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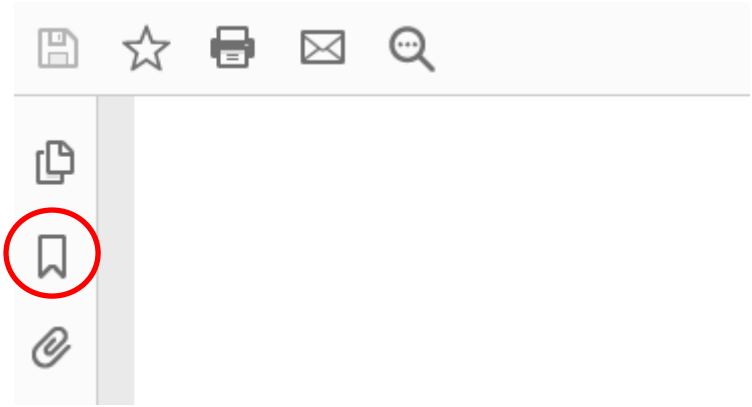

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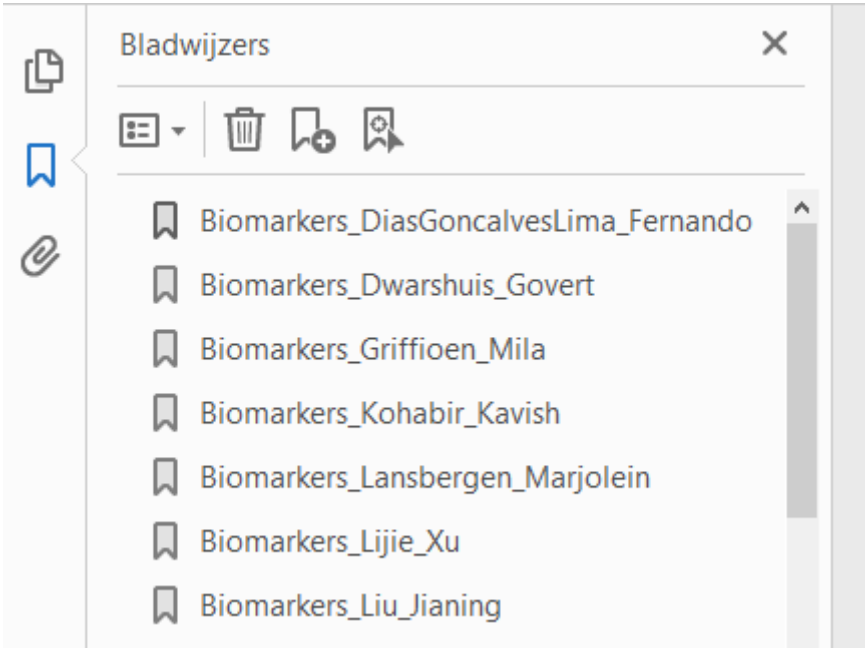

Biomarkers

Biomarkers of the Tumor Micro-Environment for Management of Anal Pre-Cancers

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Anal high-grade squamous intraepithelial lesions (HSIL) are the precursor of anal cancer. They present a dichotomy in their risk of progression to malignancy, categorized into low-risk HSIL, with minimal progression risk, and advanced HSIL, with a high risk. Currently, all HSIL is treated, which results in overtreatment of low-risk HSIL, imposing burdensome consequences on patients and the health care system. This underscores the urgent need for tailored therapeutic approaches guided by objective biomarkers.

This study aims to dissect the tumor immune micro-environment (TIME) differences between low-risk and advanced HSIL. We performed a comparative analysis through multiplex imaging mass cytometry (CYTOF), examining TIME characteristics in 9 HSIL samples that progressed to cancer, 10 that showed spontaneous regression, 4 anal cancer samples, and 1 normal tissue sample, using a targeted 40-marker panel. This investigation identified four TIME biomarkers, which are currently undergoing validation in a larger, cross-sectional series of 60 HSIL samples using multispectral fluorescence microscopy (VECTRA). These samples are stratified into low-risk and advanced HSIL, using methylation marker analysis as a surrogate for cancer risk stratification.

