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Whole genomes and transcriptomes: from clinic to research and back

Abstract book
Poster pitch

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PP01: Epigenetic, transcriptomic and genetic analyses of HR+/HER2- primary and metastatic breast cancer results in cohort stratification by aggressiveness and reveals heterogeneous mechanisms of endocrine therapy resistance

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Thursday March 20, 12:10 – 12:30 CET, Poster flash talks

Endocrine therapy (ET) is typically the first-line treatment in hormone receptor-positive, human epidermal growth receptor 2-negative (HR+/HER2-) breast cancer (BC) patients; however, almost half of the patients exhibit ET resistance after initial response. Mechanisms of ET resistance are not fully understood, and require an integrated epigenetic, transcriptomic and genetic approach.

We conducted ATAC, RNA, and whole-exome sequencing on primary and metastatic BC (pBC and mBC) from 459 HR+/HER2- patients. pBC samples were derived from tumor resections of patients, which were then treated with adjuvant ET and experienced either a relapse or sustained remission over a five-year period. Samples of mBC were obtained from metastatic lesions of ET-resistant patients. We performed analysis of chromatin accessibility, gene expression, and mutational data aiming to understand relapse mechanisms in pBC and resistance mechanisms in mBC.

PAM50 probability scoring of our cohort of HR+/HER2- BC revealed subgroups within pBC and mBC, which resembled PAM50 subtypes of BC. In pBC, the “Basal” and “Her2” subgroups displayed the highest percentage of relapse, compared to the “LumA” and “LumB” subgroups. TP53 alterations were enriched in “Basal” and “Her2” subgroups, while MAP3K1 alterations were more frequent in “LumA” subgroup. In mBC, ESR1 mutations characterized “LumB” tumors, "Her2” subgroup exhibited ERBB2 alterations, and “LumA” subgroup was enriched with FOXA1 mutations. In both pBC and mBC, the subgroups were enriched with gene sets pertaining to their associated PAM50 subtypes. “LumB” subgroup was associated with higher estrogen response. “Basal” and “Her2” subgroups showed enrichment of gene sets related to

true Basal and Her2 subtypes, and transcription factor motifs of AP-1 and TFAP2. “Basal” and “Her2” subgroups was characterized by poor survival.

PAM50 probability scoring was useful to dissect heterogeneity and to unveil insights into molecular mechanisms associated with the risk of relapse and ET resistance within HR+/HER2- BC. These findings may improve prognostic accuracy and patient stratification in HR+/HER2- BC cohorts.

PP02: Ongoing chromothripsis underpins osteosarcoma genome complexity and clonal evolution

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Osteosarcoma is the most common primary cancer of bone, with a peak incidence in children and young adults. Whole-genome sequencing (WGS) studies have revealed that osteosarcoma genomes are riddled with remarkably intricate forms of structural variants (SV), collectively known as complex genomic rearrangements (CGR). Yet,

the origin of the karyotypic complexity of the osteosarcoma genome remains unexplained.

To elucidate the mechanisms underpinning cancer genome complexity in osteosarcomas and the downstream consequences of CGR during tumour evolution, we uniformly processed existing osteosarcoma WGS data sets and performed high-depth multi-regional short- and long-read WGS for hundreds of osteosarcomas and other sarcoma types. We found that chromothripsis is an ongoing mutational process occurring subclonally in 74% of osteosarcomas. Clonal expansions triggered by the acquisition of subclonal CGR often colonise distant tumour regions, suggesting that CGR act as subclonal driver events. Furthermore, chromothripsis generates highly unstable derivative chromosomes, the ongoing evolution of which drives the acquisition of oncogenic mutations, clonal diversification and intra-tumour heterogeneity across diverse sarcomas and carcinomas.

In addition, we characterise a new mechanism, loss-translocation-amplification (LTA) chromothripsis, which mediates punctuated evolution in about half of pediatric and adult high-grade osteosarcomas. LTA chromothripsis occurs when a single double-strand break in chr17p triggers concomitant TP53 inactivation and oncogene amplification through breakage-fusion-bridge cycles. It is particularly prevalent in osteosarcoma and not detected in other cancers driven by mutations in TP53 or other tumour suppressor genes. Finally, through survival analysis using diverse genomic and clinical covariates, we identify the genome-wide loss of heterozygosity as a strong prognostic indicator for high-grade osteosarcoma.

In sum, using WGS data, we have elucidated the mechanisms underpinning the complexity and evolution of osteosarcoma genomes. Our results indicate that diverse types of CGR, such as chromothripsis, occur throughout tumour evolution, priming the cancer genome for ongoing genomic instability, rapid karyotype evolution and clonal diversification. These results have implications for our understanding of the molecular basis of intra-tumour heterogeneity and drug resistance in osteosarcomas and other cancers driven by genomic instability.

PP03: WAYFIND-R: A Global Initiative Empowering Research in Precision Oncology

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Precision oncology has become a pivotal part of cancer research and patient care, with growing numbers of approved molecularly-matched therapies.1 Real-world data (RWD) are valuable for cancer research and informing patient management; however, there is a need for high-quality RWD to address the efficacy–effectiveness gap of patient outcomes observed in real-world practice compared with trial populations.2

Developed in accordance with guidance from the European Medicines Agency, WAYFIND-R (NCT04529122) is a multicountry, multisite, prospective cancer registry that is collecting RWD from adult patients diagnosed with a solid tumour and profiled with next-generation sequencing (NGS) tests. The objective of WAYFIND-R is to make data available, interoperable and shareable for research to advance the field of precision oncology by: (1) characterising the treatments and clinical course of patients with solid tumours who underwent NGS testing; (2) providing a collaborative research platform to improve understanding of health outcomes, cancer care processes, treatment patterns and decision-influencing factors in a real-world setting, including disparities in patients' access to advanced diagnostics and therapies; (3) supporting the design/conduct of epidemiological research and clinical trials; and (4) sharing learnings from cross-country implementation of the registry and considerations for 'fit-for-purposeness' of RWD. The initiative will help inform best practices for NGS-based treatment decisions by clinicians, foster global collaborations between cancer centres and enable robust data analysis by providing academics with access to research-ready data for further analysis in molecularly-defined groups of patients.2

WAYFIND-R collects long-term RWD including patient characteristics, treatments, outcomes, biomarkers (detected by other methods than NGS, such as immunohistochemistry, polymerase chain reaction and enzyme-linked immunosorbent assay) and genomic data from NGS tests certified/validated for clinical use (i.e. any gene panel size, hotspot sequencing, whole genome sequencing, whole exome sequencing or RNA sequencing) used for tissue or liquid biopsies at enrolment and routine follow-up visits according to local standards of care using standardised data collection. Variables linked to the decision-making process, reason for NGS testing and molecular tumour board recommendations regarding treatment options, major risk factors and socioeconomic/demographic data are also collected by WAYFIND-R.2

The WAYFIND-R® Data Sharing and Collaboration Platform enables researchers to analyse anonymised oncology data from the registry transformed to the Observational Medical Outcomes Partnership Common Data Model (OMOP CDM) within a secure research environment. Researchers can access individual participant data after independent data access committee approval of their research proposal.2

As of 02 December 2024, data have been collected from 4757 patients from 119 sites in 32 countries in Europe, the Middle East and Africa, Asia–Pacific, Latin America and North America.2 The most common cancer types are lung, colorectal, breast, pancreas, liver and prostate cancer.3 Data from WAYFIND-R regarding NGS have been reported previously.3

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PP04: Unveiling the Complex and Enigmatic Biology of Cancer of Unknown Primary Through Their Genomic and Transcriptomic Landscape

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Introduction
Cancer of Unknown Primary (CUP) is a challenging and burdensome diagnosis, defined by the inability to identify the cancer's tissue of origin. This limitation significantly impacts treatment effectiveness for over 1,000 newly diagnosed patients in the Netherlands and accounts for up to 2% of all global cancer cases annually. CUP tumors typically exhibit high self-renewal, migratory capacity, plasticity, and evasion of the immune system. However, which biological mechanisms are responsible for these behavioral patterns in CUP remains to be further understood and is essential for improving diagnostic accuracy and developing effective therapies. This study examines the genomic and transcriptomic landscape of tumor biopsies from 283 CUP patients.
In addition, we used genomics data to predict whether CUP samples could reliably be assigned to a Tissue-of-Origin, including Non-Small Cell Lung Cancer (CUP-NSCLC). Furthermore, we compared the genomic and transcriptomic profiles of CUP-NSCLC to common metastatic NSCLC (mNSCLC) to identify and understand biological differences unique to CUP.

Materials and Methods
Whole-genome-sequencing (WGS; N=283) and RNA-sequencing (RNA-seq; N=79) data from CUP patients and WGS (N=615) and RNA-seq (N=304) data from mNSCLC samples were obtained through the Hartwig Medical Foundation (HMF; Data Requests DR-196 and DR-
359). The HMF WGS workflows provided data to analyze genomic features, predict mutational signatures, and assess cancer drivers using the ratio of non-synonymous to synonymous substitutions (dN/dS). Somatic copy number alterations were assessed using GISTIC 2.0. Tissue-of-Origin predictions and potential therapeutic targets were identified using the CUPPA algorithm and iClusion database, respectively. Dimensional reduction analysis and hierarchical clustering were applied to identify distinct clusters, while proliferation, immune, and fibrotic signatures were mapped to assign dominant functional groups.

Results
The genomic and transcriptomic profiles of CUP revealed substantial heterogeneity among patients. Mutational signatures identified four subgroups linked to smoking, APOBEC activity, UV radiation, and DNA-damage repair pathways. Smoking- and UV-related signatures were strongly associated with predicted lung and skin tumors, respectively. The frequently mutated genes included cell-cycle regulators (e.g., TP53, RB1, and CDKN2A) and immune-related genes (e.g., HLA-I). Notably, 66% of patients exhibited potentially actionable genetic alterations, and 58% of CUP samples could be assigned to a potential Tissue-of-Origin. Interestingly, three distinct tumor microenvironment CUP subgroups were identified using the transcriptomic data: immune-enriched, immune/stromal-enriched, and fast-proliferating. The genomic profiles of CUP-NSCLC tumors revealed a distinct group characterized by alterations similar to those of the non-oncogene-addicted subgroup of mNSCLC patients. These alterations are predominantly linked to smoking. Moreover, the CUP-NSCLC transcriptome showed less immune cell infiltration and a higher expression of transcription factors MYC and E2F targets, indicating a higher proliferation rate.

Discussion
WGS analysis revealed highly diverse genomic alterations in CUP, resembling those in other metastatic tumors. Additionally, our findings support the clinical applicability of WGS to indicate the Tissue-of-Origin and offer tumor-agnostic therapeutic strategies. Transcriptomic data suggested that immune escape and rapid proliferation contribute to the aggressive behavior of CUP tumors. Comparative analyses revealed that only a subgroup of mNSCLC tumors share characteristics with their CUP counterparts. Similar patterns may exist for other cancer subtypes, warranting further investigation.

PP05: Non-invasive proteomic biomarkers for glioblastoma stratification

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Glioblastoma (GBM) is the most malignant brain cancer. Almost inevitably fatal, it has limited treatment options and short overall survival (OS). GBM has very few molecular biomarkers, all measured in tissue after sampling material through the highly invasive procedures of brain biopsy or surgery. To address a large unmet clinical need for non-invasive GBM biomarkers, we performed in-depth plasma proteomics in two independent GBM patient cohorts by high-resolution isoelectric focusing fractionation (HiRIEF), coupled with a liquid chromatography and mass spectrometry (LC-MS/MS) analysis, for the aim of stratifying GBM patients based on the plasma proteome in relation to clinical outcomes.

We longitudinally analysed the plasma proteome in a discovery cohort of 53 GBM patients, analysing 131 plasma samples, collected before surgery and at three time points after surgery. We discovered that based on treatment-naïve plasma protein levels, GBM patients stratify into two plasma proteome patient groups (PPGs). The PPGs had large differences in the plasma proteomes, with PPG2 patients having higher plasma levels of GBM-enriched mesenchymal-like proteins and cancer-signalling proteins. The plasma proteome showed correlation with radiological parameters and survival differences, further manifested in shorter OS in PPG2 patients (hazard ratio = 2.98, 95% confidence interval: 1.47−6.05), adjusted for potential confounders. Through machine learning, we identified that a panel of 31 proteins may serve as a prognostic biomarker for determining the PPG status with high accuracy (89%). In a longitudinal follow-up, we observed a convergence in the plasma proteome of the PPGs on average one month after surgery. Finally, we confirmed the major findings in a validation cohort of 73 patients (100 plasma samples) and compared the PPG-associated proteome to 36 non-cancer controls. We further discovered that PPG2 patients might benefit from a more radical surgical resection, reflected in longer OS, paving the way for precision surgery in GBM.

In summary, based on circulating blood plasma protein levels we propose a non-invasive approach in stratifying GBM patients into two groups. We report previously undescribed plasma proteomic biomarkers that can accurately determine PPG status and predict OS, opening new opportunities for monitoring GBM.

PP06: Proteo-Transcriptomic Analyses Reveal Upregulation of the MAPK Signaling Pathway in Microsatellite Stable Early-Onset Colorectal Cancer

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The prevalence and death rates of early-onset colorectal cancer (EOCRC) have been increasing at an alarming rate since 1994. Compared to late-onset colorectal cancer (LOCRC), EOCRC is more aggressive and resistant to treatment. Despite KRAS mutations and the MAPK/ERK pathway's significant role in colorectal cancer tumorigenesis and progression, these mutations are less frequent in EOCRC patients.
In this study, we conducted proteo-transcriptomic profiling of two independent cohorts [MD Anderson and The Cancer Genome Atlas (TCGA)] comparing EOCRC and LOCRC tumors. Our focus was on sporadic, microsatellite-stable, treatment-naïve patients with early-onset (< 50 years) and late-onset (> 60 years) colorectal adenocarcinomas.
Our analysis revealed consistent findings in the enrichment profiles between EOCRC and LOCRC. The two pathways with the highest levels of significant GSEA were the epithelial-mesenchymal transition and the Wnt/β-catenin signaling pathway. Both cohorts showed a consistent enrichment in Hallmark KRAS signaling. However, using the novel KRAS-ERK gene signatures to better capture the pathway signaling functionality, we found consistent enrichment of MAPK upregulation in both cohorts. A further analysis of this enrichment was conducted in wild-type RAS GTPase (KRAS, NRAS, and HRAS) tumors, revealing a consistent enrichment of MAPK upregulation in EOCRC wild-type RAS GTPases tumors ($p < 0.001$). Proteomic analysis revealed supportive findings to the transcriptomic profilings.
This study not only elucidates the unique molecular attributes of EOCRC but also lays the groundwork for exploring MAPK targeted therapies for this age group. Given the increasing prevalence of EOCRC, this study lays the groundwork for larger investigations of MAPK upregulation in EOCRC and the potential development of precision therapeutic strategies

PP07: Phosphoproteome profiling in precision oncology

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Precision oncology approaches employing genomics-guided targeted therapies for individual patients have provided significant survival benefits in several cancer types. However, varying response rates in solid malignancies, many patients without actionable genomic lesions, and increasing evidence that non-genomic mechanisms may play an important role in tumors indicate that genomics alone is often insufficient to inform and guide the clinical care of patients. Since most targeted anti-cancer drugs inhibit the activity of protein kinases, measuring the tumor phosphoproteome as a direct readout of kinase activity is poised to enhance molecular stratification. We established a clinical (phospho)proteomic workflow for integration into the molecular tumor board (MTB) workflow of the Germany-wide INFORM (for children with relapsed cancers) and MASTER (for young adults with refractory cancers and patients with rare tumors) registry studies and profiled > 1300 tumor tissue specimen from patients enrolled in the DKFZ/NCT/DKTK MASTER study or the INFORM registry trial. To assess the aberrant activity of druggable signaling pathways, we developed a new tumor proteome activity status (TOPAS) scoring methodology using expression data of > 8,000 proteins and > 20,000 phospho-sites per patient in a heterogeneous pan-cancer cohort. TOPAS scores integrate protein expression and phosphopeptide abundance data at several levels to detect aberrant signaling pathway activities of several interdependent oncogenic signaling pathways within one tumor specimen. We show for the first time that comprehensive (phospho)proteome profiling is feasible and informative in a real-world prospective precision oncology setting. Discussion of (phospho)proteomic data of > 900 prospective patients in weekly MTB meetings revealed that adding a (phospho)proteomic layer can supply critical information for personalized therapies that is not discernable from genomic and transcriptomic data. In an independent value evaluation (329 target recommendations in 104 patients), the phosphoproteome layer was decisive in 22% of all recommended targets. Based on anecdotal cases with available clinical follow-up, we provide evidence that (phospho)proteome profiling carries important diagnostic value by detecting actionable tumor-driving kinase signaling in patients without actionable genomic lesions or by functionalizing genomic variants of unknown significance. Overall, our
work demonstrates the utility of the additional phosphoproteome data layer to enhance therapeutic recommendations in molecular tumor boards.

PP08: Comparison of diagnostic performance of optical genome mapping and whole-genome sequencing for gynecological sarcoma diagnostics

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Background:
Mesenchymal tumors in the gynecological tract are a diverse group, which have recently been recognized as a distinct entity. Some driver genetic events are known but most gynecological mesenchymal tumors have no specific genetic hallmark mentioned in the WHO Blue books, and genetics have not been a substantial part of the diagnostic process. However, the availability of whole-genome sequencing (WGS) is increasing in the clinical setting, and new technologies such as optical genome mapping (OGM) have emerged as promising approaches as substitutes for cytogenetic analyses. In our ongoing project, we aim at presenting multiomics data from gynecological mesenchymal tumors, and compare WGS to OGM.

Methods:
27 patients with gynecological mesenchymal tumors were recruited at Pathology department, Karolinska University Hospital, Stockholm, Sweden. DNA and RNA were isolated from fresh frozen tumor samples, and DNA was isolated from peripheral blood. WGS and whole-transcriptome sequencing (WTS) was performed on the Illumina platform. Ultra-high molecular weight DNA was isolated and mapped through the Bionano pipeline. WGS and WTS data was analyzed in the software Scout and Autoseq, with filtering criteria set for clinically relevant structural variants and copy number aberrations at high variant allele frequency. OGM data was analyzed in the Bionano Access software, according to recommended settings.

Results:
Out of the 27 included samples, 23 passed quality control in the OGM analysis. Leiomyosarcoma was the most common diagnosis (n=15), followed by leiomyoma (n=11). There was also one case of lipoblastoma.

All three fusion genes detected by WGS and WTS were also detected by OGM. All nine clinically relevant truncating translocations (involving the genes RB1, CDKN2A, TP53, NF1, and RAD51B) detected by WGS were also detected by OGM. In a 60 year old woman with lipoblastoma, a t(6;8) involving the PLAG1 gene was visible in the WGS data. However, based on OGM, it was clear that it was a three-way translocation, t(1;6;8). OGM detected translocations, both reciprocal and unbalanced, in all but four cases. Most of these had not been detected in the clinical filtering process of WGS data, but could on closer inspection be verified. The clinical impact of these translocations is unknown.

The CNA profiles based on WGS and OGM were identical in all cases. However, the visualizations of WGS data in Autoseq enabled detailed analysis of specific genes involved in the CNAs, such as homozygous loss of TP53 and RB1 in larger regions of subclonal or heterozygous loss.

Discussion:
In our hands, OGM complements the WGS analysis and increases the chance of defining structural variants in gynecological mesenchymal tumor samples. However, we encounter difficulties in filtering the clinically relevant translocations from the passenger events in the OGM data. Since OGM is a new technology, there are not many cases presented in the literature, and more knowledge is needed to be able to verify or discard our new findings as diagnostically relevant.

PP09: Application of Whole Genome Sequencing enhances the diagnostic yield in CNS tumours: The KCH Neuropathology Department experience

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Background & objectives: Accurate diagnosis and prognostic stratification of central nervous system tumours remains a challenge due to their molecular complexity, which often requires parallel running of diverse testing methods. We evaluated the added diagnostic value of whole genome sequencing (WGS) compared to standard-of-care molecular tests.

Methods: Fresh brain tumour samples from 255 patients (102 paediatric/TYA, 153 adult) were analysed by a multimodal NGS panel (covering 305 DNA and 76 RNA targets) and DNA methylation array (DKFZ, brain classifier). Comparative analysis with WGS data was performed including detection of small nucleotide (SNVs) and structural variants, loss of heterozygosity (LOH) and germline findings.

Results: Multimodal NGS panel detected diagnostic variants in 86.7% of the cases, with a total of 453 pathogenic SNVs and 58 gene fusions. WGS confirmed 96.7% of the known pathogenic SNVs and found an additional 90 previously undiscovered variants. All diagnostic fusions were confirmed by WGS. Twenty-four cases had no driver SNV or gene fusions, but were confidently profiled by methylation array. Germline variants were detected in 26 cases (identification rate of 10%). Four cases had high tumour mutational burden (13-189 mut/Mb). Methylation array appeared sensitive for copy number variation assessment, apart from chromanagenesis events (33 cases) and genome-wide ploidy gains (40 cases).

Conclusion: WGS offers a single-test approach with improved diagnostic utility over the standard-of-care molecular tests. The added value was most apparent in detection of previously unknown germline variants and cases with high TMB that also altered the patient management. WGS also returned superior structural variant information compared to methylation array.

