with Severus and Copy Number Variants (CNVs) with Purple. We have generated sequencing data for over 200 patients through the NHS Genomic Lab Hubs, and approximately 400 patients as part of our research cohorts, focussing on leukaemia, sarcoma and brain tumour patients.

Our ONT cancer analysis pipeline can detect clinically relevant SNVs, CNVs and SVs with precision and recall exceeding 90%. LRS offers a superior view of complex structural variation, for example phasing of SV breakpoints can distinguish multiple events occurring in cis or trans phase. Furthermore, variants in hard-to-map regions of the genome, such as repetitive sequences, that are inaccessible to SRS technology can be analysed using LRS, allowing detection of additional clinically relevant variants. Characterisation of tumour-specific DNA methylation patterns allows for classification of brain tumour subtypes with machine learning tools, with similar accuracy to data generated from methylation arrays. Our pipeline can also detect and characterise methylation of clinically relevant regions, such as the MGMT and BRCA1 promoter regions.

We have demonstrated that cancer genomes can be comprehensively and accurately characterised with ONT sequencing. This data will be made available as a resource for future research. Comparison with the current standard of care testing will be assessed in the context of the clinical impact for each patient group. This will inform recommendations for the use of ONT for standard of care testing.