In conclusion, our preliminary data indicate that variations in TIME across low-risk and advanced HSIL subclasses. TIME biomarkers that could improve HSIL management strategies, reducing overtreatment and enhancing patient outcomes.

Biomarkers

Glioblastoma therapy monitoring using platelet mRNA

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Liquid biopsies are a promising approach to monitor disease activity for difficult-to-reach-cancers, including brain cancers. Glioblastoma is the most frequently diagnosed and most lethal of malignant primary brain tumors. Patients are typically treated with tumor resection followed by chemotherapy and radiotherapy. During the course of adjuvant treatment, up to two-thirds of the patients with a glioblastoma may face a form of pseudoprogression and/or radionecrosis. Both conditions are a consequence of the tumor treatment, imply local inflammatory processes, and are marked by contrast enhancement at MR-imaging. Clinically, the patient's condition may decline. As this phenomenon can mimic true progression, it frequently results in prognostic uncertainty for physicians and patients. Routinely obtaining tissue biopsies from intracranial tumors is not desired and entails significant patient risks.
Platelets have been known to interact with tumors in multiple ways, shielding them from the immune system, affecting the tumor microenvironment and facilitating metastasis. They sequester mRNA from various sources, including extracellular vesicles. Furthermore, the RNA in the platelet is subject to alternative splicing upon external stimuli, offering opportunities for detecting tumor-specific changes through the platelet transcriptome and enabling treatment monitoring. As the lifespan of platelets is roughly 10 days, they carry an up-to-date snapshot of tumor status.
In the current study, we investigate whether we can use this platelet RNA profile to improve the distinction between true progression on the one hand, and pseudoprogression/radionecrosis on the other hand.

Biomarkers

Dynamics of methylated DNA in the urine of cervical (pre)cancer patients

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Cervical cancer arises from a persistent infection with human papillomavirus (HPV) and typically develops following cervical intraepithelial neoplasia (CIN, graded 1-3). Existing screening programs utilize HPV testing on cervical scrapings and self-swabs. However, to address the challenge of low participation rates, there is a pressing need for novel, less invasive screening methods. Detection of DNA methylation markers, such as ASCL and LHX8, in urine has emerged as a promising approach for identifying CIN3+ lesions.

This study aims to investigate the dynamics of DNA methylation markers in cell-free DNA present in urine samples. A cohort consisting of 25 cervical cancer patients, 25 individuals with CIN2/3 and 25 healthy controls will be recruited to provide six urine samples over the course of three consecutive days, collected both in the morning and evening. First findings indicate that DNA methylation levels exhibit an increasing trend with disease severity. Notably, cervical cancer patients consistently show elevated DNA methylation levels, whereas healthy controls exhibit lower levels. In contrast, individuals with CIN2 and CIN3 exhibit varying DNA methylation levels across different collection time points, potentially impacting sensitivity. This investigation will enhance our understanding of the dynamics of DNA methylation markers in the urine of cervical (pre)cancer patients, improving novel screening strategies.

Biomarkers

ARTEMIS: crRNA Engineering Sharpens Cas12a's Diagnostic Arrow to the Right Target with Single-Nucleotide Precision