PP10: NGS as standard of care diagnostics for pediatric cancer

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Despite improvements in cure rates, cancer is still the leading cause of disease-related deaths among children in high-income countries. As childhood cancers are rare in comparison to adult cancers, concerted efforts are needed to advance research and improve patient care. In The Netherlands, all childhood cancer care and most research has been concentrated in a single national center, the Princess Máxima Center for Pediatric Oncology. By performing whole-exome sequencing and RNA-sequencing as standard-of-care we provide individualized diagnosis and treatment plans for precision medicine purposes. Sequencing is performed on all diagnostic primary tumor samples with a turn around time of less than 2 weeks, equating to approximately 600 paired tumor – normal exome comparisons and 600 RNA-seq analyses per year. Resulting in integrative tumor driver identification and the identification of events otherwise likely missed through targeted approaches. Gene fusions are identified in nearly 30% of patients and a clinically relevant mutations or focal copy number changes in 40-50% of patients. Our pan-cancer RNA-seq based classifier has an accuracy of ~95% in predicting tumor (sub)types across the breadth of pediatric tumors. And as of 2025 we will be replacing exome sequencing with whole genome sequencing whilst maintaining turn around times, thereby further developing our precision oncology program. The institute's centralized position enables uniform generation of data from patients across the country for diagnostic and research purposes.

PP11: From Medical Prescription to Genome report: a Complete Software Ecosystem

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The goal of the Plan France Médecine Génomique 2025 (PFMG 2025) was to set up national high-throughput sequencing laboratories in order to enhance access to genomic medicine for diagnostic, prognostic, and therapeutic purposes, in the field of rare diseases and cancers. SeqOIA (Sequencing, Omics, Information Analysis) is one of the two national clinical laboratories. The laboratory itself is supported by the Assistance Publique des Hôpitaux de Paris (AP-HP), the Institut Curie and the Institut Gustave Roussy.

SeqOIA operates with interconnected in-house softwares and pipelines. The prescription platform, SPICE (SeqOIA PrescrIption ConnEctée), ensures seamless patient tracking throughout the entire process, from the initial consultation and sample reception to sequencing, bioinformatic analysis, interpretation, and the generation of reports.

The automated cancer diagnosis pipeline within the SeqOIA project encompasses multiple stages. The first steps lead to reads alignment files of matched normal-tumoral exome or genome as well as tumoral transcriptome if available. Then, SNVs and indels are called in matched tumor-constitutional pairs, and also separately in tumor and germline samples. Copy Number Variations (CNV) are called in tumoral genomes. Additional features are also computed, such as Microsatellite Instability (MSI), Tumor Mutational Burden (TMB), Homologous Recombination Deficiency (HRD) and mutational signatures. An optional standalone branch dedicated to the transcriptome includes table counts, fusion detection, and tumor-type prediction. Planned enhancements include the detection of oncogenic viruses and viral integrations, as well as improvements in tumor-type predictions and gene expression analysis.

The above-mentioned pipeline's outputs are automatically imported into gLeaves, the genome interpretation software developed by SeqOIA. While still under development to support fusions, the software currently displays annotated SNVs (Single Nucleotide Variants) and SVs (Structural Variations) within a user-friendly interface. Variants of interest can be efficiently filtered and visually reviewed through an integrated genomic visualization tool based on the Integrative Genomics Viewer (IGV). Finally, patient information and selected results are automatically exported into a comprehensive biological report, which is then sent back to the prescriber using SPICE.

PP12: Validation of chemotherapy resistance biomarkers using retrospective clinical trial emulation with real-world data

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Chemotherapy remains a mainstay of cancer treatment with more than half of adult cancer patients receiving chemotherapy at some point during their clinical history. However, chemotherapy resistance continues to be a major problem in providing effective treatment to cancer patients, and as a result of widespread chemotherapy use approximately 80–90% of patient mortality can be attributed to drug resistance. Despite the clear need for a precision medicine approach to help guide chemotherapy treatment decisions there are almost no clinically-approved predictive biomarkers for these drugs. To help address this issue, we have developed three biomarkers that predict resistance to a set of common chemotherapies (Thompson et al., biorXiv 2024). Our biomarker technology is based on pan-cancer mutational signatures of chromosomal instability (Drews et al., Nature 2022), which can be quantified in all tumour types using a single genomic test.

As part of their validation, predictive biomarkers are typically evaluated by prospective clinical trials, with prospective randomised controlled trials remaining the gold standard for providing clinical evidence. However, considering the often prohibitive financial costs and burden of patient suffering associated with running a randomised control trial, there has been a shift in recent years towards retrospective analysis of existing real-world data for biomarker evaluations. As cytotoxic chemotherapies are ubiquitous in cancer treatment, there is an exciting opportunity to combine existing methodologies of real-world emulation with accepted biomarker trial designs to retrospectively emulate clinical trials.

Here, we used this strategy to assess the performance of our biomarkers in identifying patients resistant to platins, taxanes and anthracyclines. We leveraged real-world cohorts to emulate a series of biomarker-stratified clinical trials across a total of 840 patients curated from The Cancer Genome Atlas Program and the Hartwig Medical Foundation. In phase III emulations where patients were pseudo-randomised to a single chemotherapy treatment or alternative standard-of-care arm, predicted resistant patients had increased risk of treatment failure for taxane in ovarian (HR=7.435, 95% CI=3.967-20.458), taxane in metastatic breast (HR=3.98, 95% CI=1.20-13.22), taxane in metastatic prostate (HR=5.46, 95% CI=2.19-13.62), anthracycline in ovarian (HR=1.88, 95% CI=1.03-3.44), and anthracycline in metastatic breast (HR=3.69, 95% CI=1.87-7.28). Non-randomised emulations showed predictive capacity for platinum resistance in ovarian (HR=1.46, 95% CI=1.12-1.90) and anthracycline in sarcoma (HR=3.59, 95% CI=1.19-10.81). We also demonstrate that implementation using gene capture panel sequencing of tissue or shallow whole genome sequencing of cell free DNA from liquid biopsies may be feasible (83% and 95% of concordance compared to gold standard prediction, respectively).

Our findings demonstrate the clinical value of these biomarkers in predicting resistance to various chemotherapies across multiple cancers through a single genomic test, transforming the current one-size-fits-all chemotherapy approach into a more precise and tailored treatment. They also highlight the potential of using real-world retrospective data to emulate biomarker-stratified trial designs without the expense of prospective trials.

PP13: Assessing defects in homologous recombination repair with a composite biomarker in a tumor agnostic setting

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Background: Homologous recombination repair (HRR) deficiency (HRD) disrupts DNA repair, causing genomic instability. HRD-positive tumors are sensitive to poly (ADP-ribose) polymerase inhibitors (PARPi), which have demonstrated significant clinical benefit in some entities. Current PARPi drug approvals use mainly BRCA1/BRCA2 variants or large-scale genomic scarring as HRD biomarkers, which can only identify subset of patients who may benefit from HRD-targeted therapies. Developing broader, more accurate composite biomarkers, integrating HRR impairments as well as the resulting genomic patterns, may improve patient selection for these therapies. Established composite HRD biomarkers are trained on cohorts with strong HRD prevalence and have stringent input requirements, limiting their clinical application. DNA-damaging agents like trabectedin may act synergistically in combination treatments with PARPi.

Methods: We developed a composite HRD biomarker, the TOP-ART score, using a cross-entity cohort of whole-genome and whole-exome sequencing data from 739 patients enrolled in precision oncology programs. The TOP-ART score integrates germline and somatic variants in 182 HRR-associated genes and incorporates genomic patterns resulting from HRD, such as mutational signature 3 and genomic instability quantified by genomic scarring events. Patient-derived cancer models were used to evaluate the association of the TOP-ART score with drug sensitivity to PARPi and trabectedin and compare its predictive capabilities to other published composite HRD biomarkers.

Results: We observed strong associations between genomic patterns of HRD and HRR gene impairments, including – but not limited to – BRCA1/BRCA2. Genomic patterns varied depending on the fraction of affected alleles and cancer type. Compared to established biomarkers, the TOP-ART score identified more HRD-positive cases and allowed absolute quantification without cohort-wide normalization in WGS and WES samples. In vitro drug testing on patient-derived cancer models showed that this TOP-ART positivity was significantly associated with favorable responses to PARPi, validating its predictive value for HRD-targeted therapies.

Conclusions: The novel TOP-ART score is an accurate and sensitive HRD biomarker with broadly applicability. It is used prospectively to determine eligibility for the multicentric randomized phase-2 interventional clinical trial TOP-ART (Trabectedin/Olaparib vs. Physician’s choice in DNA Repair Deficiency Tumors, PMO-1603, ClinicalTrials.gov Identifier: NCT03127215), which tests a combination treatment of olaparib and trabectedin vs. physician’s choice in a cross-entity setting.

PP14: Knowledge Connector: Decision support system for multiomics-based precision oncology

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Precision cancer medicine strives to improve patient outcomes by tailoring clinical management to individual molecular profiles evaluated within multidisciplinary molecular tumor boards (MTBs). The effectiveness of MTB recommendations depends on the accurate, comprehensive, and consistent interpretation of increasingly complex and multilayered molecular data. To address this critical challenge, we have developed and implemented, as part of the multicenter precision oncology NCT/DKFZ/DKTK MASTER trial, the Knowledge Connector (KC), an advanced decision support system that integrates patients' molecular and clinical data with global knowledge, facilitating the standardized generation and documentation of MTB recommendations.

The KC enhances data curation, enables seamless database integration, and supports in-depth case discussions based on multi-omics data. A key innovation of the KC is the introduction of blocks of clinical knowledge (BoCKs)—modular units of curated clinical information assembled by curators during case evaluations and subsequently reviewed by an independent team of experts. This results in a continuously growing collection known as the BoCKbase, which complements external knowledge bases, streamlines evidence from diverse sources, and significantly improves the reuse and storage of critical clinical information. By efficiently extracting relevant biomarker-drug associations and reducing reliance on external resources, the KC increases the efficacy of data interpretation in clinically meaningful ways and optimizes inter-curator concordance. Our findings demonstrate that the KC is a versatile tool supporting medical decision-making in MTBs, thereby enabling the scalability and standardization of precision cancer medicine. The BoCKbase has been successfully implemented and maintained at the National Centers for Tumor Diseases (NCT) in Heidelberg and Dresden, with plans underway to extend its deployment to additional centers in the near future. This expansion will further validate the utility of the KC and contribute to the harmonization of MTB practices across institutions.

In summary, the KC processes relevant biomarker-drug associations and increases the efficacy of data curation in a clinically relevant manner. It is a versatile tool that supports decision-making in MTBs and enables the scalability of precision cancer medicine.

PP15: Clinical utility of ctDNA versus tissue profiling for therapy selection in advanced cancer

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Background
Circulating tumor DNA (ctDNA) has emerged as a promising and minimally invasive alternative for molecular profiling in precision oncology. We prospectively investigated the clinical utility of comprehensive genomic profiling (CGP) of ctDNA, compared to tissue-based CGP, in identifying actionable biomarkers for therapy selection in patients with advanced cancer.

Methods
In the molecular screening phase of the IMPRESS-Norway trial (NCT04817956), a subset of patients with treatment-refractory advanced cancers underwent parallel CGP of plasma ctDNA and archival tissue samples. Findings were reviewed by the national molecular tumor board (MTB) for actionable biomarker identification to guide targeted therapy selection.

Results
From April 2021 to June 2023, 529 patients representing 65 different cancer types were enrolled. ctDNA results were available for 508 patients, with matched tissue results for 468. Actionable biomarkers enabling access to study treatment were identified in 27.8% of the patients (141/508). Of 137 patients with both ctDNA and tissue CGP, concordance of biomarkers was observed in 40.1% (55/137). In 48.2% (66/137) of the cases the biomarkers were identified in tissue only, while in 11.7% (16/137) they were identified in ctDNA only. Furthermore, in 40 patients who did not have matched tissue CGP, ctDNA identified treatment-decisive biomarkers in four additional cases. Patients with elevated ctDNA fractions (>10%) were associated with a higher biomarker detection rate in ctDNA (85.3%), compared to patients with lower levels (35.3%). Additionally, the concordance varied by biomarker categories and cancer types, with higher ctDNA detection rates observed in colorectal, neuroendocrine, breast, and bladder cancers. Lower detection rates were observed for copy number alterations and gene fusions, and the utility was expectedly limited for patients with CNS cancers. Median turnaround time for ctDNA CGP was
significantly shorter (16 days, IQR: 14-20) compared to tissue CGP (39 days, IQR: 33-48), with a median difference of 21 days ($p<0.001$).

Conclusions
ctDNA profiling represents a promising complementary approach to tissue profiling, particularly for patients with high ctDNA fractions, when tissue profiling is not feasible or when rapid therapeutic decisions are needed. While clinical utility varies across cancer types, ctDNA may serve as a primary diagnostic tool for therapy selection in selected patient populations.

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P01: Clinical utility of whole genome sequencing in routine molecular cancer diagnostics for solid tumours

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Molecular diagnostics is increasingly important for treatment decision making and personalising care for cancer patients. Test approaches will need to keep up with the growing and increasingly complex biomarker detection needs, to allow patients to benefit from novel drugs and insights. Whole Genome Sequencing (WGS) provides a sustainable solution for this challenge, with clinical validity and added value over existing approaches, as demonstrated in various studies. Here, we present the first systematic evaluation of the value of WGS-based molecular diagnostics for solid tumours in a routine setting in a comprehensive cancer centre.

Evaluation of 888 clinical cases showed a diagnostic success rate of 89%, up from 70% in the study setting, with mean turnaround times (TAT) of 6 working days, coming from 10. Improvements were mainly due to procedural changes in the pathology laboratory and optimised logistics associated with scaling. Molecular tumour board-guided interpretations of genomic findings identified potentially actionable biomarkers in 74% of patients, in line with previous findings by us and others. Importantly, we show that 29% of patients with a known primary tumour (n=600) have biomarkers for reimbursed targeted treatments, 26% of whom started such treatment within one year after WGS. Also, 68% of patients with cancer of unknown primary (CUP, n=123) had a WGS-resolved diagnosis or a biomarker indicating reimbursed targeted treatment, or both, with 70% starting any treatment specific to the newly resolved diagnosis. Pathogenic germline variants were detected in 6.5% of evaluated patients, with about half of these variants not previously found because patients did not qualify for clinical genetics referral.

Taken together, in real-world implementation, WGS-based diagnostics was found to meet standard requirements for success rates and TAT. Diagnostic, therapy guidance and germline predisposition information were efficiently obtained by this single test approach, accumulating to clinical relevance for 42% of all patients.

P02: Proof-of-concept of DNA methylation-based multi-cancer detection in liquid biopsies using IMPRESS

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DNA methylation is a promising biomarker for cancer detection. Previously, our research group identified a common methylation pattern, shared between the eight most common cancer types (lung, colorectal (CRC), liver, breast, pancreas, head and neck, esophageal and prostate cancer). Moreover, we developed IMPRESS (Improved Methylation Profiling using Restriction Enzymes and smMIP sequencing), a cost-effective method for methylation detection without bisulfite treatment. Using methylation-sensitive restriction enzymes (MSREs) and single-molecule Molecular Inversion Probes (smMIPs), IMPRESS allows multiplex detection of thousands of differentially methylated CpG sites. Up to now, this multicancer IMPRESS assay was optimized for use in tissue samples.

Here, we optimized the IMPRESS protocol for use in liquid biopsies (LBs) and we present a proof-of-concept study showcasing its ability to differentiate LB samples from colorectal cancer patients and healthy controls. First, we optimized the protocol for a DNA input of 5 ng, compatible with a typical cfDNA yield from plasma samples. Next, we evaluated our multicancer assay on liquid biopsies from 16 metastatic CRC and 32 healthy control samples.

Normalized read counts, representing methylation levels, of CRC patients were compared to those of healthy controls. CRC results were validated with an *NPY* (neuropeptide Y) methylation ddPCR assay, a known marker for ctDNA-based CRC detection and used in our lab. *NPY*-positive samples showed significantly higher normalized counts compared to *NPY*-negative and healthy samples (Fig.1). Moreover, in two CRC patients, monitored from pre-treatment through stable and eventually progressive disease, normalized counts correlated with disease status. We noticed a decrease during treatment and an increase at progressive disease (Fig.2).

In conclusion, we successfully optimized the IMPRESS multi-cancer assay for LBs, enabling the distinction of CRC patients from healthy individuals. We will soon expand this test to cover the eight major cancer types, which can facilitate earlier and minimally invasive multi-cancer detection.

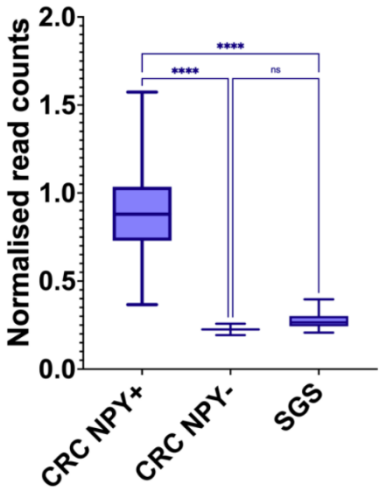

Figure 1. Distribution of the sum of normalized counts in colorectal cancer (CRC), which are positive (*NPY*+) or negative (*NPY*-) for the *NPY* methylation ddPCR assay, and healthy liquid biopsy (LB) samples. **** = p-value <0.0001; NPY, neuropeptide Y.

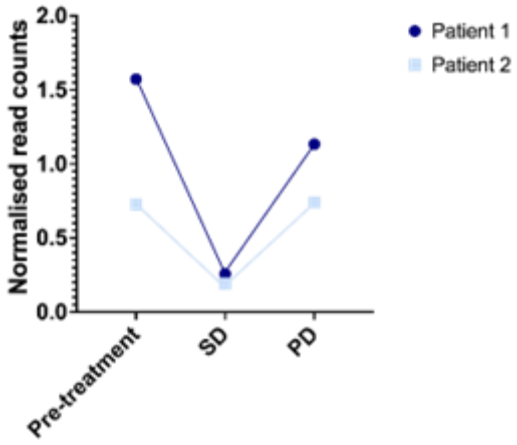

Figure 2. Scatterplot of the follow-up of the normalized read counts of two colorectal cancer patients. LBs, liquid biopsies; SD, stable disease; PD, progressive disease.

P03: Longitudinal molecular characterization identifies temozolomide-driven evolution of IDH-mutant 1p/19q-codeleted oligodendrogliomas.

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Background: Oligodendrogliomas are malignant primary brain tumors characterized by distinct IDH gene mutations and the codeletion of chromosomal arms 1p and 19q. As curative treatments are unavailable, therapy aims to delay disease recurrence through surgery, chemotherapy, and radiotherapy. At tumor recurrence, oligodendrogliomas typically exhibit a more aggressive phenotype and become life-limiting. Our longitudinal study used whole-genome sequencing and other sequencing methods to explore the molecular mechanisms underlying the increase in malignant behavior from primary surgery to tumor recurrence.

Methods: In our multicenter retrospective study, we included 89 patients with IDH-mutant 1p/19q-codeleted oligodendrogliomas who had two time-separated surgeries. Whole-genome sequencing data was obtained for 104 tumor samples from 56 patients, and whole-exome sequencing for 66 samples from 33 patients. Additionally, RNA bulk sequencing was performed on 117 samples from 45 patients, and RNA single-nucleus sequencing on 30 samples from 13 patients. Clinical data, including demographics and survival, was available for all patients.

Results:
IDH mutations, 1p/19q codeletion, and TERT promoter mutations remained stable across surgeries, indicating early driver events. However, CIC and FUBP1 mutations were less stable, with e.g. 28 of 60 CIC mutations lost at recurrence. Both the total copy number variations and tumor mutational burden increased at recurrence ($p=0.003$ and $p<0.001$, respectively). Moreover, of the 40 patients treated with temozolomide, 16 developed hypermutation (≥10 mutations/Mb). Hypermutant tumors were enriched for PIK3CA and MSH6 mutations at recurrence, while nonhypermutant tumors were enriched for NOTCH1, PIK3CA, and PIK3R1 mutations. Hypermutation at recurrence was associated with worse overall survival (HR 3.64, 95%CI 1.24-10.68), remaining an independent negative prognostic factor after correction for confounders such as age and sex ($p=0.021$).
Whole-genome sequencing data allowed us to allocate individual mutations to genome-wide mutational signatures. Temozolomide treatment effects were evident through acquired mutational signatures in both hypermutant and non-hypermutant tumors ($p<0.001$ for both). In hypermutant tumors, recurrent mutations were linked to the single-base

substitution (SBS) 11 signature corresponding with previously published datasets. In non-hypermutant tumors mutations were allocated to the SBS119 signature, a novel treatment-related association. By integrating signatures with clonality estimates, clonal timings of treatment-related mutations were correlated with treatment dates. Aging-related mutations were mostly clonal, while treatment-related mutations tended to be subclonal, suggesting these emerged late post-treatment.

Finally, associating treatment with snRNAseq-derived cellular states, we observed a significant increase in proliferating stem-like cells in hypermutator versus non-hypermutator recurrent oligodendrogliomas.

Conclusions:
These findings illuminate the molecular changes in oligodendrogliomas post-treatment with temozolomide and suggest hypermutation-driven malignant progression of recurrent oligodendrogliomas.

P04: State-Specific Cancer Gene Dynamics and Their Clinical Implications

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Metastatic cancer, a major cause of mortality, has been understudied compared to primary tumors, leaving gaps in our understanding of how cancer genes adapt between these states. We analyzed the association between mutations and copy-number alterations in 25,000 tumor samples from both primary and metastatic cancers. Our findings show that cancer genes display distinct interaction strengths across these states, with 27.45% of genes, including ARID1A, FBXW7, and MARCA4, shifting between one-hit and two-hit drivers. Interaction strengths varied by cancer state and treatment conditions, revealing seven state-specific interactions. We also identified 38 primary-specific and 21 metastatic-specific high-order interactions, enriched in cancer hallmarks, indicating unique tumor progression mechanisms. These findings highlight dynamic tumor progression mechanisms and underscore the importance of considering cancer state in research and treatment strategies for precise therapeutic interventions.