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Accurate detection of pathogenic SNPs is vital in cancer diagnostics and treatment monitoring. While CRISPR-based diagnostics (CRISPRdx) offer promising avenues for cost-effective, rapid, and point-of-care testing, achieving single-nucleotide fidelity remains challenging. We scanned the human genome to tARget paThogEnic Mutations In the Seed region (ARTEMIS), the most stringent crRNA domain, identifying over 600 targetable cancer-associated SNPs, including BRAF p.V600E, BRCA2 p.E1953* , TP53 p.V272M and ALDH2 p.E504K. Cas12a exhibited substantial tolerance to single mismatches within the seed region. We found that introducing deliberate synthetic mismatches within the seed region significantly enhanced on-target activity with single-nucleotide fidelity. Notably, both the position and nucleobase type of mismatches influenced the detection accuracy. With improved specificity, our Cas12a assay could accurately detect and even semi-quantify BRAF p.V600E in cfDNA from cell lines and patient liquid biopsies. These results provide insights into more rationalized crRNA design for high-fidelity CRISPRdx of clinically relevant SNPs, which support the road towards tailored, personalized and cost-efficient healthcare solutions in oncologic diagnostics.

Biomarkers

Integrated genomic and transcriptomic analysis to predict FOLFIRINOX response in metastatic pancreatic ductal adenocarcinoma

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Background: Predictive markers for FOLFIRINOX response in metastatic pancreatic ductal adenocarcinoma (mPDAC) patients are urgently needed. Here, we aimed to validate the benefit of whole genome sequencing (WGS) in predicting treatment outcome in a Dutch mPDAC FOLFIRINOX cohort.

Methods: 108 mPDAC patients started FOLFIRINOX and had successful WGS of their pretreatment biopsies. 51 mPDAC samples of non-FOLFIRINOX treated patients were also included in the cohort in order to explore the relation between transcriptome-based subtype and somatic mutational status with more power.

Results: 88 percent of the included samples had a KRAS mutation and 82 percent had mutated TP53. The KRAS wild-type patients showed MYC amplifications, gene fusions, and mutations in MMR proteins. Patients with a KRAS wild-type tumor had a better survival following FOLFIRINOX treatment ($p = 0.009$) compared to KRAS mutated tumors. As described before, transcriptome-based classification according to Moffitt revealed a cluster of tumors with a “Moffitt-negative” subtype, showing low expression of classical and basal-like signature genes. We found that KRAS wild-type tumors were enriched for this Moffitt negative subtype ($p < 0.0001$), while they did not have lower tumor cellularity. The gene expression of these Moffitt negative tumors correlated with neural-like and neuroendocrine gene sets.

Conclusions: We found that KRAS wild-type tumors do not fit in the classical – basal-like classification schemes. Both KRAS status and transcriptome-based subgroup were prognostic for FOLFIRINOX treatment response and should therefore be validated for incorporation in diagnostic practice.

Biomarkers

Optimizing Urine Proteomics for Cancer Biomarker Discovery

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Proteomics based on unbiased mass spectrometry is optimally suited to bridge the gap between genomic information and biological functions and disease phenotypes, as it studies the expression and/or post-translational modification of proteins, the major cellular players. Despite major improvements in knowledge and clinical management, cancer is still a deadly disease. Novel biomarkers for non-invasive and early cancer diagnosis and prognosis are urgently needed.
This Ph.D. project focuses on proteomics strategies that can pinpoint novel non-invasive cancer protein biomarker signatures in biofluids including urinary extracellular vesicles. More specifically, current projects include 1. to identify noninvasive protein biomarkers and signatures present in the urinary extracellular vesicles of both proximal and distant cancers; 2. to determine the optimal urine fraction (i.e., full urine, supernatant, pellet, and urine EVs) for cancer biomarker discovery;3. to compare and optimize the in-house urine EV extraction method (VN96 peptides) with two novel techniques (i.e., TiO2, and SAX beads).
First, we focus on identifying noninvasive protein biomarkers and signatures present in the extracellular vesicles of both proximal and distant cancers. Through mass spectrometry-based proteomics, we analyze urinary EVs from proximal (prostate and bladder) and distant (colorectal, cervical, lung) cancer types (n=60), alongside healthy controls (n=13) in a discovery cohort. Subsequently, we validate our findings using urine samples from individuals with lung cancer (n=20) and healthy controls (n=20). Our study underscores the potential of urinary EVs as a rich source for the noninvasive discovery of cancer-specific biomarkers and signatures across various cancer types.