P05: Cancer Dynamics: Position-Specific Mutant Interactions in TP53

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Protein-protein interactions (PPI) are essential to numerous cellular processes and often disrupted in cancer by mutations in key regulatory proteins such as TP53. TP53, encoding the p53 protein, is one of the most frequently mutated genes in various cancers, with most mutations occurring as missense variants that compromise its tumor-suppressive functions. This study employs protein covariation analysis to infer how mutations in p53 alter PPIs, combined with AI-based structural prediction tools like AlphaFold3 to provide detailed insight into the mutation-driven changes at specific residues. We found that mutations at key interaction interfaces with proteins such as MDM2 and MDM4, particularly within the p53 transactivation domain, were predicted to interfere with known binding dynamics. We also prioritized mutations most likely to drive altered PPIs and cancer-related pathways. These findings provide a basis for developing therapeutic strategies to restore wild-type p53 function or target the oncogenic activities of mutant p53, offering potential advancements in cancer treatment.

P06: Successful whole genome sequencing on cell-free DNA from cerebrospinal fluid

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Whole genome sequencing (WGS) represents one of the most promising advancements in molecular diagnostics, offering complete genomic information. One of the challenges using this approach is the need for sufficient tumour material. Ideally, fresh frozen samples with a tumour cell percentage of at least 20% is required, which is not always feasible in clinical practice. This case report demonstrates the potential of using WGS on cell-free DNA (cfDNA) isolated from cerebrospinal fluid (CSF).

A 43 years old female former-smoker was diagnosed with NSCLC and leptomeningeal metastases associated with a bad prognosis of 1-2 months in the absence of therapy. Her relative young age and low amount of pack years smoked, suggested the presence of non-smoking-associated targetable mutations. However, molecular cancer hotspot profiling identified no EGFR mutations, but only a TP53 G245A mutation and loss of STK11. Furthermore, no rearrangements were indicated for RET (FISH) or ALK, ROS1, and NTRK (immunohistochemistry). Notably, PD-L1 expression was high (90%). Due to the lack of targetable mutations, WGS was desired for more extensive screening for therapy options. As a fresh primary tumour biopsy was not available for WGS due to prior radiotherapy, cfDNA from the CSF was the only available material to use for WGS. Even though the amount of cfDNA is low for WGS standards, it is of high quality and predominantly tumour cell-derived.

Cell-free DNA was isolated from 5 mL CSF and prepped for WGS using whole blood as a reference. The analysis of the WGS data, using standard Hartwig somatic pipeline results, passed standard quality controls. As expected, tumour purity was high (91%), and tumour origin was identified as NSCLC with 99% certainty. Besides TP53 G245A and loss of STK11, CDKN2A P81L, KEAP1 E134*, and SMARCA4 M272fs, were observed. Despite no targetable mutations, WGS revealed a strong smoking signature and a high tumour mutational burden (37.1 mutations per Mb). Together with the known high PD-L1 expression, these findings supported initiating immuno therapy.

This case report illustrates a promising potential of using WGS on cfDNA in a clinical setting where conventional tumour material is unavailable. Further research on the applicability of WGS on cfDNA from CSF and other types of liquid biopsies could provide significant clinical benefits, enabling less invasive biopsies and more therapy options.

P07: Characterizing rare cytogenetic subgroups of acute myeloid leukemia by whole genome and transcriptome sequencing

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Acute myeloid leukemia (AML) is a genetically heterogeneous disease, with this variability impacting its classification and clinical outcomes. Whole genome sequencing (WGS) offers the potential to detect all relevant sequencing variants in a single approach, covering single nucleotide variants (SNVs) and insertions/deletions (indels), as well as structural variants (SVs) or copy number variants (CNVs). In this study we aim to show how WGS can be used to characterize two rare subgroups of AML.

Two AML cohorts, one characterized by translocation t(6;9), resulting in a DEK::NUP214 fusion (n=14), and tetraploid AML (n=10), were sequenced at the DKFZ Genomics and Proteomics Core Facility (Illumina HiSeq X Ten) with a mean coverage of 78x and analyzed by the ICGC variant calling pipeline. Moreover, transcriptome sequencing was performed for n=6 AML samples with DEK::NUP214 fusion and analyzed with RIFTT, a fusion gene detection pipeline developed in house. Functional SNVs and indels were selected based on confidence scoring, SVs were selected based on event score. Comprehensive clinical data were used for validating the findings in both directions.

On average, 4.37 million SNVs, 984,977 Indels and 201 SVs were found per patient sample. The majority of SNVs and indels were intergenic (48%) or intronic (36%) and less than 0.1% of SNVs and indels were called somatic, as well as functional and highly confident. In total 23/25 structural alterations (92%) detected in routine diagnostics were confirmed by WGS and/or RNA-seq, including translocations, deletions and FLT3-ITDs. Importantly, the t(6;9) translocation was detected in every patient of the DEK::NUP214 cohort. In addition, in tetraploid AML a deletion del(9), resulting in a SET::NUP214 fusion, was found in n=2 samples and an inversion inv(16), resulting in a CBFB::MYH11 fusion, was found in one sample. Overall, n=10 genes are recurrently mutated by SNVs, indels and/or SVs.

Our study confirms that WGS is a sensitive approach for the detection of sequence variants as well as structural variants in AML. We were able to confirm most alterations identified in routine diagnostics and additionally detected clinically relevant variants such as a CBFB::MYH11 gene fusion. In addition, WGS offers the possibility of detecting these variants, as well as intronic and intergenic variants, in one single approach. We conclude that WGS can be a powerful complementary approach to existing techniques and opens new insight into the molecular landscape of AML.

P08: Patient-derived adenoma organoids as preclinical model for early cancer evolution

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Large-scale DNA copy number alterations (CNAs) are present in >80% of human cancers and arise from chromosomal instability. The ongoing missegregation of chromosomes during cell division has been associated with poor prognosis, metastasis and therapy resistance. But the timing and order by which CNAs arise and impact tumorigenesis remains unknown.

To uncover the dynamics and phenotypic consequences of early CNA evolution, we generated a panel of patient-derived adenoma (PDA) organoid cultures of colonic polyps resected during a colonoscopy procedure. During long-term culture up to 150 population doublings we find that a large subset spontaneously acquires and maintains CRC-recurrent chromosome gains, including 1q, 7pq, 8q, 13pq and 20pq.

Frequent single-cell CNA profiling and genetic lineage tracing with DNA barcodes reveals ongoing clonal selection and patterns of deterministic evolution. Competition experiments demonstrate that late-passage organoids have increased fitness and outcompete early-passage organoids. Interestingly, we also find that p53-deficiency is not a prerequisite for the acquisition and maintenance of CNAs and large-scale structural alterations. Lasty, live-cell imaging surprisingly shows that low mitotic error rates are sufficient to facilitate CNA evolution in premalignant cells.

Taken together, PDA organoids spontaneously evolve different genomic and phenotypic features analogous to in vivo colorectal cancer (CRC). These findings establish PDA organoids as in vitro model for CNA evolution in premalignant cells and allows for detailed characterization and modeling of CNA dynamics in relation to CRC.

P09: Performance Assessment of CGI-Clinics for Genomic Alteration Classification at Centre Léon Bérard and Gustave Roussy

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Background: Precision oncology requires precise genomic profiling to guide the orientation to targeted treatments. The Cancer Genome Interpreter (CGI, Muiños et al. 2021) uses multiple models including machine learning to better infer pathogenicity of the molecular alterations found by high throughput genomic profiling technics. We aimed to evaluate CGI-Clinics Version 1 on patients' data from two French Cancer Centers: Centre Léon Bérard and Gustave Roussy.

Methods: We analyzed the genomic data of patients with any solid tumor types from the following programs: 1. PROFILER (NCT01774409) analyzing SNV, CNV and fusions on FFPE tumor material, and 2. PRISM (Bayle et al. 2023) analyzing SNV and fusions on circulating tumor DNA. We evaluated CGI-Clinics V1 classification, with a special focus in variants of uncertain significance (VUS), in comparison to the classification performed by each institutions: prospectively at Centre Léon Bérard using Alamut Visual Plus (including Cosmic, ClinVar, OncoKB), version 1.11, and systematically reviewed by a molecular pathologist at the local Molecular Tumor Board and retrospectively at Gustave Roussy, relying on both an expert-curated internal knowledge base and public databases (ClinVar, OncoKB). We assessed the two mutation classification components of CGI-Clinics V1: 1. BoostDM (Machine Learning models), and 3. OncodriveMUT (rule-based method).

Results: We analyzed molecular alterations from the tumor tissue of 148 patients included in PROFILER, and from the ctDNA of 2622 patients in PRISM. In PROFILER, CGI-Clinics V1 identified 93.3% of oncogenic and 81.8% of non-oncogenic mutations. There were 33.3% of VUS reclassified to oncogenic by CGI-Clinics V1. In PRISM CGI-Clinics V1 identified 87,3% of oncogenic and 66,7% non-oncogenic mutations. There
were 31,3% reclassified to oncogenic mutations. In PROFILER and PRISM cohort, OncodriveMUT were responsable of most of the VUS reclassified as oncogenic (respectively 87% and 91,3%), but not validated by humans.

Conclusions: CGI-Clinics V1 shows promising potential to improve genomic analysis and clinical decision-making from two French cancer centers. We identified that BoostDM showed a good performance and most errors were due to OncodriveMut, that will be replaced with new algorithms in CGI-Clinics V2.

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P10: Genomic and transcriptomic predictors of resistance to anti-PD1 monotherapy in patients with advanced melanoma

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Background
Anti-PD1 therapy has improved survival of patients with advanced melanoma. However, a substantial number of patients does not benefit due to tumor resistance. To understand failure of anti-PD1 therapy in advanced melanoma, the primary objective of this study was to identify genomic and transcriptomic characteristics associated with resistance to anti-PD1.

Methods
In this prospective multicenter study (NCT01855477), tumor biopsies and matched whole-blood samples were collected from 279 patients with advanced melanoma prior to the start of first-line systemic therapy. Whole genome sequencing (WGS) and high-quality RNA sequencing (RNA-Seq) were performed. To identify immune-predictive biomarkers, the cohort was split into two independent cohorts, a training cohort with in-depth clinical data (N=76) and a testing cohort (N=203). After tumor biopsy, 114 previously untreated patients were treated with anti-PD1 monotherapy. Based on their tumor response after anti-PD1 therapy, patients were categorized as good or poor responders.

Results
Overall, the two cohorts were similar based on clinical, genomic, and transcriptomic features. Unsupervised hierarchical clustering of the RNA-seq data revealed two
distinct immunogenic gene expression patterns in the advanced melanoma transcriptome, reflecting low and high expression of immune cell-related genes. Patients with a poor tumor response after anti-PD1 generally had a lower number of specific immunogenic signatures and were categorized into a cluster with low expression of different immune cell-related genes, including signatures of IFN-gamma, effector T-cells and antigen presentation pathways. The cluster of patients with the low immunogenic gene expression score was also associated with a poor overall survival compared to the cluster with high immunogenic gene expression scores.

Conclusions
Based on different immune signatures, a cluster with low immune-related expression patterns was found in patients with a poor response after anti-PD1 monotherapy. This specific cluster may contribute to better understand resistance to anti-PD1 and identify patients with melanoma who need alternative treatment strategies.

P11: Topoisomerase 1 poisoning-dependent mutational signatures in colorectal cancer

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DNA topoisomerases are essential nuclear enzymes that regulate DNA topology, chromatin structures, and basic processes such as transcription and replication. DNA Topoisomerase 1 (Top1) reduces torsional tension of DNA duplexes by coupling DNA strand cleavage-ligation with strand rotation. During catalytic activity, a transient Top1-DNA cleavage complex (Top1cc) forms, where Top1 is covalently linked to the 3' end of the broken strand. Top1ccs can be stabilized by factors like base mismatches and antitumor Top1 poisons, such as camptothecin (CPT) analogs. CPT targets Top1 in cells, increasing Top1cc levels, affecting RNA and DNA synthesis, and activating ubiquitin-dependent Top1 degradation. Stabilized Top1ccs also increase Transcription-Replication Conflicts (TRCs), leading to DNA double-strand breaks (DSBs) at replication forks. We have recently disclosed that CPT-stabilized Top1ccs favor transcription/replication conflicts (TRCs) in cancer cells triggering extensive DNA double-strand breaks (DSBs) at highly transcribed genes of early replicating regions and leading to chromosome instability. Thus, we hypothesize that Top1ccs may significantly alter genome structure by promoting structural rearrangements and specific mutations at DNA damage sites. Since the mutational burden and profiles related to Top1 poison therapy have not been defined yet in human cancers, here we present an exploratory analysis of distinct mutational signatures in metastatic colorectal tumor samples of the Hartwig Medical Foundation (HMF) collection. Using mutational signatures present in COSMIC and "de novo" datasets, we evaluated the "absolute signature contribution" calculated for each sample across 3 groups of patients: 1) patients treated with the Top1 poison, Irinotecan; 2) patients who were administered with drugs other than Irinotecan; 3) patients who have not received a systemic drug therapy. We found that some insertion/deletion (ID), single base substitutions (SBS) and double base substitutions (DBS) appear to occur specifically in Irinotecan-treated patients. We further validated the specific contribution of Irinotecan by selecting patients who received Irinotecan together with anti-VEGF, pyrimidine antagonists, and platinum-based therapies, as they were more frequently administered together in the Hartwig cohort. We compared them with a group of patients who were administered with the same combination of drugs, excluding Irinotecan, and with untreated patients. Interestingly, the results show that only ID denovo 5, of unknown origin, highlights a more specific contribution of Irinotecan.
Furthermore, we performed WGS sequencing and mutational signature profiling in a colorectal cancer cell line treated with CPT and compared to untreated sample to validate the results in patients. We can conclude that current regimens containing Irinotecan seem to more likely induce IDs. However, other specific analyses are needed to establish fully the effects of Top1 poisons on genome mutations/alterations.

P12: Unveiling General and Subtype-Specific Methylation Profiles in Neuroendocrine Tumors

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Neuroendocrine tumors (NETs) are a heterogeneous and relatively rare group of malignancies originating from neuroendocrine cells, most commonly arising in the lungs and gastrointestinal tract. While extensive research into the genetic background of these tumors has provided valuable insights, it has revealed that NETs typically exhibit a low tumor mutation burden (1.09 mutations per Mb), suggesting the involvement of alternative drivers in their development. In pancreatic NETs, the few recurrently mutated genes identified such as MEN1, DAXX, and ATRX are crucial for proper chromatin remodeling, pointing to a significant role for the epigenome, and specifically the methylome, in NET pathogenesis. Despite its potential to deepen our understanding of tumor development and to serve as a powerful tool for biomarker discovery, the NET methylome remains poorly characterized. This gap limits both our biological understanding of these tumors and our ability to identify meaningful and clinically relevant biomarkers. To address this, we have comprehensively investigated the NET methylome through a differential methylation meta-analysis, incorporating site-specific controls and comparisons with other cancer types.

We combined our own and publicly available methylation array data generated using Illumina's Infinium HumanMethylation450 and EPIC BeadChip, including 150 pancreatic (PNET), 46 small intestine (SINET), and 80 lung NETs (LNET) into a comprehensive beta value matrix. Beta values were derived from the raw data, normalized using the Beta-Mixture Quantile function, and underperforming probes were filtered out. Differential methylation analysis was then performed using a modified linear mixed regression model implemented in the ChAMP package in R. For each NET subtype, three subanalyses were conducted: NET vs. healthy tissue (NT, n=381), NET vs. blood (HB, n=808), and NET vs. 14 other tumor types (TP, n=5735). For each subtype, the differentially methylated probes (DMPs) identified in all subanalyses (NT, HB and TP) were identified by intersecting the DMPs. Next, the intersection of these DMPs was taken to identify the DMPs that are common between all NET subtypes and can thus be considered the general NET DMPs. We then performed a negative selection to correct for the intrinsic difference in tissue of origin between the NET subtypes. Ultimately, the general NET DMPs as well as the subtype-specific DMPs were used as input for overrepresentation analysis (ORA) performed with the methylGSA package.

A total of 2.608 PNET, 4.349 SINET, 14.605 LNET and 592 general NET DMPs were identified. The majority of highly-informative DMPs were located within the gene body and intergenic regions, areas often overlooked in numerous methylation studies. Overrepresentation analysis revealed several significantly upregulated gene sets among multiple profiles, while other only showed up for the general NET or subtype-specific profiles.

Taken together, our comprehensive characterization of the NET methylome revealed the existence of both a general NET methylation profile and subtype-specific profiles. These profiles demonstrate the possibility to differentiate NETs from normal tissue, healthy blood, and 14 other tumor types, as well as the classification of NETs based on their tissue of origin, highlighting their potential as a source of clinically relevant biomarkers.

P14: Germline determinants of aberrant signaling pathways in cancer

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Cancer is a complex disease influenced by a heterogeneous landscape of both germline genetic variants and somatic aberrations. While there is growing evidence suggesting an interplay between germline and somatic variants, and a substantial number of somatic aberrations in specific pathways are now recognized as hallmarks in many well-known forms of cancer, the interaction landscape between germline variants and the aberration of those pathways in cancer remains largely unexplored.

Utilizing over 8500 human samples across 33 cancer types characterized by TCGA and considering binary traits defined using a large collection of somatic aberration profiles across ten well-known oncogenic signaling pathways, we conducted a series of GWAS and identified genome-wide and suggestive associations involving 276 SNPs. Among these, 94 SNPs revealed cis-eQTL links with cancer-related genes or with genes functionally correlated with the corresponding traits' oncogenic pathways.

GWAS summary statistics for all tested traits were then used to construct a set of polygenic scores employing a customized computational strategy. Polygenic scores for 24 traits demonstrated significant performance and were validated using data from PCAWG and CCLE datasets.

These scores showed prognostic value for clinical variables and exhibited significant effectiveness in classifying patients into specific cancer subtypes or stratifying patients with cancer-specific aggressive phenotypes.

Overall, we demonstrate that germline genetics can describe patients' genetic liability to develop specific cancer molecular and clinical profiles.

Dalfovo, D., Scandino, R., Paoli, M. et al. Germline determinants of aberrant signaling pathways in cancer. npj Precis. Onc. 8, 57 (2024). https://doi.org/10.1038/s41698-024-00546-5

P15: Rapid methylation profiling for classification of central nervous system tumors in adults using Nanopore-sequencing and AI for implementation in daily clinical practice

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Tumors of the central nervous system (CNS) are clinically and biologically highly diverse, ranging from benign neoplasms to highly malignant tumors. Therefore, accurate characterization of CNS tumor (sub)types is crucial for optimal therapeutic management, as different types of tumors require different treatment approaches. Nowadays, many neoplasms are defined by both histological features and molecular characteristics, in accordance with the latest WHO 2021 classification of CNS tumors.

Genome-wide methylation patterns are routinely examined as part of clinical diagnostic procedures and combined with methylation-based tumor classifiers like the Heidelberg CNS tumor classifier. While this diagnostic procedure accurately categorizes CNS tumor subtypes and provides high-resolution insights into diagnostically-relevant copy number variations (CNVs), this procedure relies on microarray-based DNA methylation profiling. Although effective, this platform is both time-consuming and costly, rendering implementation of this procedure into daily clinical practice infeasible for many pathology laboratories across the world.

Nanopore-sequencing can address these limitations, as demonstrated by the recently developed ‘Sturgeon’ CNS tumor classifier that is able to accurately predict the molecular CNS tumor (sub-)class in less than 40 minutes based on sparse Nanopore methylation profiles, at a significantly lower cost.

Here, we exploit a well-characterized cohort of patients with adult-type diffuse gliomas and meningiomas (glioblastoma, IDH-wildtype; astrocytoma, IDH-mutant; oligodendroglioma, 1p/19q co-deleted; meningiomas; n=200) of which snap-frozen tumor samples are readily available and that are all already characterized by the microarray platform and the Heidelberg classifier. These samples are used to generate sparse DNA methylation profiles by Nanopore-seq and are subsequently classified by Sturgeon. Our study assesses the equivalence between the Sturgeon/Nanopore-seq platform and the currently established Heidelberg classifier/microarray platform.

If equivalence is established, our study provides evidence supporting implementation of the Sturgeon approach in routine diagnostic practice, which will substantially reduce the turnaround time for molecular diagnostics of CNS tumors, reduce costs and has the potential to transform surgical procedures by enabling real-time adaptation of intraoperative management.

P16: Integrating KRAS mutation status improves transcriptome-based prognostic modelling in FOLFIRINOX-treated metastatic pancreatic cancer patients

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Background: First-line chemotherapy (FOLFIRINOX) benefits few metastatic pancreatic ductal adenocarcinoma (mPDAC) patients. Prognostic markers for treatment-related survival are needed. This study validated the added benefit of whole genome sequencing (WGS) to transcriptome-based classification in modelling FOLFIRINOX-related survival.

Methods: mPDAC patients planning to start FOLFIRINOX were included in a prospective nationwide cohort. Pretreatment biopsies were submitted to WGS and RNA sequencing. Samples of non-FOLFIRINOX treated patients were included for exploratory analyses.

Findings: WGS was performed in biopsies from 108 FOLFIRINOX-treated patients and 51 non-FOLFIRINOX-treated patients. 12% of the tumors was KRAS wild-type. These tumors had more targetable alterations (42% vs 17%) and were associated with a longer median overall survival (mOS) than KRAS mutant tumors (7.8 months in KRAS mutant vs. 17.7 months in wild-type tumors, $p = 0.0024$). Transcriptome-based clustering revealed a tumor subgroup showing low classical and basal-like gene expression, enriched for KRAS wild-type status ($p < 0.0001$), the so-called 'classifier negative' subtype. The gene expression of these classifier negative tumors correlated with neural-like signatures. For patients with a homologous recombination deficient (HRD) tumor, mOS was not improved (8.0 months in HRP vs. 13.3 months in HRD tumors, $p = 0.21$).

Conclusions: KRAS wild-type tumors are a distinct PDAC subgroup with a better prognosis. Consequently, KRAS status assessment before transcriptome-based subtyping can stratify patients into three prognostic molecular subgroups (KRAS wild-type, KRAS mutant classical and KRAS mutant basal-like). This integrative way of classification should be validated prior to incorporation in diagnostic practice.

P17: Investigating modulatory effects of IFN-ε on tumor microenvironment in astrocytoma patients

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Gliomas are the most common primary brain tumors and are highly heterogeneous. Their prognosis varies widely, with 5-year survival rates ranging from 10% to 70% depending on the subtype. Glial and progenitor cells are commonly identified as the cells of origin; however, glioma behavior is significantly influenced by its microenvironment. Tumor microenvironment (TME) promotes tumor growth, invasion, and therapy resistance through complex cellular and molecular interactions. Among the modulatory factors of the TME, interferon epsilon (IFN-ε), has been linked to immune activation and tumor suppression. Its loss is frequently observed in glioma patients and is associated with poor outcomes in pancreatic and ovarian cancer. Therefore, our aim is to investigate the modulatory effect of IFN-ε on astrocytoma TME.

In this study, we integrated publicly available single-cell RNA sequencing data from 71 glioma patients, including 8 astrocytomas, 3 oligodendrogliomas, and 60 glioblastomas, covering 97 samples and 479,819 cells. These datasets were preprocessed to identify cell clusters, with initial annotations sourced from the original studies. Marker gene expression analysis further refined these annotations, revealing 16 distinct cell phenotypes, including immune, glial, stromal, and malignant populations.

Specifically, we identified various immune cell types (CD4+ T cells, CD8+ T cells, T regulatory cells, NK cells, B cells, plasma B cells, and dendritic cells), alongside monocytes, microglia, macrophages, oligodendrocytes, fibroblasts, endothelial cells, pericytes, and malignant cells. This high-resolution atlas provides a foundation for generating single-cell gene signatures, enabling large-scale deconvolution analyses of bulk glioma samples.

To inspect the astrocytoma TME, bulk RNA sequencing data from the GLASS-NL cohort (n=100 patients) will be deconvoluted using Statescope, a multi-modal deconvolution framework that integrates scRNAseq-derived gene signatures together with prior knowledge of tumor proportion for an accurate deconvolution and cell state discovery. Application of this method to longitudinal RNA-seq data from GLASS-NL cohort enables us to trace the changes in the TME details hidden within the bulk RNA. By analyzing the output data together with the IFN-ε status derived from available DNA-seq data, we can correlate these findings to clinical data.

Studying the effect of IFN-ε on astrocytoma TME brings us a step closer to better understand the underlying disease processes and thereby ultimately to precision medicine for astrocytoma patients.

P18: Multi-modal data integration for personalised breast cancer care: the Personalised Breast Cancer Programme & SYNERGIA

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The Personalised Breast Cancer Programme (PBCP) provides whole-genome sequencing (WGS) and whole-transcriptome sequencing results within a clinical timeframe. WGS is incorporated into standard clinical care for breast cancer, in order to personalise treatment and assess its impact and utility within the NHS.

Since 2016, the programme has grown to incorporate five hospital sites across England, for patients diagnosed with Stage I-IV invasive breast carcinoma. To date, 1,700 of 2,250 patients have been recruited onto the study (age range: 21-94 years). PBCP demonstrates the feasibility of integrating germline and fresh-frozen tumour WGS into standard clinical care pathways, with all results discussed at a multi-disciplinary Omics Review Board and reports returned to patients in a clinically actionable timeframe (median time from sample collection: Cambridge = 6.1 weeks, other sites = 11.1 weeks). Patients are followed for 10 years with a further blood and tumour biopsy taken in case of relapse. Eight percent of patients have had a breast cancer familial risk variant discovered through PBCP, whilst 70% of patients have had a clinically actionable somatic variant and/or mutational signature identified. Around a third of patients have been enrolled into further studies or clinical trials. In addition, PBCP derived data has been used in collaborative research projects investigating; i. ways to improve the accessibility and utility of WGS in the NHS and ii. the use of multi-modal data integration to improve prediction and prognostication.

A key problem for implementing WGS in routine clinical care is the availability of fresh tissue biopsies for WGS. PBCP contributed to work by the Nik-Zainal group comparing WGS data derived from fresh-frozen versus formalin-fixed paraffin-embedded (FFPE) tumour1. In this study, 578 FFPE cancer datasets from the 100,000 Genomes Project were analysed with 11,014 fresh-frozen samples in order to characterise three artefact signatures (SBS57, SBS FFPE and ID FFPE) and develop an “FFPEImpact” score that is able to quantify sample artefacts. This work demonstrates the feasibility of preserving actionable information from tumour FFPE WGS, increasing opportunities for personalised medicine where the logistical demands of fresh-frozen collection can act as a barrier to many.

Finally, data accrued through PBCP will combine with those from other breast cancer clinical studies to form SYNERGIA: a large, multi-modal data repository that will integrate longitudinal clinical information with WGS, transcriptomics, ctDNA, spatial- and single cell- omics, radiology imaging and digital pathology. Machine learning will be used to automate currently manual and time-consuming processes such as radiology image segmentation and pathology residual cancer burden calling, in addition to the development of prediction algorithms and improved prognostic tools. SYNERGIA aims to improve patient stratification and better personalise breast cancer therapy through identifying early indicators of disease relapse and refining prediction of treatment response.

1. Shadi Basyuni, … & Serena Nik-Zainal. Large-scale analysis of whole genome sequencing data from formalin-fixed paraffin-embedded cancer specimens demonstrates preservation of clinical utility. Nature Communications (2024).

P19: Quantifying immune cell telomere content at single-cell resolution in context of PD-1 checkpoint immunotherapy

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Introduction: Biological processes such as aging, carcinogenesis, and immune response rely on the ability to maintain or rapidly expand cell populations. The fitness of the involved cells is constrained by their replicative potential, which is reflected in the cellular telomere content. Average leukocyte telomere length drops from 10 kbp in newborn to 4 kbp in centenaries, and also displays substantial inter-individual heterogeneity among individuals of the same age. To study the impact of telomere length on immunotherapy, we describe a novel workflow for the inference of telomere content from scATAC-seq data. In a pilot study, we then characterize the response of the T-cell compartment to programmed cell death protein 1 (PD1) blockade in basal cell carcinoma on the telomerome-level.

Method: We apply the TelomereHunter software to scATAC-seq data to determine telomere content on single-cell level in a publicly-available hematopoietic dataset consisting of 35,139 cells in total. Integrating information from open-chromatin-based signatures to assess cell identity, we characterize the heterogeneity of telomere length for individual cell populations pre- and post-immunotherapy.

Results: The extracted telomeric reads from the scATAC-seq data reflect the expected telomereome to genome fraction. Telomere content distributions differ significantly between cell populations. We observe that the median telomere content in intermediate and terminal exhausted CD8+ T-cells prior to treatment onset is significantly correlated to response to PD-1 checkpoint blockade. Likewise, telomere content correlates with post-treatment cell proliferation in terminally exhausted and T follicular helper cells from patients responding to immunotherapy.

Conclusion: Telomere content measurement from scATAC-seq data has a sufficiently high signal-to-noise ratio to detect significant differences between cell types and states. Furthermore, the telomere content of CD8+ exhausted T-cells at treatment

onset is a putative biomarker for successful PD-1-based immunotherapy. If confirmed on a larger cohort, more cost-efficient assays can be used to directly measure telomere length in different populations of exhausted T-cells, and thus, translate these results into a clinical application.

P20: Multimodal Foundation Models in Oncology: From research to clinical impact

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The advent of foundation models (FMs) represents a transformative shift in artificial intelligence, enabling these large-scale AI models to learn and generalize from the underlying structure of data without being confined to specific tasks. By capturing intricate patterns and relationships, FMs have unlocked new possibilities for solving complex problems across a range of domains. In oncology, their capacity to integrate and interpret multimodal data is poised to new clinical applications. This presentation explores five key innovation steps showcasing the efforts to develop and deploy multimodal foundation models within real-world clinical settings, emphasizing technical achievements and experimental results.

Oncology workflows rely on an intricate tapestry of data modalities, including textual reports, electronic health records (EHR), medical imaging, and genomics data. Each of these data types contains rich, modality-specific information, but integrating them into a cohesive representation remains a challenge. Therefore a first stage comprises of training modality specific encoders transforming this diverse data into a unified embedding space. These encoders capture the unique features of each modality while preserving their underlying complexity, enabling alignment across modalities and addressing challenges like sparse and temporally distributed datasets.
Secondly, self-supervised learning (SSL) underpins the pre-training strategy, enabling the extraction of meaningful representations from data without relying on labels. For text, token prediction tasks effectively model semantic structures, while for images, contrastive methods leverage spatial locality to learn feature-rich representations. Genomic data, however, presents unique challenges for SSL due to the lack of inherent spatial or sequential locality in its features. Unlike text or images, where relationships between neighboring elements can provide valuable context, genomic sequences often exhibit long-range dependencies and sparse patterns of relevance. Despite these complexities, we present promising initial results demonstrating how SSL can be adapted to genomic data by designing tasks that capture both local sequence variations and global genomic context. These advancements highlight the potential of SSL to unlock meaningful embeddings for this highly structured yet non-local modality.
Upon successful pre-trianing, the robustness and clinical utility of our models are ensured through rigorous, continous evaluation across a diverse range of downstream tasks. This multi-tiered evaluation encompasses the direct assessment of embeddings, task-specific metrics, and results from real-world clinical scenarios.
Stronger evaluation runs hint at more powerful patient embeddings —high-dimensional representations that unify multimodal data into a holistic view of each patient's clinical journey. These embeddings capture complex relationships across modalities, enabling the inference of missing data points and the integration of incomplete longitudinal records. as a result.

Lastely a clinical application in form of a patient focused AI assistant for medical experts from diverse disciplines is presented as the key validation point for the FM. This assistant supports clinicians across disciplines by automating routine tasks, offering diagnostic recommendations, and enabling natural, contextual interactions tailored to specific patient profiles. Iterative feedback from clinical users not only validates the assistant's utility but also strengthens the foundation models through real-world application. Ultimately, the integration of generalist AI applicatios into clinical workflows exemplifies how foundation models can have a strong impact in the clinical diagnostic workflow in oncology.

P21: GEN-BioDORA: A digital FAIR genomics biobank

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Aim: Creating a FAIR genomics data storage infrastructure enabling data use and reuse.

The ADORE (Amsterdam Oncology and Neuroscience Research) initiative at AmsterdamUMC offers a platform for collaborative, interdisciplinary research aimed at understanding the biomolecular profiles of cancer and neurodegenerative diseases. The initiative involves high dimensional Next Generation Sequencing data as well as methylation array data, requiring specialized infrastructures for efficient storage, management, and analysis. This initiative also presents a challenge: the need for a robust data storage infrastructure that complies with GDPR regulations and Amsterdam UMC's data protection standards, while allowing the findability, accessibility, interoperability and reuse (FAIR) of the data. The proposed infrastructure GEN-BioDORA, will make it so that the diverse datasets can be processed and compared in an interoperable manner utilizing standard bioinformatic pipelines and a user-friendly interface to browse the various neuro- and onco genome catalogs with controlled access. The GEN-BioDORA database is set to facilitate cases in which, for example, neuroscience studies could use matched normal germline whole exome sequencing (WES) data from cancer patients, to identify genetic variants associated with neurodegenerative disease risk; Or germline WES data from healthy controls in neuroscience research could be used to filter and highlight true somatic mutations in tumor material. Through the ADORE initiative, the genome data will be made interoperable through the Research Data Platform of ADORE/AmsterdamUMC in the parallel BioDORA project, for research applications including multi-modal Artificial Intelligence.

P22: Systematic Assessment of the Applicability of Comprehensive Genomic Testing Towards Better Cancer Clinical Diagnostics in the Czech Republic

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Recent technical advancements have allowed extensive implementation of sequencing technologies in clinical cancer research. However, their introduction in daily diagnostic use is hampered by multiple factors, such as the need for a systematic evaluation of their diagnostic yield over the routinely used, mostly low-throughput methods, financial aspects related to the healthcare reimbursement system, and setting standards of best laboratory practice for a wet-lab as well as dry-lab analytical part. We have decided to approach some of these aspects in the collaborative study Applicability of Comprehensive Genomic Testing 2wards Better Diagnostics (aka ACGT2) involving several centers across the Czech Republic. Here, we present the initial steps of our efforts.
To evaluate the benefits of high-throughput methods, we implemented short-read whole-genome sequencing (WGS, Illumina), long-read WGS (Oxford Nanopore Technologies, ONT), and optical genome mapping (OGM, Bionano Genomics) to evaluate a consecutive cohort of cases with lymphoid malignancies as model diseases. We plan to test liquid biopsies as well as tissue specimens, depending on the specific diagnosis.
For pilot experiments, we chose ten chronic lymphocytic leukemia cases with complex karyotypes and applied the three methods, aiming initially to investigate their genomic structural and copy number variants (SVs and CNVs, respectively). Short-read WGS was performed on tumor-normal paired DNA samples of every patient, whereas ONT and OGM on tumor samples only. Complementary data from routine diagnostic and experimental methods were available, including chromosomal banding analysis (CBA), multicolor FISH, chromosomal microarray (CMA), and chromatin conformation capture Micro-C. All these data types were used to evaluate chromosomal numerical and structural abnormalities detected in the samples against the hg38 genome reference. We implemented the Delly tool for SV and CNV calling from the sequencing methods. OGM data were analyzed and visualized using Bionano Access software.
When comparing CNVs from short-read WGS, ONT, OGM, Micro-C, and CMA, we found that 65.5% of all changes were detected by at least three different methods. The remaining findings were mainly from genomic short-read WGS and CMA, which showed the highest accuracy and resolution, including the detection of minor clones. ONT proved very sensitive in SV analysis, detecting 65% of all breakpoints detected by at least one of the other methods. When comparing the results further with multicolor FISH and CBA, the short-read WGS, ONT, and OGM detected most findings in major clones (>20% mitoses) but repeatedly failed to detect specific rearrangements, such as dicentric chromosomes and derived chromosomes involving more than two chromosomes. For this purpose, the analysis against the T2T reference would be beneficial.
Overall, we uncovered significantly greater SV complexity in all ten cases by combining short-read WGS, ONT, and OGM than identified by low-throughput methods. These pilot data will serve to adjust the analytical approaches for further larger patient cohorts.
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P23: A Computational Approach for Phylogenetic Tree Reconstruction from Bulk Sequencing Data

Lea Kowsky, Prof. Dr. Martin Peifer, Dr. Nima Abedpour

Understanding an exploring the evolutionary history of cancer is key for developing new targeted therapies. A phylogenetic tree reveals important information on how the cancer has evolved. However, the reconstruction of a phylogenetic tree from bulk tumor samples is a quite challenging task as such a sample contains a mixture of normal cells, tumor cells and sub clonal tumor cells. Here, we present a computational approach to reconstruct phylogenetic trees from bulk tumor data by using methods from graph theory.

The data is clustered according to the calculated cancer cell fraction (CCF). In a second step, a statistical test is used to compare the different clusters and to determine a nesting matrix and a truncal cluster. The nesting matrix is a one-to-one correspondence to a directed graph. From this directed graph one can enumerate spanning arborescences which are potential candidates for a phylogenetic tree. The possible tree candidates are used in a matrix factorization problem to identify the tree which best fits the data.

P24: Statescope: Revealing cellular states in the tumor microenvironment using standard bulk RNA-seq data

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Background:
Precise identification of cell type fractions and their states in the tumor microenvironment is crucial for biomarker discovery in immuno-oncology. Although single-cell RNA sequencing (scRNAseq) has made it feasible, technical limitations and cost considerations restrict its application to large patient series and clinical applications. Deconvolution algorithms can infer cell fractions and gene expression profiles from standard bulk RNA sequencing (RNAseq) data. While fraction estimations are well-established, accuracy in cellular state identification remains unclear. Prominent inter-patient gene expression heterogeneity in malignant cells leads to less precise cell signatures required by deconvolution algorithms, making its application to cancer RNAseq particularly challenging.

Results:
We introduce Statescope, a framework utilizing a multi-modal deconvolution strategy to accurately identify hidden cell type-specific RNA profiles and cell states from standard bulk RNAseq data. We rigorously evaluated Statescope's performance using bulk RNA profiles with CyTOF-determined cell fractions from 46 individuals' blood samples, and in silico using lung cancer single cell atlas. In benchmarking analysis, Statescope significantly outperformed state-of-the-art approaches (CIBERSORTx, BayesPrism, and TAPE) in both cell fraction estimation and cell type-specific gene expression profiling. Notably, Statescope demonstrated the most accurate and complete identification of cellular states in scRNA-seq data.
We then established a lung cancer cell state atlas for 15 cell types by applying Statescope to the TCGA lung cancer cohort (n=1,025; **Figure 2a**). This cell state landscape confirmed previously reported distinctions between lung adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC) identified by single-cell analysis:

1. The cell state landscape pinpointed that most distinctions in bulk gene expression profiles between LUAD and LUSC originated from differences in malignant cell states, as expected for distinct histology types (**Figure 2b**).
2. Statescope accurately distinguished alveolar fibroblast and myofibroblast states and their differential enrichment in LUAD and LUSC.
3. Statescope profiled neutrophil states, often underrepresented in standard single-cell sequencing, across all TCGA samples. It accurately captured the higher abundance and tumor-associated state of neutrophils in LUSC compared to LUAD, as previously demonstrated in single-cell studies with dedicated protocols.

Finally, we applied Statescope to RNA-seq data from metastatic lung cancer samples (from HMF, those with available clinical information and RNA-seq data; n=67) and a randomized clinical trial for PD-L1 blockade (POPLAR/OAK; n=891 samples), distinguishing states of 15 cell types using the TCGA-derived cell state landscape as a reference. Survival analysis revealed that B cell states are significantly prognostic for metastatic lung cancer patients, with the fourth state significantly predictive of patient benefit from immunotherapy.

Conclusion
We demonstrate that a dedicated deconvolution framework for standard bulk RNA-seq data can accurately reveal hidden cell states in large sample series, which is not possible with single-cell sequencing approaches.

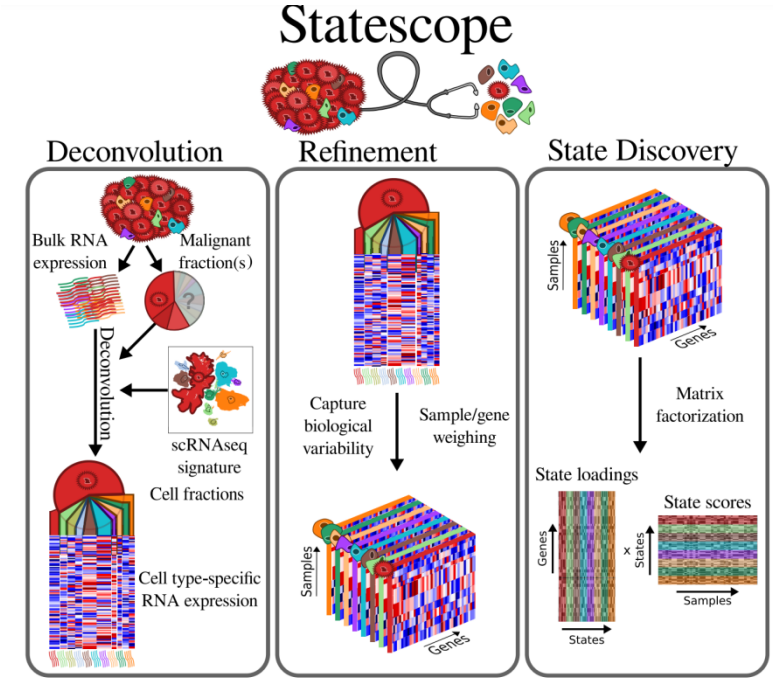

Figure 1. Overview of Statescope framework

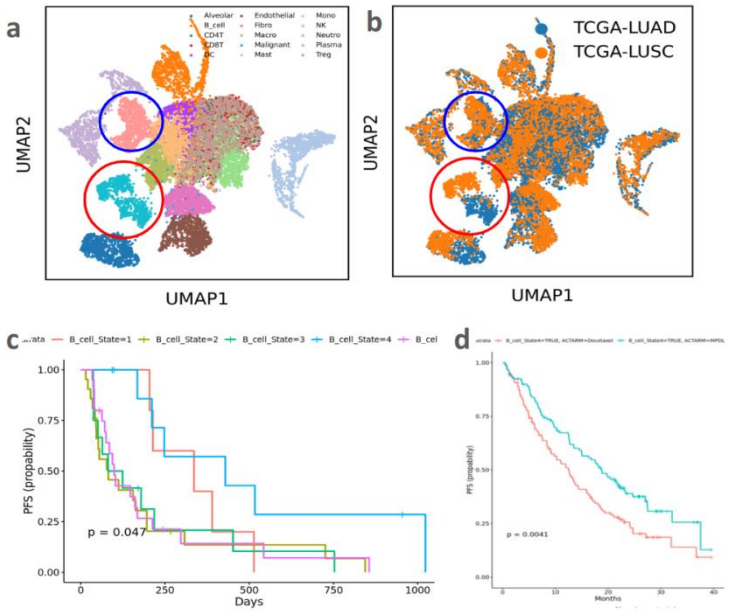

Figure 2. TCGA lung cancer cell state atlas inferred by Statescope.
Cell states landscape of TCGA lung cancer cohort, elucidated by Statescope. Each cell states colored by cell type (a) and histological subtype (b), where malignant cells (red circle) and neutrophils (blue circle) are highlighted. c) Kaplan-Meier plot compares progression-free survival between metastatic lung cancer patients with distinct B cell state (HMF cohort; n=67). d) Kaplan-Meier curve compares the progression-free survival of patients with 4th B-cell state treated with chemotherapy (red) and immunotherapy (blue) from POPLAR/OAK cohort.