Biomarkers

Different methods of plasma proteomics

Jianing Liu[1]

[1] CCA

Plasma proteomics, the systematic study of proteins in blood plasma, offers a comprehensive view of the dynamic interplay between proteins and physiological processes in health and disease. And a wealth of information for understanding disease mechanisms, identifying biomarkers, and guiding therapeutic interventions. This review provides an overview of recent advancements and applications in plasma proteomic analysis, highlighting its significance in understanding disease mechanisms and identifying potential biomarkers for diagnosis, prognosis, and therapeutic monitoring. We discuss the methodologies employed in plasma proteomics, including mass spectrometry-based techniques, emphasizing their strengths and limitations, and enable high-throughput profiling of protein expression and modifications. Additionally, we explore the challenges associated with plasma proteomic research, such as sample preparation variability, data analysis strategies, and the need for robust validation of biomarker candidates. Future directions in plasma proteomic research, such as the integration of multi-omics data and the development of novel computational tools, hold promise for further advancing our understanding of disease biology and improving patient care. Overall, plasma proteomics holds immense promise in revolutionizing personalized medicine by facilitating precision diagnostics and therapeutics tailored to individual patient profiles.

Biomarkers

Limit of Quantification as an objective cutoff value for flow cytometry-based measurable residual disease assessment in acute myeloid leukemia

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Background
Measurable Residual Disease (MRD) after two cycles of intensive chemotherapy, assessed by Multi-Parametric Flow Cytometry (MFC), is a prognostic indicator in Acute Myeloid Leukemia (AML).
Based on the European Leukemia Network (ELN) expert group's recommendations, patients are considered MRD-positive when more than 0.1% of white blood cells (WBC) have a leukemic phenotype. However, about a third of MRD-negative patients experience a relapse. To reduce these false-negative results an alternative cutoff has been proposed based on Limit of Quantification (LOQ).

Aim
To explore the prognostic relevance of LOQ as an MRD cutoff in our international HOVON-SAKK trials.

Methods
We retrospectively examined MRD data from 873 AML patients. The LOQ was defined at 50 Events (smallest quantifiable cluster of cells) for samples with more than 500.000 WBC.

Results
Patients classified as LOQ-negative (26%), LOQ-positive/MRD-negative (57%), and MRD-positive (17%) had an overall survival (OS) of 63.2%, 60.3% and 44.1% and an event-free survival (EFS) of 55.1%, 50.2% and 36.5% after 5 years, respectively.
In univariable OS analysis being LOQ-positive/MRD-negative had a hazard ratio (HR) of 1.19 [95% confidence interval] [0.92-1.53] and MRD positive of 1.87 [1.39-2.54] compared to LOQ-negative.
After multivariable adjustment including ELN risk group, time to complete

remission and consolidation type, both subgroups were independently prognostic: HR = 1.40 [1.07-1.84] and 1.80 [1.30-2.49], respectively.

Discussion

A subset of MRD-negative patients with worse OS can be defined using LOQ in a multivariable model. Although this threshold below 0.1% seems to have potential for prognostic relevance, the clinical relevance warrants further investigation.

Biomarkers

Enhancing Cancer Detection: Machine Learning Insights from Fragmentation Patterns of Plasma Cell-free DNA

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Cell-free DNA (cfDNA) consists of DNA fragments released in the bloodstream following cell death. cfDNA analysis presents a patient-friendly alternative in oncology, particularly for cancer detection. The fragmentation patterns encoded in cfDNA molecules offer a valuable resource that can be harnessed through machine learning methods. However, despite the potential for early cancer detection and intervention, existing methods suffer from limited sensitivity and flexibility. We aim to enhance cancer detection sensitivity through machine learning classifiers by gaining insights into the characteristics of cfDNA molecules.

Our study focuses on developing machine learning classifiers using cfDNA fragment-end patterns. These patterns serve as distinctive genomic signatures, distinguishing cancer from control samples. We specifically extract these patterns from open-chromatin regions across both cancer and control samples to construct a dataset. This dataset enables training classification models to predict cancer status of an unknown sample based on its observed fragment-end patterns.

In our initial analysis, we examined a cohort of 28 B-cell lymphoma and seven control samples employing logistic regression, achieving 100% specificity and 85.7% sensitivity. To broaden the scope of our research, we intend to increase the scale of our cohort, incorporating a broader range of cancer types, including brain cancers.

Our preliminary results underscore the potential of our method for enhancing cancer diagnosis and uncovering cancer-specific DNA regions, which may indicate tumor-derived signals. The development of a robust diagnostic classifier based on cfDNA analysis could have significant implications for early detection of cancer and intervention, leading to improved patient outcomes and reduced healthcare costs.

Biomarkers

Early detection of oral cancer and premalignant lesions with a non-invasive genetic assay

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Background and aim: Oral squamous cell carcinoma arises from the oral cavity epithelium. Early stage tumors are locally excised and the prognosis is favorable. However, most patients present with advanced stage of disease and despite highly invasive resections with reconstruction and postoperative chemoradiotherapy, 5-year survival remains a disappointingly 50%. Hence early diagnosis is key. Oral cancer is preceded by precancerous changes in the mucosal lining, most of which harbor cancer-associated mutations. These precancerous changes are sometimes visible as lesions, such as oral leukoplakia, but mostly remain invisible to the human eye. To detect early malignant conversion of the precancerous fields, an alternative method is required since biopsies cannot be performed when the precancerous changes are not visible. This project aims to develop and evaluate non-invasive methods for early oral (pre)cancer detection and monitoring.

Methods: Oral rinses and brush samples from oral leukoplakia patients are collected at baseline and longitudinally. Genetic alterations are identified by capture-based sequencing on corresponding biopsy samples. Sensitivity of a newly developed genetic assay will be evaluated using oral rinses and brushes obtained at baseline. Value for monitoring will be determined from longitudinal samples.

Results: We will expand on our previous findings that non-invasive samples accurately detect oral (pre)cancer. This will be accompanied by data from an alternative genetic assay.

Discussion: Initial experiments were performed on a small sample set without longitudinal follow-up sampling, and exclusively using brush samples. This research will explore the potential of oral rinses for more comprehensive and convenient oral cavity surveillance.

Biomarkers

Computational measurable residual disease assessment in acute myeloid leukemia using mixture models

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Measurable residual disease (MRD) assessed by multiparameter flow cytometry (MFC) is an important prognostic factor for both overall survival and risk of relapse after chemotherapy in acute myeloid leukemia (AML). Current practice focuses on manually identifying CD45dim blasts expressing a leukemia-associated immunophenotype (LAIP). Computational identification of these cells could remove the manual analysis's complicated and labor-intensive aspects.

Here, we evaluated the possibility of computational AML-MRD assessment in single samples using Gaussian mixture models (GMMs). First, healthy and leukemic CD45dim blasts expressing the primitive markers CD34 or CD117 are identified using a GMM-based classifier. Next, predicted blasts are compared against a GMM-based reference model trained on blasts from disease-free regenerating bone marrow. In this comparison, the aberrancy of individual cells was quantified by leveraging the statistical foundation of the GMM. An aberrancy cut-off allowed for extracting a leukemic cell count in each sample, which was used to obtain a computational MRD percentage in combination with a predicted white-blood cell count as a denominator.