P25: Improving early phase clinical trial inclusion for patients with advanced cancer using whole genome sequencing and algorithmic trial matching

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Introduction: Patients with advanced cancer with no standard-of-care treatment options, who are in relatively good clinical condition, may opt for treatment in early phase clinical trials. With an increasing availability of early clinical trials investigating targeted treatments, comprehensive molecular diagnostics play an increasingly important role in this setting. Yet, availability of large amounts of molecular data also adds complexity to trial allocation procedures due to the need for molecular interpretation. This procedures are already complex and labour intensive, due to multiple in- and exclusion criteria, specific for each individual trial.
Here, we evaluate the diagnostic yield and subsequent clinical trial inclusion when routinely applying whole genome sequencing (WGS) diagnostics and whole transcriptome sequencing (WTS) in a Phase I unit setting. In addition, we explore the value of a novel algorithm ACTIN ('Algorithmic Cancer Treatment Initiative') for automated trial selection in this population.

Results: WGS could be arranged with a turnaround time (TAT) of eight working days and success rate of 91%. A potentially actionable marker based on WGS was identified in 171 of 192 patients with successful WGS (89%). Only 25% of patients actually started WGS-informed treatment. The main reason for this was a deteriorating patient clinical condition and unavailability of treatment.
Three patients had a potentially actionable marker based on WTS. None of the patients started WTS-informed treatment.
In the first evaluation phase of the automated trial allocation algorithm, the report was compared after discussion in the MTB within a team of experts. During this phase, ACTIN is further developed in a continuous process. At the time when the algorithm functioned sufficiently, the second evaluation phase was initiated.
In this phase, ACTIN could be used by the MTB during the meeting. In comparison, 77% concordance was found between the treatment allocation by the MTB and the ACTIN report, meaning that the same trial was proposed. In all disconcordancies, this was due to incorrect data input and not based on faulty matching, meaning that the algorithm interpreted the data correctly.

Conclusion: Overall, routine application of WGS opens up potential targeted treatment opportunities for over half of last resort patients, but in practice only 25%
actually started. Main reason for not starting treatment was a rapidly detoriating condition, a well-known situation in this patient population.
In addition, automated molecular and clinical data collection and processing provides a promising solution for replacing increasingly complex parts of the labor intensive trial matching procedures and may increase efficiency in Phase I unit settings. Also, the development of ACTIN showed promising results and opportunities to expand the use to other hospitals in the country, in order to match patients to the best suitable clinical trial independent of the treating hospital.

P26: gOS: a microscope for whole genome oncology

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Abstract (500 words max)

A dramatic recent reduction in sequencing costs has made whole genome sequencing (WGS) inevitable for oncology. While WGS holds the promise of being the "iPhone of molecular oncology" — a single, simple, stable assay enabling a wide range of algorithmic biomarker "apps" — its clinical adoption is hindered by the complexity of WGS data. Pathologists and oncologists are intimidated by the challenge of integrating familiar driver alterations with the vast ocean of (structural, noncoding, passenger, and germline) variation that WGS reveals.

To address this, we developed gOS (genome oncology system), a platform for expert genomic interpretation and biomarker development. gOS supports a paradigm shift in molecular oncology from companion diagnostics to a comprehensive genomic diagnosis model akin to radiology or surgical pathology. Central to this vision is a tool for multi-scale genome assessment, serving as a "microscope" for WGS, turning the enormity of genomic data from a liability into an asset.

We built gOS on the premise that the most significant added value of WGS lies in its ability to analyze copy number and structural variation (SV) with unmatched accuracy. For example, precise zygosity inference is critical for identifying responders to synthetic lethality therapies targeting biallelic gene inactivation (e.g., MTAP). Existing targeted sequencing panels perform poorly in assessing copy number, particularly in aneuploid and rearranged cancer genomes, while WGS enables superior detection through its uniform genome coverage and rearrangement resolution. Mass balance-based algorithms directly integrate copy number and SV into a genome graph for state-of-the-art inference of segmental, allelic, and adjacency dosage.

At the heart of gOS is genome graph navigation, implemented as a non-linear genome browser. This visualization paradigm enables users to intuitively interpret WGS data across discontiguous reference regions linked by rearrangements and across zoom scales — from base-level alignments to chromosome-scale dosage. Such visualization facilitates confident assessments of actionable alterations like homozygous MTAP deletions (Fig. 1), distinguishing ecDNA amplifications from chromosomally integrated events, and resolving complex karyotypes.

A critical strength of gOS is its ability to precisely infer purity and ploidy, genome-wide parameters essential for interpreting subclonal variation and minimizing false negatives in admixed samples. Targeted sequencing struggles in this area, but gOS uniquely visualizes and enables vetting of purity-ploidy fits, allowing pathologists to confidently sign out biallelic alterations and evaluate variant detectability.

Beyond copy number and SV, gOS facilitates deep assessments of signature-based biomarkers like HRD and MSI while providing a platform for novel algorithmic biomarker development. Numeric features (e.g., TMB, FGA) are visualized against population reference densities, conditioned on tumor type, to highlight deviations. gOS also integrates state-of-the-art annotation databases (e.g., OncoKB, AlphaMissense) and leverages GPT-4o API to generate citation-supported, tumor-specific variant interpretations in an EHR-ready PDF format.

In conclusion, gOS enables intuitive, multi-scale exploration of WGS data, empowering pathologists and oncologists to make confident integrative diagnoses. At NYU, we are deploying gOS to interpret WGS on clinical tumor samples. As WGS transitions into mainstream oncology practice, we anticipate that gOS will set the standard for clinical-grade whole-genome interpretation, unlocking the full potential of this transformative technology.

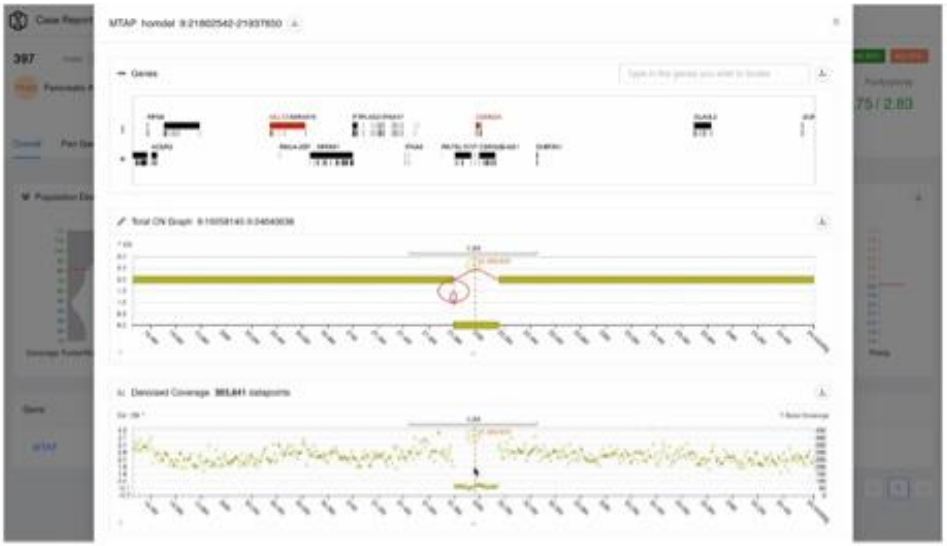

Figure 1: Snapshot of gOS genome graph browser showing nodes (segments) and edges (junctions) affected by a homozygous loss of CDKN2A and MTAP.

P27: A multi-sample study reveals the evolution and heterogeneity in high-grade serous ovarian cancer

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Ovarian cancer is a heterogeneous disease and high-grade serous carcinoma (HGSC) accounts for 70% of them. The pathogenesis of HGSC had remained obscure until the identification of its tubal origin; however, the carcinogenesis process remains largely underexplored. Meanwhile, targeted therapy using poly(ADP-ribose) polymerase (PARP) inhibitors have revolutionized the treatment of HGSCs and emphasizes the importance of patient stratification. Nonetheless, current clinical trials use different assays and a consensus approach for patient stratification is still lacking. In this study, whole genome sequencing technique was used to profile tumor-normal sample pairs, including some related tumors collected from different anatomical sites. This yields a multi-sample cohort comprising 55 pre-treatment samples from 33 HGSC patients and is suitable for addressing questions about molecular stratification and tumor pathogenesis.
We developed a simple classification scheme to stratify patients into HRD-like and FBI-like subgroups and validated our methodology in PCAWG ovarian cohorts. Comprehensive analyses on the two subgroups were conducted on genomic scars ranging from mutations, indels, structural variants and copy numbers. Our data suggests that the genomic subgroups are characterized by different extent and onset timing of homologous recombination repair defect (HRD) as well as different forms of focal amplifications. Of note, HRD-like group showed higher levels of copy number-based HRD score, which often serves as a surrogate biomarker for PARP inhibitor treatment response.
On the other hand, tumor evolutionary trajectory was reconstructed based on in silico methods capable of inferring relative timing of genetic alterations. This highlights an early bifurcation of carcinogenesis paths in this HGSC dichotomy, despite a common scenario of a very early TP53 mutation, an often early whole genome duplication and a chromosomal instability phenotype seen eventually.
Given the cohort stratified into two DNA-based subgroups, we further use the bulk RNA-sequencing data from the same samples to explore the heterogeneity in terms of the known RNA-based molecular subtypes (mesenchymal, immunoreactive, differentiated and proliferative subtypes), as well as differences in their tumor microenvironment compositions.
Overall, these findings corroborate the concept of tumor-intrinsic genomic phenotypes by providing mechanistic underpinnings from the aspect of tumor evolution. This provides the rationale for studying HGSC disease biology in different contexts and formulating questions about subtype-specific pathogenesis and vulnerabilities.
Translationally, it also holds the promise for better identifying the patient subset that might benefit from PARP inhibitor treatment.

P28: Comprehensive Multimodal Profiling to Advance Sarcoma Precision Oncology and Clinical Care

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Background: Sarcomas represent 1-2% of adult cancers but encompass over 100 subtypes, posing unique challenges for diagnosis and treatment. Current genetic testing relies on multiple sequential assays, each providing limited insight while delaying clinical decisions. Notably, up to 20% of soft tissue sarcomas show no known molecular markers with standard approaches. There is an urgent need for comprehensive molecular profiling to improve diagnosis, guide therapy selection, and advance precision oncology for sarcoma patients.

Methods: We are implementing an innovative multimodal profiling approach leveraging three complementary technologies: (1) Fiber-Seq for simultaneous genome-wide detection of mutations, DNA methylation, and chromatin accessibility; (2) Nanopore direct RNA-seq to capture full-length transcripts and their modifications; and (3) data-independent acquisition mass spectrometry for deep proteome quantification. We are aiming to profile 375 sarcomas, prospectively collected at diagnosis. Containerized analysis workflows will be implemented to enable reproducible processing of this complex data, with results delivered through standardized clinical reporting templates designed for molecular tumor boards.

Results: Our pilot Optical Genome Mapping and Fiber-Seq data on 50 undifferentiated sarcomas demonstrates the feasibility of comprehensive multiomic profiling, revealing previously undetectable alterations with potential therapeutic relevance. We anticipate identifying clinically actionable insights in over one-third of cases, impacting both diagnosis and treatment selection. We will also strive to accelerate sarcoma research through responsible data sharing and providing open-source analysis workflows.

P29: The 1000 NEN Genome

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Introduction: Neuroendocrine tumors (NET) arise from neuroendocrine cells in various organs. Once considered rare, their incidence and prevalence are rising. Most NET are diagnosed at advanced stages, limiting curative options and causing significant morbidity and mortality. Despite a low somatic mutational burden, NET are linked to >8 inherited predisposition syndromes and familial clustering, suggesting unidentified germline drivers. Estimates of pathogenic germline variant prevalence in NET vary (4.5%-45%), and previous GWAS have significant limitations. Addressing these gaps could enable early detection through targeted screening, personalized risk stratification, and insights into disease mechanisms to guide innovative therapies. The '1000-NEN-Genome project' aims to address these gaps by consolidating a large European cohort, generating high-quality WES data, and performing comprehensive analyses to identify (likely) pathogenic germline variants, develop a polygenic risk score (PRS), and advance understanding of NET.

Materials and Methods: Buffy coat samples from > 1100 European NET patients diagnosed with (i) NET from pancreatic, small intestinal, or pulmonary origin of TNMstage (I-IV), and (ii) without previously diagnosed genetic syndrome, have been collected in 17 study sites. Associated pseudonymized clinicopathological data is registered into a centralized database, to allow pooled analyses, while strictly adhering to ethical/legal guidelines. gDNA will be extracted and subjected to WES after enrichment of the exome with the Twist Human Comprehensive Exome kit (Twist Bioscience).

Planned analyses: A primary list of variations will be generated using an in-house pipeline following GATK Best Practice Guidelines, will be annotated using VariantDB, and classified per ACMG criteria. Initially, mutation status of genes included in the oncopanel, containing genes previously linked to NET and other tumor types, will be analyzed. Next, a hypothesis-free approach will then assess (likely) pathogenic germline mutations using literature and pathway analysis with GeneMania to determine their relevance. Then, a first-of-its-kind digenic variant exploration using the Variant Combination Pathogenicity Predictor (VarCoPP) from IBsquare Toolbox for Oligogenic Analysis will follow to predict the potential pathogenicity of any bi-locus variant combination. Finally, a GWAS will be performed with 7000 healthy controls, to identify loci linked to sporadic NET phenotypes and additional disease risks, with data analyzed for clinicopathological associations via PLINK. Results will include p-values, effect sizes, and visualisations using Manhattan and quantile–quantile plots. A PRS model will then be developed through regression and cross-validation to estimate an individual’s genetic risk for developing NET.

Conclusion: Ultimately, this project will facilitate early disease detection among highrisk individuals and improve disease management by revealing the true prevalence of (likely) pathogenic mono- and digenic germline mutations, providing a PRS model, and deepening our understanding of NEN etiology. This project is a trial in progress, with first results expected from 2026 onwards.

P30: Error reduction in leukemia machine learning classification with conformal prediction

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Recent advances in machine learning (ML) have led to the development of classifiers that predict molecular subtypes of acute lymphoblastic leukemia (ALL) using RNA sequencing (RNA-seq) data. While these models have shown promising results, they often lack robust performance guarantees. The aim of this study was three-fold: to quantify the uncertainty of these classifiers; to provide prediction sets that control the false negative rate (FNR); and to perform implicit error reduction by transforming incorrect predictions into uncertain predictions.

Conformal prediction is a distribution-agnostic framework for generating statistically calibrated prediction sets whose size reflects model uncertainty. In this study, we applied an extension called conformal risk control to ALLIUM, an RNA-seq ALL subtype classifier. Leveraging RNA-seq data from 1042 patient samples taken at diagnosis, we developed a multi-class conformal predictor, ALLCoP, which generates statistically guaranteed FNR-controlled prediction sets.

ALLCoP was able to create prediction sets with specified FNR tolerances ranging from 7.5-30%. In a validation cohort, ALLCoP successfully reduced the FNR of the ALLIUM classifier from 8.95% to 3.5%. For cases whose subtype was not previously known, the use of ALLCoP was able to reduce the occurrence of empty predictions from 37% to 17%. Notably, up to 34% of the multiple-class prediction sets included the PAX5alt subtype, suggesting that increased prediction set size may reflect secondary aberrations and biological complexity, contributing to classifier uncertainty. Finally, ALLCoP was validated on two additional RNA-seq ALL subtype classifiers, ALLSorts and ALLCatchR.

Our results highlight the potential of conformal prediction in enhancing the use of oncological RNA-seq subtyping classifiers and also in uncovering additional molecular aberrations of potential clinical importance.

P32: The origin of clonal hematopoiesis after hematopoietic stem cell transplantation can be traced to prenatal development

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Background: Clonal hematopoiesis (CH) is a prevalent, premalignant state in aging individuals that is driven by somatic mutations, often in chromatin regulators such as DNMT3A. In addition to its progression into hematologic malignancies, it is associated with an increased risk of age-related diseases through an inflammatory state, such as diverse autoimmune diseases and osteoporosis. In aged recipients of hematopoietic cell transplantation (HCT), phylogenetic analysis suggested that CH driver acquisition can already occur during prenatal development. However, CH also occurs in younger individuals, particularly under selective pressures, such as HCT. The origin and clonal trajectory of CH in young HCT recipients remains unknown.

Aims: To characterize the mutation accumulation and clonal dynamics in individual hematopoietic stem and progenitor cells (HSPCs) belonging to DNMT3A mutant CH and their wildtype counterparts in three recipients of HCT in childhood.

Methods: Three HCT recipients with DNMT3A mutations at variant allele frequencies >5% were selected from a cohort of long-term childhood HCT survivors. For each recipient, we performed whole-genome sequencing (WGS) after clonal expansion on 9-10 single CD34+ HSPCs, including 4-5 wildtype and 4-6 mutant HSPCs. The rate and spectrum of mutation accumulation was compared to those of healthy individuals. We constructed phylogenetic trees based on shared and private somatic mutations and converted the molecular time scale to real time. Mutational signature analysis was performed on individual driver mutations and the clonal versus subclonal mutations derived from the topologies of the phylogenetic trees.

Results: Overall, the rate and spectrum of mutation accumulation in DNMT3A-mutant HSPCs and their matched wildtype counterparts were indistinguishable, reflecting normal aging. These results justify the construction of phylogenetic trees and conversion of mutation time into real time, based on a postnatal linear mutation rate. We identify donor-derived CH in all three recipients, supported by mutational signature analysis of both the CH-driver and the catalog of pre- and post-expansion mutations. Our findings in confirm that DNMT3A mutations can originate very early in life, including prenatally. In addition, our cohort includes neonatal donor material, showing that age-related expansion of a CH clone is not a prerequisite for donor-derived CH.

Conclusion: Our results underscore the role of early somatic mutations and environmental factors like HCT in promoting CH. Future work comparing age-related and post-HCT CH may help understand the gene- and age-dependency of CH dynamics and identify potential targets for intervention.

P33 : Transforming Cancer Data Exploration: Towards Intelligent Navigation and Analysis Using a cBioPortal Co-Pilot

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SE4BIO

The cBioPortal for Cancer Genomics is an open-source platform for visualizing and analyzing large-scale cancer genomics data. The public instance of cBioPortal hosts molecular and clinical data from > 200,000 tumor samples collected from > 400 published cancer studies. Based on usage metrics and scientific citations, it is the most popular cancer genomics resource, serving thousands of cancer researchers on a daily basis. With its intuitive analysis and visualization interface, the cBioPortal has enabled the translation of complex cancer genomics data into groundbreaking research and insights, demonstrated by >30,000 citations to date in the scientific literature.

One of the key reasons for the success of cBioPortal is its ability to provide access to large and complex genomic data sets without requiring bioinformatics or computational skills. Thanks to a vibrant open source development community, we have witnessed rapid growth in data volume and features in cBioPortal. As a result, the growing complexity of data and interface options threatens to outpace the very researchers it aims to empower. Without intervention, we risk a situation where the abundance of data and features slows down discovery rather than accelerating it, known as the paradox of plenty. Addressing this UI/UX challenge is not just an improvement; it is imperative to sustain the pace of innovation in cancer research. With the introduction of Large Language Models (LLMs) and the integration of LLMs into user interface co-pilots, we are developing an interface that can understand user questions in their own language, and actively navigate them to the analysis results that they are looking for. In doing so, we believe that the integration of an AI co-pilot into the cBioPortal will allow it to continue its critical role in serving the cancer research community.

P34: The Circulating Cancer Catalog as basis for personalized cancer therapy

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For most cancer types the primary tumor is not by itself life-threatening, the metastases are. These are caused by Circulating Tumor Cells (CTCs), which are shred by the primary tumor and circulate through the blood (in some cases saliva or urine). Ongoing attempts to treat cancer, even if they are fully personalized, are commonly based on biopsies of the primary tumor. While these can provide valuable information, they do not represent the full heterogeneity. In addition only a subset of the primary tumor cells are able to enter the blood and migrate to new sites, making those cells much more relevant. Therefore, we generate a personalized Circulating Cancer Catalog that describes the Whole Genome Sequence as well as the mRNA content of a collection of single CTCs isolated from the blood of the patient. This catalog can underlay any treatment modality; our current focus, of which the progress will be described, is on personalized immunotherapy.