Using the model, we were able to correctly classify MRD positivity in 34 out of 35 AML follow-up samples (97%), with one false negative. Lastly, the model allowed for the detection of several LAIPs unreported in expert gating, illustrating the usefulness of computer-assisted MRD analysis. Our results demonstrate a proof-of-principle for combining automated blast detection with a reference-based approach for fully automated MRD assessment in AML. The reported approach uses little training data, limiting human bias, facilitating applicability across clinical centers and the transition to novel cytometry platforms.

Biomarkers

Urinary miRNAs as non-invasive biomarkers for cervical cancer detection: a feasibility study

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Introduction: Cervical cancer (CC) is the 4th leading cause of women's mortality worldwide, resulting in more than 300,000 deaths annually. The disease dysregulates a variety of cellular pathways that correlate with disease progression and cancer development, including RNA pathways. The use of RNA biomarkers therefore represents a promising strategy to timely detect CC and support the current screening and triage programs. MicroRNAs (miRNAs) are small RNA molecules that regulate gene expression and associate with tumor development. These molecules are released into bodily fluids, such as urine. This study aimed to assess if urinary miRNAs might be valuable non-invasive biomarkers for CC detection. Methods: We optimized the extraction and profiling of urinary miRNAs by testing four (mi)RNA isolation kits and performing digital PCR analyses in 7 urine samples from healthy controls and their urine fractions (whole urine, supernatant, and sediment). We also performed miRNA-specific sequencing on a small series of urines from women with CC and healthy controls to explore the potential of urinary biomarker discovery by IsoSeek. Results: Here we show that the miRNeasy serum/plasma and Norgen (mi)RNA isolation kits performed best for isolating miRNAs in urine sediment and whole urine/supernatant, respectively. Urine from women with CC showed a higher expression of let-7 miRNAs when compared to healthy controls. Conclusion: We describe a protocol for miRNA analysis and biomarker discovery in urine, which will be valuable for research on CC and overall women health. Our results suggest that specific urinary miRNAs may be useful biomarkers for CC.

Biomarkers

CD34+CD38- Leukemia Stem Cells are Associated with Prognosis in Non-Intensively Treated Acute Myeloid Leukemia

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Background:
Acute myeloid leukemia (AML) patients unfit for intensive therapy receive non-intensive treatment with hypomethylating agents (HMA). No prognostic factors have been identified yet.

Aim:
To assess the prognostic value of leukemia stem cell (LSC) load in unfit AML patients at diagnosis and after three cycles of HMA.

Methods:
In bone marrow samples of patients treated in the HOVON-SAKK 135 trial, we applied a one-tube flow cytometry LSC assay to determine the LSC load, immunophenotypically characterized as CD45dimCD34+CD38- and LSC marker positive cells.

Results:
Of the 144 patients included in the trial, LSC were measured in 121 patients at diagnosis and 49 after cycle 3 of which 38 were responsive to therapy; 57 patients did not reach cycle 3 and of 38 patients we did not have (enough) material to perform the assay. The optimal cut-off values (maximally ranked statistics) for LSCpositivity were 0.01% at diagnosis and 0.001% after cycle 3. Patients who were LSCpositive at diagnosis had significantly worse OS (hazard ratio (HR) (95% CI): 2.0 (1.3-3.3), p = 0.002) and EFS (HR (95% CI): 2.0 (1.3-3.1) p = 0.002), when adjusting for age, sex, and WBC count. After cycle 3, the prognostic effect of LSC was larger for both OS (HR (95% CI): 2.9 (1.3-6.5); p = 0.02) and relapse-free survival (RFS; HR

(95% CI): 5.1 (1.9-13.5) $p < 0.0001$), when adjusting for age, sex and WBC at diagnosis.

Conclusion:
LSC assessment was significantly associated with prognosis in non-intensively HMA-treated patients, and clinical implications will be further investigated.