P35: Bioinformatics field standard for Genetic Medical Laboratories

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In the past years bioinformatics has become a crucial part in several medical laboratories, such as Genetics, Pathology and Medical Microbiology. Hence, the work of bioinformaticians within these laboratories needs to comply to both the ISO-15189 and the IVDR. Where the IVDR contains requirements for software development, “software shall be developed and manufactured in accordance with the state-of-the-art taking into account the principles of development life cycle, risk management, … ” , the ISO-15189 does not include requirements for bioinformatics or software development processes. However, these processes are described in the IEC-62304, a harmonized standard for “Medical Device Software – Software life cycle processes”. The use of this standard is voluntary and gives good guidance to demonstrate compliance with legal requirements.

Bioinformaticians from all the genetic medical laboratories in the Netherlands gathered to discuss the processes used to develop bioinformatic workflows, i.e. software development. The processes within the different centers and the IEC-62304 were used as a guidance to create a field standard for bioinformatics.

The field standard is available within GitHub, https://vkgl-kwaliteit.github.io/BioinformaticaVeldnorm/. The main goal of this document is to give guidance to what should be documented and registered to comply to the ISO-15189 and the IVDR. The document could also serve as a guidance for (internal) auditors.

P36: Implementation and validation of gene fusion detection in routine cancer diagnostics

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Gene fusions play an important role in the oncogenesis and progression of many tumors. Being able to accurately detect fusions can inform diagnostic, prognostic and therapy selection. Unlike traditional methods, whole transcriptome sequencing (WTS) allows for comprehensive detection of both known and novel fusions. However, the nature of the data makes fusion detection challenging.

With healthcare moving towards large-scale sequencing and precision oncology, there is a need for robust, scalable and portable pipelines that can operate in clinical settings.

Here we describe the implementation and validation of the nf-core/rnafusion pipeline (https://nf-co.re/rnafusion) for gene fusion detection using WTS data. The pipeline is built within nf-core, a community-based framework to build and maintain bioinformatics analysis pipelines. The nf-core/rnafusion pipeline uses multiple callers (STAR-Fusion, FusionCatcher, Arriba, SQUID, and pizzly) to generate a combined comprehensive report of all fusion events, which increases the confidence of the calls and aid in their interpretation.

nf-core/rnafusion has an easily maintainable codebase assembled into a continuous integration environment. Containerisation ensures reproducibility and portability of the analyses. Combining results grants confidence in fusion events repeatedly identified by different tools and increases the chances of identifying novel fusions.

To validate the pipeline we used both commercially available RNA samples known to contain a wide range of gene fusions, as well as clinical samples for which fusions have previously been detected with alternative methods. We evaluate the robustness of sample handling and library preparation as well as the sensitivity and performance of the pipeline. The validation covers the detection of known fusion events between two different genes when transcripts are highly expressed (at gene and transcript level) and the correct identification of breakpoints. Other categories of RNA fusions, such as exon-skipping events or lowly expressed gene fusions, were outside the scope of this validation and will need to be addressed separately.

Our results show that we can consistently detect all gene fusions (19/19) present in commercial samples, at both gene and transcript level when multiple fusion transcripts were present, as well as, the exact breakpoints. Our analyses show that integrating results from multiple fusion callers can considerably reduce the number of false positives. Additionally, we consistently observed high recall in highly-expressed fusion transcripts from clinical samples, while improvements are still needed in order to consistently detect lowly-expressed fusions.

P37: Converging on a novel class of neoantigens for vaccines through extensive sequencing and immunopeptidomics

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The development of therapeutic cancer vaccines is contingent on the discovery and validation of antigens presented by tumors. An effective immune response requires extracellular presentation of MHC-bound antigenic peptides on tumor cells and subsequent recognition by T-cell receptors to ensure cytotoxic T cell-mediated tumor killing. While the identification of potential antigenic peptides is challenging, their subsequent immunological validation is even more intricate.

By leveraging whole genome sequencing (WGS), in combination with short and long read RNA-sequencing in our discovery pipeline FrameScan, we can identify neo-open reading frame peptides (NOPs) on a per-patient basis. Hidden NOPs are a previously undescribed class of cryptic neoantigens that derive from somatic structural variants in which coding upstream regions are fused to non-coding intronic or intergenic parts. As such, hidden NOPs are highly dissimilar from wildtype peptides, thereby potentially having increased immunogenicity over other, more conventional antigens, such as missense mutations and overexpressed tumor associated antigens (TAAs).

We previously conceptually validated hidden NOP translation through ribosome profiling and proteomics, and validated hidden NOP immunogenicity through immunopeptidomics, TNFa and INFg response of CD8+ T-cells using three cancer cell lines and three lung cancer patient PBMCs#. In the current study we analyzed a glioblastoma cohort of 20 patients, where FrameScan detected neoantigens from 184 NOPs of which 72 hidden NOPs. Since mass-spectrometry coupled with HLA affinity chromatography is sensitive and on-target, many HLA-bound peptides can theoretically be identified in a run. As such, immunopeptidomics is uniquely suited to provide compelling evidence for antigen presentation in tumor samples, but spectral peptide identification is complicated by immunopeptidomics' large theoretical search space, requiring sound testing of target-decoy assumptions underlying its statistics.

In this work, we show that at least four FrameScan-identified NOPs (and two missense mutations) in a limited cohort of glioblastoma patients could be validated in an independent glioblastoma immunopeptidomics dataset, providing further evidence that rare and lowly expressing antigens resulting from somatic structural variants can be exploited for personalized cancer therapeutics. Whether patient-residing hidden NOPs can be harnessed for oncological vaccination strategies requires larger-scale discovery and clinical validation efforts, including orthogonal methods such as immunopeptidomics.

 # Part of this work is published as Michael V. Martin et al. Cancer Immunol. Res. 2024

P38: How to assess the outcomes of High-Throughput Sequencing technologies in oncology for economic evaluation? A systematic review

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Background: High-throughput sequencing technologies, (HTS) such as whole genome sequencing (WGS), whole exome sequencing (WES), and whole transcriptome sequencing (WTS), have revolutionized medicine, particularly in oncology. These techniques enable the detection of genetic alterations, guide therapeutic decisions, and improve patient outcomes by tailoring treatments or refining patient diagnoses. However, their value remains measured through various criteria, including biological criteria (e.g., type of molecular alterations, disease mechanisms…), therapeutic criteria (e.g., access to targeted therapies, inclusion in clinical trials…), or other health outcome criteria (e.g., overall survival, quality-adjusted life years, QALYs…). This works aims to report the outcomes available in the literature to measure the effectiveness of HTS for the purpose of economic evaluation. This will provide a robust value framework for enhancing transparency and guiding informed decision-making when assessing these healthcare technologies in oncology.

Methods: A systematic literature review following the PRISMA guidelines was conducted between April and October 2024 to assess the clinical and economic impacts of HTS technologies in oncology. Data were collected from PubMed and Scopus using comprehensive search strings combining Mesh terms related to health outcomes, clinical utility, economic outcomes, and specific sequencing technologies such as WGS, WES and WTS. Studies between 2014 and 2024 were reviewed. Exclusion criteria were: non-oncology studies, non-human research, other techniques than WGS/WES/WTS, studies without efficacy results, and those outside the scope (e.g., case reports, position papers, reviews).

Results: In total, 1,777 articles were identified and 91 of which were finally retained after detailed screening. The selected articles predominantly focused on somatic oncology (94%), with a minority addressing constitutional oncology. WES was the most studied technique (71%), followed by WTS (33%) and WGS (27%). Applications of HTS were categorized between research application (79%) and clinical application (21%) studies.
The first category focused on molecular characterization of tumors, carcinogenesis processes, and identification of therapeutic targets or predictive biomarkers without direct patient care implications. Key efficacy criteria here were biological and could be expressed from a health economic evaluation perspective as the cost per number of additional molecular alterations detected. On the other hand, clinical application articles focused on diagnostic, therapeutic, predictive and prognostic criteria affecting patient management. Diagnostic applications included refining or modifying cancer diagnoses, while oncogenetic applications identified individuals at risk for hereditary cancers. Prognostic and predictive applications informed treatment strategies and orientation to targeted therapy, even though our review reveals that limited data were available on intermediate or final clinical outcomes (e.g response rate, overall survival). Key efficacy criteria in this setting were clinical and could be expressed from a health economic evaluation perspective as the cost per additional diagnosis, or the cost per additional targeted therapy orientation.

Conclusion: This review highlights the heterogeneity of criteria used to assess the value of HTS technologies and emphasizes that current economic evaluations remain limited and that the traditional cost per QALY framework may not fully capture the value of these technologies.

P39: Comparative Analysis of Hartwig Medical Foundation's Oncoanalyser and DKFZ's Variant Calling Pipelines for Cancer Genomics

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Accurate identification of genetic variants—single nucleotide variants, insertions/deletions (indels), copy number variants and structural variants—is crucial in precision oncology for identifying driver genes, characterizing tumors, and guiding treatment. This study compares two bioinformatics workflows: the variant calling pipelines developed at the German Cancer Research Center (DKFZ) and operated by One Touch Pipeline (OTP), and Oncoanalyser, created by the Hartwig Medical Foundation. OTP is an automated platform that manages and processes Next-Generation Sequencing (NGS) data, overseeing the entire digital workflow from raw sequence data import to genomic event identification. In contrast, Oncoanalyser is a recently published nf-core Nextflow implementation designed to detect a comprehensive range of variant types and key tumor characteristics from various short-read platforms. Both workflows are specifically optimized for paired whole genome samples. This comparative analysis aims to evaluate the performance of both pipelines, generate metrics such as F1 score, precision, and recall, and investigates the variants exclusive to either workflow in detail. We aim to contribute to ongoing efforts to improve and standardize bioinformatics workflows in cancer genomics, ultimately enhancing the accuracy and utility of genetic information in personalized cancer treatment.

P40: Timing the Origin of Pediatric Rhabdoid Tumors

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Rhabdoid tumors are very aggressive rare soft-tissue pediatric cancers affecting very young children (mainly 0-3 years of age) with poor survival. They are genomically very simple, usually characterized by the bi-allelic loss of SMARCB1. Despite the simplicity of their genome, little is known about the origin of such rare tumors. In this study, we analyzed 83 rhabdoid tumor samples from 74 children, 18 of them diagnosed in Sant Joan de Déu Barcelona Hospital and sequenced in house, and 56 sequenced by the TARGET program. We used whole-genome sequencing data across these cases to characterize the genomic landscape of rhabdoid tumors. In cases with multiple tumor samples per patient, we studied the evolutionary trajectories of the different tumor lesions. An unexpected fraction of these tumors (40%) with a loss-of-function mutation in one of the two SMARCB1 alleles underwent a Copy Number Neutral Loss of Heterozygosity (CNLOH) event resulting in the deletion of the wild-type SMARCB1 copy and the duplication of the defective allele. We have been able to approximate the timing of the clonal expansion of the tumors through the quantification of the aging mutations (SBS1, SBS5 and SBS40); and the timing of the driver event (SMARCB1 loss) through this CNLOH. The results point to a very early SMARCB1 loss (likely during embryonic development) while the clonal expansion can occur several years later during the child's infancy, up to the age of 15 years in one of the cases. These results indicate that probably other factors, besides the genetic driver event, are required to trigger the appearance of the tumor. This study represents the first in-depth genomic overview of rhabdoid tumors that attempts to explain the origin and development of such rare and deadly tumors

P41: An Analysis Application for a Paediatric Somatic Whole Genome Sequencing Service for Wales

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Almost a quarter of childhood deaths in the UK are caused by cancer, and there are around 80 new cases annually in Wales. Current standard of care testing for these patients requires multiple tests including invasive procedures, each of which targets only a limited number of genes or specific type of variant. Introduction of a whole genome sequencing (WGS) service for paediatric cancer patients would allow for the detection multiple variant types over a much wider virtual gene panel or even the entire genome. WGS also allows for the testing of germline and somatic variants simultaneously through sequencing matched tumour and germline DNA.

Here we discuss the development of a paediatric somatic WGS service for Wales. As part of the development of this service, we are coding major updates to an integrated application that allows for the prioritisation and analysis of both somatic and germline variants for a given patient. This application will streamline the analysis process by collating information, filtering and tiering variants and storing variant classifications. Additionally, by integrating the classifications from other in-house cancer assays such as TSO500, we will build a unique resource for Welsh clinical genomics.

P42: Independent component analysis of cancer genomic data suggests an additional mechanism of action of Olaparib

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Genomic cancer data are often derived from a mix of various cell types, including both tumor and non-tumorous cells. This is a major challenge is using genomic data to understand or predict therapy response. Even when derived from single cells, these data require unmixing to enhance their potential in improving patient outcome. Genetic features governing response to treatment rarely act alone, but act in concert, and it is in understanding this concerted action that we gain mechanistic insight of treatment response. Moreover, mechanistic insight of treatment response is crucial to understand how resistance can be overcome. The PARP inhibitor olaparib is a prime example of a successful drug with known biomarkers, such as loss of homology-directed DNA repair due to the absence of functional BRCA1 or BRCA2. While restoration of BRCA1/2 function is a well-established mechanism of drug resistance in the clinic, it does not explain all cases of resistance. Moreover, there are also patients that respond to olaparib who do not carry a BRCA1/2 mutation in their tumors. To investigate mechanisms of response to olaparib we aimed to produce hypotheses about PARP functionality from large scale cancer data. To do so, we applied independent component analysis (ICA) on transcriptome and essentialome data. ICA is a computational approach to separate a mixed signal to find the independent components by maximizing the statistical independence of the estimated components. This may produce useful hypotheses that can be tested in the laboratory to check causality.

We applied ICA using publicly available TCGA transcriptomes (n = 10,817) and DepMap essentialomes (n = 1,046) data. This created two new sets of matrices: one of transcriptional components (TCs) and one of essentiality components (ECs), and their activity in each sample. We recently found that loss of TAOK1 confers resistance to olaparib (manuscript in preparation), and we therefore selected TCs and ECs in which TAOK1 had an extreme weight. We identified a specific genetic interaction between TAOK1, TEAD1, YAP and PARP2, which produced the hypothesis that PARP2 ADP-ribosylates TEAD1 and thereby alters YAP/TEAD1 transcription factor activity and finally cell proliferation.

We then showed that TEAD1 is indeed ADP-ribosylated, and that in PARP1KO, PARP2KO and PARP1KO-PARP2KO TEAD1 ADP-ribosylation levels are markedly reduced. Further, we found that this loss of ADP-ribosylation concurs with a decrease in TEAD TF activity, where olaparib greatly reduces TEAD TF activity in sparsely cultured cells. Functionally, the PARP1KO-PARP2KO cells exhibit slowed growth when seeded sparsely, which could indicate that loss of TEAD1 ADP-ribosylation slows cell proliferation. This is of particular interest, since olaparib is used in a maintenance setting where minimal residual disease mimics the in vitro setting of sparsely seeded cells. Thus, applying ICA on genomic data yields laboratory-testable hypotheses that may be useful to shed light on an additional mechanism of action of olaparib, enabling precision medicine through the synergy between large-scale data mining and laboratory experimentation.

P43: Whole Genome Sequencing of Childhood Cancer at GMC West

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Despite cancer being considered a rare disease, approximately 35,000 children in Europe are diagnosed with cancer every year, making it the leading cause of disease-related deaths among children and adolescents. When compared with adult tumors, pediatric tumors present unique challenges. In addition to the low number of samples available to study, they are often characterized by low tumor mutation burden and the presence of complex genomic rearrangements, including whole genome duplications or gene fusions. Whole genome sequencing (WGS) has emerged as a powerful tool for a comprehensive characterization of tumors by better capturing these complex genomic rearrangements, leading to more precise and accurate diagnosis of pediatric patients.
Every year, around 350 children are diagnosed with cancer in Sweden, of which approximately 100 are diagnosed at the Sahlgrenska University Hospital (SU). To treat these patients, WGS has recently been implemented into clinical routine in Sweden. We, at the Bioinformatics and Data Centre of Gothenburg University, collaborate closely with the laboratory technicians and hospital geneticists at SU to bring high quality personalized care into routine diagnostics and treatment.
To streamline the analysis of the pediatric tumors with WGS, we have been developing a fully automated pipeline (since 2018) which analyzes sequencing data from paired germline and tumor samples.
The pipeline is containerized, making it highly efficient, portable, and reproducible, and runs on our local cluster. It maps the sequenced reads to the reference genome, and performs germline and somatic variant calling to identify variants with potential diagnostic, prognostic, and therapeutic significance. It also performs copy number calling, and structural variance calling. The pipeline ensures a rapid turnaround time of less than two days from the end of sequencing to sharing the results with the clinical geneticists.
Recent improvements have been made to further streamline and automate the molecular clinical reporting done by the SU geneticists. For this purpose, an interactive web application that simplifies the creation of a molecular report was created. The application compiles and presents the results from the pipeline, enabling the geneticists to view and select the relevant aberrations they wish to report. The final product is a patient report containing general patient information, selected variants of clinical
significance and information on how the analysis was done. In addition, there is a possibility for the geneticist to add more comments and information.
To conclude, we present a highly automated and efficient pipeline for the analysis of WGS data from pediatric tumors, with a bioinformatics turnaround time of under two days. Moreover, ongoing improvements to streamline the creation of molecular reports aim to further reduce the overall processing time of WGS samples, improving patient care.

P44: NGS-based Aspergillus detection in plasma and lung lavage of children with invasive pulmonary aspergillosis

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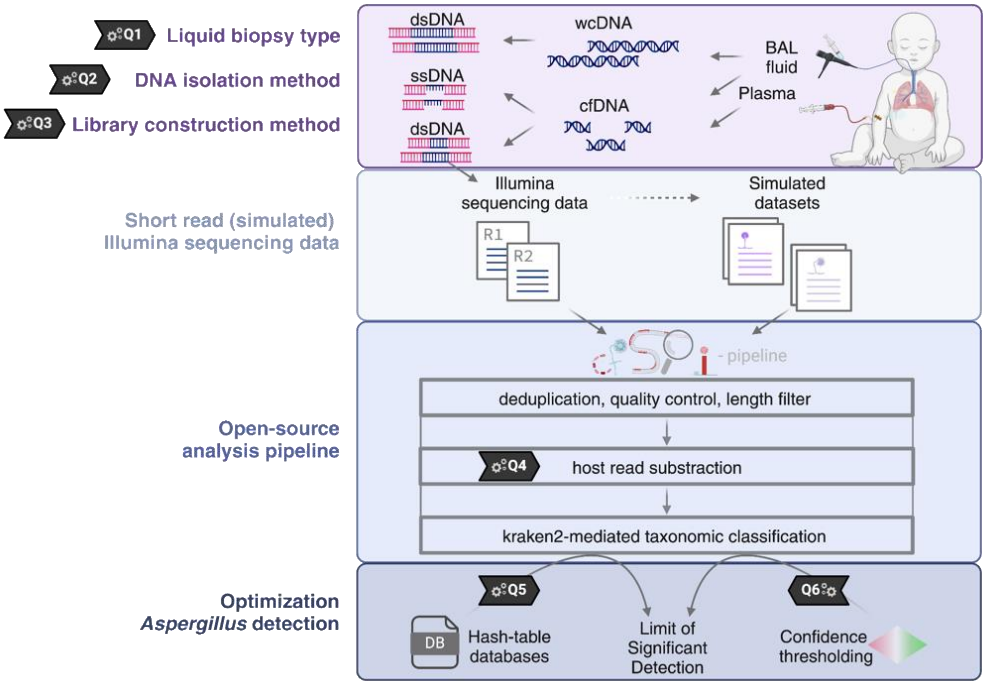
In immunocompromised pediatric patients, diagnosing invasive pulmonary aspergillosis (IPA) poses a significant challenge. Next-Generation Sequencing (NGS) shows promise for detecting fungal DNA but lacks standardization. This study aims to advance towards clinical evaluation of liquid biopsy NGS for Aspergillus detection, through an evaluation of wet-lab procedures and computational analysis. Our findings support using both CHM13v2.0 and GRCh38.p14 in host-read mapping to reduce fungal false-positives. We demonstrate the sensitivity of our custom kraken2 database, cRE.21, in detecting Aspergillus species. Additionally, cell-free DNA sequencing shows superior performance to whole-cell DNA sequencing by recovering higher fractions of fungal DNA in lung fluid (bronchoalveolar lavage [BAL] fluid) and plasma samples from pediatric patients with probable IPA. In a proof-of-principle, A. fumigatus was identified in 5 out of 7 BAL fluid samples and 3 out of 5 plasma samples. This optimized workflow can advance fungal-NGS research and represents a step towards enhancing diagnostic certainty by enabling more sensitive and accurate species-level diagnosis of IPA in immunocompromised patients.



P45

Joseph Usset

Anti-cancer therapies show variability in patient response, motivating the search for biomarkers to guide personalised treatment strategies. In particular, the ability to predict patient non-response to therapies could prevent over-treatment and reduce unnecessary side effects. Here, we use over 3,000 patients from the Hartwig medical foundation database to conduct a systematic study of molecular and clinical biomarkers associated with non-response across a broad range of anti-cancer therapies. We compute a rich set of molecular biomarkers using the broad and diverse output from the Hartwig onco-analyser pipeline. Systematic logistic regression and fisher exact tests are then ru to detect associations to lack of durable patient benefit. A simulation study of the statistical power i also performed to clarify what signals are able to be captured. The findings highlight some potentia molecular markers for non-response that merit follow-up (e.g. B2M loss in Melanoma immune checkpoint inhibitor patients), and also provide a framework for discussing current limitations of biomarker analysis for non-response.

P46: Studying the mutational and functional consequences of cancer therapy in the hematopoietic system of children

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The constant advancements of cancer treatment have improved the overall survival of cancer patients worldwide. Due to the growing number of cancer survivors, the recognition of late-onset chronic health conditions has increased. While this affects approximately 25% of adult cancer survivors, 60-90% of childhood cancer survivors are reported to suffer from chronic health complications post treatment. This discrepancy is possibly owed to the higher doses of chemo- and radiotherapy doses relative to the body surface of children, as well as the naturally longer life expectancy after treatment compared to adults. Therefore, exploring the origins of late side effects in childhood cancer survivors represents a particularly important opportunity to improve their quality of life. Typically, the observed late complications of children resemble aging phenotypes and include cardiovascular diseases, infertility, and secondary cancers. We hypothesize that chemotherapy-induced mutagenesis in tissue-specific stem cells are the origin of these late side effects in childhood cancer survivors and want to study this to help prevent this additional health burden.
Therefore, we collected bone marrow samples from children with acute lymphoblastic leukemia (ALL), which is the most common form of childhood cancer, before initiation of treatment, after completion of treatment, and after a follow-up period. We want to apply combined single-cell whole genome and transcriptome sequencing to investigate chemotherapy-induced molecular alterations in the hematopoietic stem and progenitor cells (HSPCs) of these patients and their functional consequences. Using genomic analyses, we have found that chemotherapy-induced mutagenesis is induced by clock-like processes that are also active during normal life. This enhanced mutational aging varied significantly among HSPCs, pointing to the presence of a protective mechanism in a subset of these cells. To expand on these findings, we are leveraging the transcriptome data of the same cells to identify potential protective mechanisms, tie somatic mutations to gene expression profiles, and investigate a potential induction of allele-specific expressions in HSPCs of childhood cancer survivors.
Overall, this research will give in-depth insights into the consequences of chemotherapy on the hematopoietic system and their relevance to late side effects. By identifying the molecular and cellular processes involved, we additionally gain novel knowledge about the mechanisms of mutational aging. This can contribute to the development of novel intervention strategies to mitigate late side effects and promote healthy aging.

P47: Expression profiling-based radio-pharmaceutical treatment in cases of metastatic breast cancer from the CATCH precision oncology program

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The evolving landscape of precision oncology emphasizes the need for comprehensive, tumor-agnostic approaches to maximize therapeutic efficacy. The CATCH program (Comprehensive Assessment of clinical feaTures and biomarkers to identify patients with advanced or metastatic breast Cancer for marker driven trials in Humans) integrates genomic, transcriptomic, and proteomic data to enable personalized treatment recommendations via a multidisciplinary molecular tumor board. Pilot-analysis (n=200) revealed therapy implementation-rates of about 50% with >30% of patients showing a significant improvement in PFS on the molecular-guided treatment compared to PFS of the previous treatment line.

Here, we explore the pivotal role of RNA analysis in refining treatment decisions within the CATCH framework. We highlight its significance through patient case studies where radioligand therapy targeting somatostatin receptor 2 (SSTR2) or prostate-specific membrane antigen (PSMA) was recommended based on tumor expression profiling. These cases underscore the importance of RNA-based expression analysis in identifying targets for radioligand therapy, particularly in tumors lacking actionable genomic alterations.

Between Sep 2023 and Dec 2024, we processed 208 cases (tumor and matched control). RNA sequencing was performed on 169 of these cases, with 161 samples passing quality control. Among these, 8 patients received tumor-agnostic recommendations for a SSTR- or PSMA-coupled peptide-receptor-radionuclide-therapy with 177Lu-DOTATOC or 177Lu-PSMA-617 according to their RNA expression profiles, with 5 targeting SSTR2, based on evidence from neuroendocrine tumors, and 3 targeting PSMA, based on the evidence from castration-resistant prostate cancer.

Of the 5 patients recommended for anti-SSTR2 treatment, 3 underwent DOTATATE-PET/CT imaging. In two of these DOTATATE-PET/CT showed a high uptake (Krenning Analog-Score (KAS) ^{3} 2) and the patients were deemed eligible for therapy, however one passed away before treatment initiation. The other patient, previously progressing fast on standard-of care therapy (3 months on CDK4/6- and aromatase-inhibitors, less than 3 months on either capecitabine, or peg. lip. doxorubicin), received 177Lu radioligand therapy, achieving a partial response (PR) in liver metastases (KAS = 4), with tumor reduction from 106mm to 66mm. However, disease progression was observed after 4 months in the lymph node lesion with lower DOTATATE uptake (KAS = 2) leading to treatment discontinuation and a therapy switch. Despite the challenge of variability in radioligand target expression across different lesions, integrating transcriptomic data into a multimodal analysis enables the CATCH program to show the potential of expression-based tumor-agnostic strategies in uncovering new therapeutic opportunities

P48: In silico prioritization of ultra-rare non-coding somatic mutations using sequence-based models highlight putatively functional promoter SNVs in cancer

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Background
The identification of somatic driver mutations in non-coding regions of tumor genomes has lagged behind coding regions due to their lower frequency and limited functional annotation. Recent advances in sequence-based models provide an opportunity to prioritize ultra-rare non-coding mutations for functional relevance, enabling novel discoveries in the non-coding tumor genome.

Methods
We developed a computational framework that leverages sequence-based models to assess the functional impact of (ultra) rare somatic single nucleotide variants (SNVs) in promoter regions. Somatic variant data from 24,529 tumor genomes across three cohorts (Genomics England, Hartwig Medical Foundation, and ICGC) were curated and analyzed. Promoter regions were defined around transcription start sites (TSS), and variants were scored using the PARM and Borzoi models. Enrichment of functional variants was evaluated using novel background models, followed by replication and cross-cohort validation.

Results
We re-identified known coding driver genes using sequence-based model predictions. We then applied PARM (trained on massive parallel reporter assay data) to promoter regions and prioritized 247 promoter-like regions enriched for functional SNVs, including well-known cancer drivers such as TERT. Established cancer driver genes were enriched for these functional promotor mutations (p=1.49e-3). Notably, recurrent putatively functional SNVs in the promoter of the mismatch repair gene PMS2 were identified and replicated across independent datasets, with melanoma samples showing significant enrichment. Another sequence-based model (Borzoi), trained on different types of data, supported the functional predictions for 53.85% of the prioritized regions. These findings demonstrate the utility of sequence-based models in dissecting the non-coding genome.

Conclusions
This study establishes a framework for prioritizing non-coding somatic mutations using sequence-based models. The results highlight a path forward for uncovering
functional elements in the non-coding genome, particularly in hypermutated tumors and contexts with limited statistical power. Further development and application of these models promise to gain better insight into the functional landscape of the non-coding tumor genome.

P49: Whole Genome Sequencing advances aiding clinical care of patients with Acute Lymphoblastic Leukaemia

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The NHS Genomic Medicine Service (GMS) was launched in 2019 to enable equitable access to comprehensive genomic testing using a standardised National Genomic Test Directory (NGTD). Building on the work of the 100,000 Genomes Project, WGS is now offered as routine care through the GMS for specific clinical indications including Acute Lymphoblastic Leukaemia (ALL). Here we discuss challenges and success of providing “one stop shop” genomic test for patients with ALL.

A standard experimental design for tumour WGS analysis requires individual tumour samples to be matched with a reference “normal” sample from the same patient. For ALLs, obtaining a high-quality normal sample not contaminated with tumour cells may require a long time. To provide faster access to the results, we created a “tumour first – germline late” pipeline, where tumour only results can be produced quickly and highlight clinically relevant variants in the mixture of somatic and germline variants, and a high-precision tumour-normal analysis would be produced when a high-quality normal sample is received.

Despite high overall concordance between the results of the genomic tests commissioned through the NGTD and WGS analysis, calling variants in some genomic regions remains challenging. Recent advances in genome alignment allowed us to start detecting DUX4 rearrangements from WGS data, which is a clinically relevant rearrangement activating expression of DUX4 transcription factor associated with favourable outcomes, and describe its prevalence across 100,000 Genomes and GMS cohorts.

Minimal residual disease (MRD) monitoring targeting individual immunoglobulin and T-cell receptor (IG/TCR) gene rearrangements is crucial for risk stratification and treatment of patients with ALL. In standard-of-care targeted IG/TCR high-throughput sequencing, only rearrangements covered by the panels can be detected, and the techniques are labour intensive and expensive.

We show that WGS has the potential to discover all IG/TCR rearrangements in a single assay which can be utilised for follow up MRD monitoring.

To summarise, WGS delivers all germline and somatic findings that provide diagnostic and prognostic information for patients with ALL.

P50: Development and clinical implementation of a cellular functional assay to determine the pathogenicity of variant DNA mismatch repair genes

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The rapid expansion of diagnostic sequencing in clinical genetics yields a growing pool of so-called Variants of Uncertain Significance (VUS) in disease genes. These VUS are often single base-pair alterations that substitute a single amino acid or a residue outside gene coding regions. In Lynch Syndrome (LS), patients are predisposed to develop colorectal and endometrial cancer due to a germline mutation in one of the DNA mismatch repair (MMR) genes (MSH2, MSH6, MLH1 or PMS2). Sequencing of the DNA MMR genes, often reveals a VUS rather than a clearly pathogenic variant. As long as its functional consequences cannot be ascertained, the diagnosis Lynch Syndrome cannot be established or excluded, which precludes the identification of family members at risk or not at risk and therefore installment of targeted surveillance or curative treatment.

The KWF-sponsored consortium INVUSE ("Investigating Variants of Uncertain Significance for USE in clinical practice") unites the expertise of basic scientists, clinical laboratory geneticists, pathologists, gastroenterologists, gynecologists and surgeons from all Dutch academic medical centers and the Netherlands Cancer Institute-Antoni van Leeuwenhoek hospital. The aim of INVUSE is to develop and implement into routine diagnostics two complementary functional assays to determine the activity of DNA MMR VUSs. We here present the results of a cellular assay: oligonucleotide-directed mutation screening (ODMS) that interrogates VUS activity in a normal genomic and cellular environment. The assay results, in combination with all available functional and clinical data, will help drawing a robust conclusion on the phenotypic consequences of MMR VUS.

With the recent advances in (whole genome) sequencing technologies, we expect that the VUS problem will continue to expand. Apart from VUS detected in cancer predisposed individuals, tumor DNA sequencing can reveal somatically acquired variants with uncertain significance, that could have implications for therapy choice. E.g., phenotyping MMR variants found in sporadic cancers may be critical, as MMR deficiency is a counter indication for certain chemotherapeutics but enhances the response to (neo-adjuvant) immunotherapy. Similarly, a missense variant in BRCA1 may or may not disrupt gene function, sensitizing or not sensitizing tumor cells to cisplatin or PARP-inhibitor treatment.

We anticipate that functional assays will become indispensable for accurately classifying both inherited and somatically acquired VUS, ultimately improving personalized healthcare.

P51: A Global Multiple Site Reproducibility Evaluation: AVENIO Tumor Tissue CGP Automated Kit

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Introduction
The AVENIO Tumor Tissue CGP Automated Assay (Research Use Only, not for use in diagnostic procedures) offers a fast and automated solution for comprehensive genomic profiling (CGP) using FFPE solid tumor specimens for in-house testing. The assay automates the workflow from tissue lysate through library preparation, quantification, and pooling to sequencing and post-sequencing data processing and analysis, ensuring ease of implementation and consistent performance. A global multi-site study was conducted to assess the reproducibility of the assay in real-world settings. The study focused on the reliability and effectiveness of detecting relevant genomic alterations (SNV, InDel, CNA, and rearrangement) and signatures (TMB, MSI, gLOH, and HRDsig) across 4 sites.

Methods
Four independent laboratory sites (3 non-Roche labs and 1 Roche lab) located in the US, UK, and Belgium were included in the study. Each site presented various levels of lab readiness, environment, and user experience. A set of 48 unique tissues across 14 tumor types were distributed to the 4 sites in duplicate, totaling 384 specimens for the analysis. The samples contained various genomic alterations and signatures. Sequencing QC and variant reporting were compared across all sites to evaluate reproducibility. The key variants reported were compared to those from the FoundationOne®CDx test to assess agreement. In addition, commercial reference materials were tested at each site to confirm that the assay performance met the expected benchmarks.

Results
All 384 FFPE tumor specimens completed the end-to-end workflow from tissue-derived DNA to report in all sites. The sequencing QC metrics, including computational tumor purity, insert size, and coverage noise metrics, were consistently reported across 8 replicates of 48 unique samples with a CV <10%. All samples had >99.8% of exons with over 100x coverage, median coverage >1000x, and on-target rate >80%. No cross-contamination was detected suggesting robust liquid handling in automated library preparation. Pairwise comparisons for all detected variants across 4 sites confirmed high precision, with APA and ANA >99%.
Genomic signatures with positive status were consistently reported with a score CV <14%. Compared to the reference FoundationOne®CDx test, all 4 sites showed a high positive agreement of known variants >90% with the reference results. Additional testing on commercial reference standards showed reproducible results with a 100% call rate for the positive genomic alterations. No false positive variants were detected in GIAB reference cell line DNA suggesting high analytical specificity.

Conclusions
The cross-site reproducibility study with the AVENIO Tumor Tissue CGP Automated Assay demonstrated consistent assay performance in various laboratory settings. The results confirmed the assay's adaptability and robustness, enhancing user confidence and supporting its application in cancer research.

P52: PTEN depletion is insufficient to confer resistance to vemurafenib in melanoma

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Melanoma, a cutaneous malignancy often driven by BRAF mutations, has benefited from targeted therapies like vemurafenib, which specifically inhibits BRAFV600E. However, therapy resistance and relapse remain major challenges. Mutations in PTEN, a negative regulator of the PI3K pathway, frequently co-occur with BRAF mutations. In particular, PTEN loss, which leads to PI3K hyperactivity, is associated with resistance development and poor outcomes.

We have previously demonstrated that PI3K/AKT pathway hyperactivation contributes to vemurafenib resistance in patient-derived melanoma cell lines by enabling independence from MAPK/ERK signaling. This phenotype was associated with a de-differentiation, characterized by high AXL and low MITF levels, and increased invasiveness. To explore whether PTEN loss is sufficient to induce this signaling shift via PI3K/AKT hyperactivity, we employed CRISPR/Cas9 to ablate PTEN in the vermurafenib-sensitive BRAFV600E-mutant cell line MaMel63a. In parallel, we generated the MaMel63aR cell line by prolonged vemurafenib exposure to mimic drug-induced resistance. While all MaMel63a-derived PTEN-deficient clones exhibited enhanced PI3K activity, most of them did not consistently develop resistance. One notable exception was the clone 1D8, that displayed a resistant and invasive phenotype characterized by elevated AXL and reduced MITF levels, a pattern also seen in MaMel63aR cells and the vemurafenib-resistant control cell line MaMel21.

Co-treatment with vemurafenib and the AXL inhibitor bemcentinib significantly reduced viability of the resistant cell lines. Transcriptomic analysis confirmed the high AXL/low MITF signature, and identified an AXL- and EGFR-enriched regulatory network common to resistant cell lines. Preliminary experiments with combined bemcentinib and EGFR-inhibitor afatinib further demonstrated a dramatic reduction in cell viability, highlighting the therapeutic potential of co-targeting AXL and EGFR in vemurafenib-resistant melanoma.

P54: Streamlined transcriptome profiling unlocking for challenging samples in precision medicine

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Introduction: In cancer dedicated precision medicine, sequencing (NGS) plays a crucial role in diagnostic and therapy decisions and transcriptome profiling is progressively supporting stratified medicine efforts. RNA analysis by whole transcriptome sequencing (WTS) enables detection of fusion transcripts, gene expression levels and signatures, useful for primary identification, immune infiltration assessment or prognosis. It is crucial to ensure that library preparation protocols capture all molecules of interest, excluding rRNA, regardless of the quality of the total RNA available. Moreover, in the context of cancer, tumoral material is limited and sparing sample is a crucial point to consider.

As part of the national cancer sequencing organization, the PFMG SeqOIA lab must enable fast and reliable high throughput analysis of tumors. Therefore, automated solutions for library preparation have become important to increase throughput, reduce time for result availability for lower costs, high reproducibility and reduced contamination risk. We demonstrate successful integration of Magelia® (Inorevia) automated platform for WTS with wide range of RNA input.

Methods: High integrity RNA samples from different cancer types were processed in four different conditions: manually or Magelia® automated protocol, crossed with Illumina® Stranded mRNA Prep or Total RNA Prep kits. On the mRNA workflow the same input (100 ng) was compared across conditions, with reduced PCR cycles in Magelia® to limit duplicates ; on the ribodepletion workflow, lower inputs were tested in Magelia® (25-100 ng) compared to manual preparation (300 ng), due to the interest to unlock this method on challenging samples. Paired-end sequencing (2x100 cycles) was performed on Flowcell S1/S2, NovaSeq6000 (Illumina®).

We report different QC metrics to assess quality on library preparation, sequencing and secondary analysis.

Results: mRNA library prep using 100 ng enabled us to secure 3x more, high quality material for Magelia® treated samples, showing no adapter dimers. This translated into securing more sequenceable material, impacting quality scores, depth and diversity of the libraries.

Efficient ribodepletion generates a diverse and suitably abundant RNA population which is then preserved and accurately represented with negligible material loss during library preparation.

Efficient reaction kinetics in confined and optimized volumes have enabled a reduction in the recommended number of amplification cycles to secure enough material for sequencing, bypassing duplication issues and preserving library complexity. Indeed, one cycle below the manufacturer's recommendations have been applied in Magelia®.

Overall wetlab operations significantly gained efficiency with Magelia® system: launching overnight runs requires minimal hands-on time, automation drastically reduce risks of error and cross-contamination; optimized turn-around time is now compatible with urgent samples sequenceable on the second day after sample reception. These data highlight improved transcriptome resolution for Magelia® treated samples.

Conclusion: The automation platform Magelia® addresses critical challenges for WTS on tumor samples, such as limited quantities and quality of available RNA, the necessity to maintain library diversity and to obtain enough quantity of library with limited PCR biases. This is of particular relevance when FFPE samples need to be analyzed reliably. The protocols are optimized to run overnight allowing continuous wetlab, with minimal operator intervention. The Magelia® platform currently offers mid-throughput automated workflows for walk-away RNA-seq library preparations that streamline a fast implementation, corresponding to the needs of the SeqOIA laboratory.

P55: The DKFZ/NCT software ecosystem for precision oncology and its potential for integrated data analysis

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In order to improve patient outcomes by tailoring clinical management to individual molecular profiles in multidisciplinary molecular tumor boards (MTBs), precision oncology requires a complex underlying software ecosystem. At the German Cancer Research Center (DKFZ), this ecosystem is composed of (i) primary analysis for molecular omics data, (ii) data bases and resources for clinical data, (iii) a visualization and decision support system integrating clinical and molecular data with world knowledge, the Knowledge Connector, (iv) systems and data structures for data aggregation and integration, and (v) a zoo of software tools and pipelines for cohort analyses and pattern recognition.
Here, we introduce functionality of these respective components as well as of interfaces between them and present results obtained from integration of these resources, with a special focus on neuroendocrine neoplasias and soft tissue sarcomas, as they are use cases for early line precision oncology in a setting of competing established therapy regimen but absence of clear biomarkers for chosing between them. We assess response to various treatments, including anthracyclines, immune checkpoint inhibitors, multi-kinase inhibitors, mTor inhibitors and platinum-based treatments, exemplify genes differentially mutated and differentially expressed between responders and non-responders and report models to predict therapy response.

P56: Improved Differential Methylation Profiling Using Copy Numbers Derived from Methylation-Dependent Sequencing for cfDNA Biomarker Discovery

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Abbreviations:
CRLM Colorectal liver metastases
MeD-seq Methylation Dependent Sequencing
sWGS shallow Whole Genome Sequencing
CN Copy Number
TF Tumor Fraction
DMM: Differential Methylation Model
DMR Differentially Methylated Region

Over the past decade, liquid biopsies, the use of bodily fluids for diagnostics, have gained prominence in cancer research. In particular, the analysis of cell-free DNA (cfDNA) fragments released into the bloodstream by dying cells has emerged as a non-invasive tool for cancer diagnostics (1,2). Tumor-derived cfDNA (ctDNA) can be identified by hallmark features such as mutations, chromosomal alterations, and patterns of DNA methylation and fragmentation (fragmentomics), which have been effectively applied to diagnose and subtype cancer, discover biomarkers, and make clinically relevant predictions (3–7).

Here, we present an enhanced data analysis pipeline for our previously described Methylation Dependent Sequencing (MeD-seq) assay (8–10). We demonstrate how MeD-seq can serve as a versatile platform for comprehensive cfDNA analysis, integrating methylation profiling, chromosomal copy number (CN) profiling, and tumor fraction (TF) estimation.

First, we used MeD-seq data from 38 colorectal liver metastases (CRLM) and 5 ovarian cancer patients to generate CN profiles and TF estimates. MeD-seq-derived CN and TF estimates strongly correlated with shallow whole-genome sequencing (sWGS) data from the same patients ($\rho = 0.97$, $p < 0.001$) with minimal error (mean relative difference: -0.43%, 95% CI: [-1.5%, 0.71%]). CN profiling showed high agreement, with an average Cohen's kappa of 0.92 for samples with non-zero TF and perfect concordance for samples without detectable TF. Additionally, to streamline CN profiling and TF estimation, we developed a nextflow analysis pipeline following nf-core standards, incorporating preprocessing, alignment, and bias correction.