Biomarkers

Methylation and copy number profiling of HPV+ high-grade cervical intraepithelial neoplasia to predict clinical outcome

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Background. Human papillomavirus (HPV)-induced high-grade cervical intraepithelial neoplasia (CIN2/3) is the direct precursor of cervical cancer. While treatment of these lesions is a measure of cervical cancer prevention, a relevant percentage of CIN2/3 can spontaneously regress, resulting in overtreatment. Insight into the determinants of progression to cancer can help reduce overtreatment. Accordingly, methylation of genes FAM19A4 and miR124-2, and copy number aberrations (CNAs) are recognized as cancer-driving alterations in cervical cancer. Here, we aimed to evaluate the prognostic role of FAM19A4/miR124-2 methylation and copy number profiles in clinical regression and progression of CIN2/3.

Methods. Tissue biopsies were collected at baseline from 114 women with CIN2/3, followed for 24 months with a wait-and-see policy to determine regression or progression of their lesions (Kremer et al., JCO 2022). Seventy-seven (77) and 33 biopsies were selected for, respectively, FAM19A4/miR124-2 methylation analysis via Methylation-Specific qPCR (qMSP) and copy number analysis using mFAST-SeqS.

Results. Women with a FAM19A4/miR124-2 methylation-negative biopsy showed higher CIN2/3 regression rates (81.9%) compared to women with a methylation-positive biopsy (41.3%, P<.005). Methylation levels in tissue biopsies positively correlated to those previously determined in cervical scrapes. Preliminary data on copy number profiles showed few chromosomal alterations in progressive lesions, including gains of chr3q and losses of chr2q.

Conclusion. FAM19A4/miR124-2 methylation testing as a prognostic tool for CIN2/3 clinical regression can be efficiently applied to tissue biopsies as well as cervical scrapes, which are reliable representatives of CIN2/3 lesions. Further characterization of CIN2/3 copy number profiles could provide insight in malignant progression.

Biomarkers

DNA methylation marker and HPV analysis in the follow-up of cervical cancer

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Introduction: This study aims to explore the clinical performance of DNA methylation markers and high-risk human papillomavirus (HPV) in urine and cervicovaginal self-samples for the detection of recurrent cervical cancer.

Methods: Cervical cancer patients (n=47) collected urine and cervicovaginal self-samples pre- and post-treatment. Additionally, 20 cervical cancer patients collected urine and cervicovaginal self-samples at time of recurrence. All samples were collected at home and tested for DNA methylation and for HPV. DNA methylation levels were compared by the Wilcoxon rank-sum test and the paired Wilcoxon signed-rank test. Methylation positivity and HPV presence were compared between patients with and without recurrence by Chi-squared tests.

Results: In patients with no recurrent cervical cancer, methylation levels decreased post-treatment compared to pre-treatment in both sample types ($p<0.0001$). Methylation positivity in cervicovaginal self-samples was higher for patients with recurrence (78%) than for patients without recurrence (25%) ($p=0.0004$). Also in urine, methylation positivity was higher for patients with recurrence (65%) compared to patients without recurrence (33%) ($p=0.038$). Similarly, HPV positivity in both cervicovaginal self-samples and urine was more frequent (53% and 55%, respectively) in patients with recurrence compared to patients without recurrence (15% and 9%, respectively) ($p=0.004$ and $p=0.0001$).

Conclusion: This study shows the potential of post-treatment monitoring of cervical cancer patients by DNA methylation and HPV testing in at home collected cervicovaginal and urine samples. The highest recurrence detection rate was achieved using DNA methylation in cervicovaginal self-samples.

Biomarkers

Functional assessments including sarcopenia better detect frailty than the IMWG frailty score and improve prediction of outcome in multiple myeloma

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The International Myeloma Working Group frailty index (IMWG-FI) is the current gold standard to assess frailty in non-transplant eligible (NTE) patients with multiple myeloma (MM). However, it is unknown whether the IMWG-FI reflects functional impairments. This study compared the prevalence of geriatric impairments and sarcopenia in intermediate-fit and frail patients and determined its prognostic value.