Second, after validating CN profiles and TF estimates, we used an improved Differential Methylation Model (DMM) to analyze the methylation status of 27,931 CpG islands in 113 CRLM samples compared to 31 healthy blood donors (HBDs). Our DMM incorporates an offset matrix to include CN information, improving sensitivity and robustness. The model identified 3,577 differentially methylated regions (DMRs) (FDR corrected, $p<0.05$), 37% of which were hypermethylated and 63% hypomethylated in CRLM samples. Bootstrapping experiments showed the offset DMM outperformed the standard DMM, maintaining higher robustness (Jaccard index: 0.77, $p < 0.01$) even with smaller datasets.

Third, we linked the DMRs to associated genes for pathway enrichment analysis, identifying 3,269 gene-associated DMRs. GO term and KEGG pathway analyses highlighted 13 and 22 enriched pathways, respectively, including Wnt, TGFB, PI3K-Akt, AMPK, and Rap1 signaling. The offset DMM reliably detected pathways even in datasets with as few as 10 cancer samples.

In conclusion, MeD-seq provides a cost-effective, robust approach for simultaneous methylation profiling, CN analysis, and TF estimation directly from plasma cfDNA. This multimodal capability enables the detection of cancer-specific signals without prior tissue-based information. By integrating CN and TF data into methylation profiling, MeD-seq facilitates the precise identification of CRLM-specific DMRs, making it a valuable tool for non-invasive cancer diagnostics and cfDNA biomarker discovery. Expanding MeD-seq datasets to additional cancer types will further validate its utility as a versatile platform for cfDNA-based cancer research and diagnostics.

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P57: Interactive visualization of whole-genome data using Chromoscope and its application to analysis of DNA repair deficiencies

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A thorough analysis of whole-genome sequencing (WGS) data from tumors and matched normal tissues reveals cancer-specific mutations, including copy number and structural alterations. However, many somatic variants, especially structural variants, are difficult to identify accurately and often require visual inspection at the individual read level. Additionally, once the structural variants are correctly identified, effective interpretation can benefit from visualization tools that offer multi-scale navigation, from chromosome-scale to base-pair resolution.

We developed Chromosope (https://chromoscope.bio/) for interactive visualization of cancer genomes. It allows users to view the data at multiple scales—from global patterns to read-level visualization—to facilitate variant interpretation. To showcase its capabilities, the Chromoscope website hosts 2,778 cancer genomes from the Pan-Cancer Analysis of Whole Genomes (PCAWG) project. It has also been integrated into cBioPortal (http://www.cbioportal.org) for PCAWG data analysis.

As an example, we used Chromoscope to examine the genomic scars caused by inactivation of DNA homologous recombination genes, revealing the separation of BRCA1- and BRCA2-loss phenotypes. We also identified distinct patterns of somatic structural variants in cancers with alterations in cell cycle-related genes, such as CCNE1 amplifications and biallelic loss of CDK12.

Clinical implementations of whole-genome sequencing may benefit from Chromoscope's light-weight software architecture, its interoperability with existing data infrastructure, and its engaging visual interface for guiding structural variation analysis.

P58: Understanding radiobiology – reducing side effects

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Ionizing radiation presents a significant hazard to all living organisms by causing damage to proteins and DNA. The relative contributions of protein and DNA damage to the overall toxicity of these effects are still being actively studied. Gaining a comprehensive understanding of the mechanisms involved could improve medical applications of irradiation, especially in therapeutic treatments and diagnostic imaging. Ionizing radiation causes damage to DNA directly and indirectly through the generation of reactive oxygen species (ROS) via radiolysis of intracellular water. These ROS can further interact with neighboring molecules, extending harm beyond the initial targets. Despite advances in research, the precise mechanisms of how irradiation damages cells remain unclear. Proposed explanations emphasize DNA damage responses and defenses against ROS.

Understanding these intricate mechanisms is crucial, as it would allow targeting specific aspects of radiation's effects for medical benefits. Improved knowledge of these processes could enhance radiotherapy by maximizing treatment efficacy while minimizing collateral damage to healthy tissues. To achieve this, a bioinformatics pipeline is employed to analyze exomes from cancer patients who have experienced severe side effects (classified as at least RTOG grade 3 in acute and/or late response) following radiation therapy. The analysis concentrates on Gene Ontology Overrepresentation Analysis and rare variants common across all patients in the dataset. Since radiation susceptibility may be influenced by various genetic patterns, efforts are underway to identify key molecules affected by different genetic variants that lead to similar cellular reactions. These factors could be combined into a genetic fingerprint to assess individual cellular responses to radiation.
Interestingly, initial analyses did not show a clear association with either DNA damage repair mechanisms or oxidative stress responses. These preliminary findings underscore the diverse genetic backgrounds involved, suggesting a complex regulatory network yet to be deciphered.

An analysis of bacterial adaptation to irradiation from a previously published dataset [1] indicated an early adaptation involving cell cycle modulation, which results in slower cell growth and the utilization of regulatory G-quadruplex regions [2].
These insights could be integrated into the analysis of human exomes, offering new perspectives on variants found in regulatory and untranslated regions. Ultimately, a deeper understanding of cellular stress responses to irradiation can lead to improved therapeutic outcomes. By elucidating these mechanisms, we can refine treatment strategies, optimizing therapeutic benefits while reducing adverse effects.

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P59: TiNDA: An R Package for Detecting Tumor-in-Normal Contamination and CHiP Clusters in Genomic Studies

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Accurate classification of somatic and germline variants is essential for understanding the molecular basis of cancer based on whole genome sequencing of pairs of tumors and matched normal controls. However, the contamination of normal control samples with tumor-derived DNA and the presence of clonal hematopoiesis of indeterminate potential (CHiP) in blood samples pose significant challenges that can lead to the misclassification of variants. To address these issues, we developed TiNDA (Tumor-in-Normal Detection Analysis), an R package designed to rescue misclassified somatic variants and detect CHiP clusters based on variant allele frequency (VAF) patterns. TiNDA uses clustering algorithms to identify somatic clusters by leveraging distinct VAF profiles from tumor and control samples. This approach not only recovers variants that are misclassified due to tumor-in-normal contamination but also identifies CHiP clusters by distinguishing germline variants from genuine somatic mutations in blood samples. By incorporating cluster stability scoring and visualization, TiNDA ensures reliable results, even in datasets with noise. Users are encouraged to assess cluster stability and data quality before including rescued variants in further analyses. We recommend TiNDA as a valuable tool for regular use in genomic studies to address contamination issues, whether from tumor-in-normal scenarios or CHiP clusters. By integrating TiNDA into variant-calling workflows, researchers can significantly enhance the accuracy and reliability of somatic and germline variant identification, thereby supporting robust cancer genomic analyses.

TiNDA: https://github.com/NagaComBio/TiNDA/

Keywords: Tumor-in-Normal contamination, CHiP clusters, somatic variant rescue, R package, variant allele frequency, genomic analysis, quality control.

P60: The Data Access Procedure at Hartwig Medical Foundation

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The Hartwig Medical Foundation (HMF) is dedicated to advancing cancer research by providing access to the extensive database of pseudonymized clinical and molecular data it manages on behalf of patients. The data access request procedure is designed to ensure that data can be shared and is used ethically and in compliance with privacy legislation. Researchers can request access to data for scientific studies that aim to improve cancer treatment, support the common good and further our understanding of the molecular mechanism operating in tumors.

Researchers begin by submitting a detailed Data Access Request form, which is reviewed by the independent Data Access Board (DAB). This review process includes a scientific assessment to ensure that the proposed research can be carried out. The external DAB evaluates the ethical and legal aspects, including the terms of the informed consent signed by patients. Upon approval, a License Agreement is signed by the researcher, the Licensee, and a representative of Hartwig, outlining the terms and conditions for data use. Researchers are then granted access to the data through their institutional Google Cloud Platform account, ensuring data security through multi-factor authentication. The researchers agree to notify and acknowledge Hartwig of their findings when they publish their results.

This structured process ensures that data is shared responsibly, fostering collaboration and innovation in cancer research while protecting patient privacy and adhering to legal requirements.

P61: Cancer Vignettes: The genomic and actionability landscape per tumor entity in the Hartwig Medical Database

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Hartwig Medical foundation

The Hartwig Medical Database contains whole genome sequencing (WGS) and clinical data from more than 7000 patients with metastatic cancer in the Netherlands.

Vignettes are one-page overviews of the key genomic features for each tumor type in the Hartwig Medical Database. Sections include the mutational landscape, copy number alteration profile, mutational processes, the cancer driver landscape, actionable events and germline predisposition genes. Furthermore, they offer insights on the added value of WGS over a targeted panel for finding actionable targets for a given tumor type.

https://www.hartwigmedicalfoundation.nl/en/data/vignettes/

As an exemplary use case we will highlight individual sections of the Cancer Vignette that we generated for non-small cell lung cancer (NSCLC) – an indication for which broad molecular testing (eg WGS) is recommended in the Netherlands as of July 2024. We show that for 82% of patients in the Hartwig database molecular targets were found for FDA approved drugs - while this is only the case for 45% of all patients when a targeted panel would have been applied.

The Cancer Vignettes are generated in a semi-automated manner and freely available for researchers. We have created Cancer Vignettes for more than 40 tumor entities in the Hartwig Medical Database – including relatively rare tumor entities for which whole genome characterization has been scarce. We encourage exploration by and feedback from clinical and non-bioinformatic researchers to maximize translational insights from the data.

P62: Clinical application of tumour whole genome sequencing in routine molecular diagnostics for solid cancer patients

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ABSTRACT

Molecular testing is increasingly relevant for enabling precision medicine for cancer patients. Whole genome sequencing (WGS) provides a tumour-agnostic solution for the growing complexity of DNA-based biomarker detection, with promising results demonstrated in various studies. Here, we present real-world data of 888 patients to demonstrate the clinical value of routine use of paired tumour-normal WGS-based diagnostics for solid tumours in a comprehensive cancer centre setting.

WGS was successful in 89% of cases with a median turnaround time of 6 working days. Potentially actionable biomarkers were identified in 74% of patients, including biomarkers for reimbursed and experimental targeted therapies in 30% and 64% of patients, respectively. Importantly, 38% and 24% of these patients did start biomarker-guided therapy within one year. For cancers of unknown primary (n=123), WGS aided in solving diagnosis or identified reimbursed treatment options in 68% of cases, with 70% starting a tumour type-specific treatment after WGS. Finally, clinically relevant pathogenic germline variants were identified in 6.5% of all patients.

Together, routine WGS-based diagnostics outperformed previous study results and had clinical consequences in 42% of all patients tested. WGS thus provides a versatile and future-proof test approach for supporting clinical care for patients with solid cancers.

P63: Leveraging a WGS database to build an HRD classifier for targeted NGS panels

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The homologous recombination (HR) pathway is critical for accurate DNA double strand break (DSB) repair and genetic alterations in HR-related genes such as BRCA1 and BRCA2 leads to genomic instability. HR deficiency (HRD) causes cells to heavily rely on more error-prone DNA repair pathways resulting in a distinctive mutational signature across the genome. HRD is most frequently observed in ovarian and breast cancer and is indicative for increased sensitivity to targeted therapy with poly ADP-ribose polymerase inhibitors (PARPi). Therefore, an accurate HRD classifier is essential to identify patients that might benefit from PARPi. However, it is challenging to collect a large dataset of samples sequenced with sufficient genomic coverage to develop and validate a new classifier. Here, we leveraged Hartwig Medical Foundation's WGS database to develop an HRD classifier for targeted next-generation sequencing (NGS) panel data based on 1300 samples that were HR proficient (HRP) or HRD based on the genome-wide mutational scar-based pan-cancer Classifier of HOmologous Recombination Deficiency (CHORD). In this approach, only the reads that overlap the NGS panel regions are extracted from WGS BAM files, effectively simulating targeted NGS panel data in-silico. The genetic alterations in the simulated panel samples were then visualized in circosplots and subsequently used in a convolutional neural network (CNN), which is a machine learning algorithm, to recognize patterns in HRD and HRP mutational profiles. After training and testing this image-based CNN HRD classifier called visual CHORD (vCHORD) on simulated panel data, a real world targeted NGS panel validation set of 62 formalin-fixed paraffin-embedded (FFPE) breast and ovarian tumors with matching WGS data was used to test the vCHORD classifier. In this cohort vCHORD showed 92% concordance with their WGS CHORD classification and a true positive and negative rate of 85% and 97%, respectively. This approach highlights the strength of using an existing WGS database to develop new tools and creates the potential to develop a universal HRD classifier for different targeted NGS panels, such as TSO500, that are commonly used in routine diagnostics.

P64: WiGiTS adjustments to enable universal comprehensive cancer genomics analyses for sequencing data from Illumina, Ultima and Roche platforms

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Cost is one of the limiting factors for the broad adoption of Whole Genome Sequencing (WGS) in routine diagnostics for oncology. High throughput, short read sequencing technology is therefore the most used in diagnostics and is today predominantly delivered by the Illumina Novaseq 6000 and the Illumina Novaseq X. Recently, two other platforms, Ultima UG100 and Roche SBX have emerged with the potential to lower cost at comparable quality.

Hartwig Medical Foundation has developed and maintained a comprehensive cancer genomics data analysis toolset, WiGiTS. WiGiTS consists of an integrated collection of tools for genomic and oncology. Functionality includes small variant and structural variant calling. Examples of downstream tools are CUPPA (Cancer of Unknown Primary Prediction Algorithm) and NEO (identification of neoisotopes). Here we present how we are adapting it to deliver uniform performance and output independent of the utilized sequencing platform. Differences in chemistry and measurement of these platforms and their DNA preparation lead to different quality characteristics, including error profiles and duplicate rates which can be introduced by amplification.

We are introducing Redux as a key component of our pipeline to address these issues and enable standardized downstream processing. The main purpose of Redux is to generalize duplication marking. For Illumina sequencing platforms PCR-duplicates are commonly marked by flagging reads with identical start and end positions of fragments, which is not applicable to other platforms. For example, the end of the fragment is not always reached for molecules (SBX, Ultima). Alternatively, low-quality bases and indels can cause alignment issues near the end of reads (SBX, Illumina & Ultima) leading to differences in positions. Redux handles these cases by with platform-specific routines. In addition, Redux natively supports Unique Molecular Identifiers (UMIs) and amplification-free methods.

In addition to adaptations in redux, some features in other components were implemented to correctly handle each different platform. An illustrative example is q-score recalibration, performed by the variant caller SAGE. The q-scores assigned to each base by the different platforms are not directly comparable and not always accurate since they do not account for sequence context and DNA damage that might have occurred prior to sequencing. The included variant caller, SAGE, performs a quality recalibration that is based on empirical error rates in data for each sample. These empirical qualities can be higher or lower than the sequencer-provided quality. It also incorporates two platform-specific features in the variant calling – whether a read is simplex or duplex (SBX) and the indel-specific quality score (Ultima).

We have compared variant calls between Illumina and SBX and Illumina and Ultima and find clinically equivalent results for small somatic variants. We are continuing work on structural variants and copy numbers with initial results looking promising. Our pipeline is open source and actively maintained. Tools are best run as a pipeline to utilize the integrations between tools. To facilitate this a Nextflow implementation, named Oncoanalyser is available on nf-core.

Relevant tools

WiGiTS: https://github.com/hartwigmedical/hmftools

Oncoanalyser nf-core: https://nf-co.re/oncoanalyser/

P65: Implementation of a tumor-informed WGS-based ctDNA test for monitoring tumor burden on Ultima UG100

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The use of liquid biopsies for cancer disease monitoring is increasingly being explored in clinical research, with numerous ongoing trials evaluating its potential for widespread clinical implementation. Detection of cancer through a non-invasive blood draw, or liquid biopsy, allows the evaluation of novel aspects of disease progression, which are not captured in current tissue-based TNM classifications. Using these novel biomarkers for clinical decision-making has been proven to allow de-escalation of systemic chemotherapy when possible and escalation when necessary for colorectal cancers and diffuse large B-cell lymphomas.

Cells in the body, including cancer cells, release cell-free DNA (cfDNA) and can be obtained during a liquid biopsy. Tumor-derived cfDNA or circulating tumor DNA (ctDNA) is a small fraction of the total cfDNA, distinguished by specific genomic alterations such as small variants, mutations, and structural changes that reflect the genetic makeup of the tumor.

Here we show our implementation of a whole-genome sequencing (WGS) test for detecting ctDNA. This test uses a tumor-informed approach, where WGS data from tissue biopsies are used to guide ctDNA detection. Genome-wide tumor-specific mutations identified in tissue samples are utilized to accurately determine the tumor DNA fraction in plasma samples with high sensitivity and specificity.

Our methodology utilizes the unique paired plus-minus sequencing (ppmSeq) technology to detect tumor-derived alterations using the UG100 sequencer by Ultima Genomics. This duplex sequencing method is PCR-free, does not come with additional costs for library preparation or sequencing depth and results in very high-quality single nucleotide variant (SNV) calls.

To estimate the tumor fraction in cfDNA, we developed WGS-Informed Sample Purity (WISP), a tool that calculates the tumor fraction by comparing genomic alterations found in the tumor tissue DNA versus the cfDNA. These genomic alterations include the detection of single nucleotide variants (SNVs), copy number aberrations (CNAs) and loss of heterozygosity (LOH). WISP corrects for non-tumor-derived cfDNA, thus refining tumor burden estimates and improving the precision of ctDNA measurements.

As part of our ongoing research, we are setting up the CTD-SCAN study (ctDNA scanning for monitoring tumor burden), designed to assess the clinical utility of this WGS-based ctDNA test in monitoring tumor burden across various tissue types. The study, focusing on lung, breast and colorectal cancer, involves longitudinal ctDNA sampling to monitor ctDNA dynamics during and after treatment. This study aims to assess the clinical utility of WGS-based ctDNA detection for monitoring tumor burden and evaluate its performance compared to current standard-of-care methods, such as medical imaging. By integrating tumor-informed sequencing, advanced ctDNA analysis technologies, and tumor fraction estimation, we aim to provide a more accurate and clinically relevant tool for the management of cancer patients. A tumor-informed approach enhances detection sensitivity and specificity, potentially improving the test's utility. The CTD-SCAN study will evaluate the clinical impact of WGS-based ctDNA monitoring in real-world settings and determine its broader applicability in routine clinical practice.

P67: WiGiTS: a universal suite of open-source tools for decoding genomics data for cancer precision medicine research and diagnostics

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Genomic analyses are central to cancer diagnostics and research. Experimental techniques are gradually moving from targeted gene panels and exome sequencing to complete whole genome sequencing with the goal of maximizing information captured on a patient's tumor. Over the past decades, many, mostly single purpose, computational analytical tools have been developed for converting sequencing data into clinically and biologically relevant information. However, with maturation of the field, there is a growing need for comprehensive cancer genome data analysis solutions suitable for both genomics research and routine diagnostics. Here, we present WiGiTS, a suite of state-of-the-art actively maintained open source bioinformatic tools that is extensively validated for diagnostic and research purposes. WiGiTS supports comprehensive calling and extensive annotation of variants of all types from large panel, exome and whole genome sequencing data of tumor only and tumor-normal paired samples. Output includes advanced biomarkers and tumor characteristics like microsatellite instability, homologous recombination deficiency, HLA typing, tissue of origin prediction, viral presence and pharmacogenetic analyses. When available, RNA-seq data is integrated in the analyses and annotation. WiGiTS is vendor-agnostic and supports processing of data from multiple platforms (Illumina, Ultima) on different reference genome builds (hg37 and hg38). WiGiTS is available as a Nextflow pipeline at nf-core, named OncoAnalyser, allowing for portable multi-platform implementation, including public clouds and private compute clusters. Taken together, WiGiTS enables rapid and cost-efficient processing of diverse and large genomics datasets to enable decoding cancer genomes in research and diagnostic settings.

P68: CUPPA3.0: A machine-learning classifier for predicting tumor of origin for Cancer of Unknown Primary (CUPs)

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Cancer of unknown primary (CUP) affects an estimated 3-5% of all patients diagnosed with metastatic cancer. Prognosis for these patients is poor, partially because of the typically long diagnostic odyssey and lack of registered treatments. It has been shown that Whole Genome Sequencing (WGS) can assist in a (more) detailed classification and diagnosis of tumor types. [Nat Rev Clin Oncol 8, 701–710 (2011), Nat Commun 13, 4013 (2022)]

We have previously demonstrated the added value of using WGS for simultaneously determining the tissue of origin and finding potentially actionable mutations for targeted treatments by a WGS-based 'Cancer of Unknown Primary Prediction Algorithm' (CUPPA). The algorithm is based on previously described principles and developed and validated using a large pan-cancer WGS database of metastatic cancer patients (Hartwig Medical Database). [ESMO Open. 2022 Dec;7(6):100611] In the Netherlands, WGS is now part of standard of care procedures for patients with CUPs.

Here, we present further improvement efforts on CUPPA – implemented or to be implemented over the course of next years. We will describe our approach to obtain structured information on tumor location and histology for all samples in the Hartwig Medical Database (currently over 7000) to improve the training data for the CUPPA algorithm. Furthermore, we will capitalize on large datasets generated internationally (i.e. Master DKFZ and genomics England) to expand the training set with clinically relevant but rare tumor entities. Furthermore, we will explore novel visualizations that maximize insights from the algorithm for clinicians. Finally, we will explore hierarchical models for main types and subtypes.

By implementing these changes, we expect increased diagnostic yields and higher accuracy of the algorithm for prediction of tumor type. In addition, we expect improved utility for differential diagnosis for hard to diagnose tumor (sub)types (e.g. sarcoma's).