The HOVON 123 study was a prospective phase II trial, in which patients aged ≥75 years with newly diagnosed MM were treated with nine cycles of melphalan-prednisone-bortezomib (MPV). Of the 214 included patients, 71 were intermediate-fit and 143 were frail. Compared to the intermediate-fit, frail patients had higher rates of cognitive dysfunction (MMSE <24 15% vs 27%, p=0.052), depression (GDS >6 13% vs 35%, p<0.001), malnourishment (MNA <8 7% vs 20%, p<0.001), and low physical functioning (QLQ-C30 <47 15% vs 45%, p<0.001). Frail patients more often had (pre)sarcopenia (13% vs 31%, p=0.011), low gait speed (<0.8m/s in 29% vs 55%, p<0.001) and low grip strength (27% vs 47%, p=0.009).

Comparing predictive values, the combination of depression and ISS-stage outperformed the IMWG-FI in predicting progression-free survival (PFS). For overall survival (OS), the combination of WHO status, ISS and gait speed had superior predictive value over the IMWG-FI.

This is the first study to demonstrate that the IMWG-FI indeed detects functional frailty in NTE-NDMM patients. Geriatric assessments better predicted PFS and OS compared to the IMWG-FI, supporting their incorporation to determine the level of frailty in older patients with multiple myeloma.

Biomarkers

NanoCMSer: A Clinically Applicable Tool for Stratification of Colorectal Cancer Samples

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Colorectal cancer (CRC) is a significant contributor to global cancer-related mortality, emphasizing the critical need for advanced predictive biomarkers to guide treatment decisions. In our pursuit of identifying novel biomarkers, as part of an international consortium, we have previously categorized CRCs into four consensus molecular subtypes (CMS1-4). These subtypes show promise for predicting outcomes and facilitating biological investigations. To streamline the integration of CMS classification into routine clinical practice, where routinely formalin-fixed paraffin-embedded (FFPE) samples are used, we developed NanoCMSer. This NanoString-based CMS classifier reliably stratifies both fresh-frozen (FF) and FFPE samples using a concise panel of just 55 genes. Employing domain adaptation methods enhanced the generalizability of the classifier. For FF NanoString-based datasets, an accuracy of 95% and for FFPE NanoString-based datasets an accuracy of 92% was achieved, which is the highest reported for FFPE tissues. Furthermore, a 96% accuracy across a comprehensive collection of 1,976 RNAseq-based samples from 23 datasets was determined.

Additionally, our findings highlight that even with a limited number of genes, the biological relevance of predictions at the individual sample level can be still identified, with distinct CMS biology readily recognizable upon conducting enrichment analysis. Furthermore, our observations revealed a substantial decline in 5-year recurrence free survival (RFS) for CMS2 (21%) and CMS3 (31%) patients in stage III vs II, while this decline is less pronounced for CMS1 (11%) and CMS4 (9%). We posit NanoCMSer as a robust tool with potential applications in both tumor biology and clinical practice, accessible through the NanoCMSer R package.

Biomarkers

Disease burden in high-grade vulvar intraepithelial neoplasia

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Objectives: To assess disease burden in high-grade vulvar intraepithelial neoplasia (HG-VIN) patients.
Methods: From a population-based study on HG-VIN, 578 HSIL patients and 46 dVIN patients were selected. Disease burden was assessed using pathology data from the Dutch Nationwide Pathology Databank (Palga) as well as clinical data from the medical records. Kaplan-Meier analysis was used to estimate the recurrence risk. In addition, duration of disease and number of invasive procedures were determined. Follow-up time was censored at time of vulvar cancer development.
Results: The median follow-up time was 15.4 years in HSIL and 5.5 years in dVIN ($p<0.001$), with recurrence and/or cancer in 297/578 (51.4%) and in 30/46 (65.2%), respectively. Cancer developed in 62/578 (10.7%) HSIL patients and 25/46 (54.3%) dVIN patients. The 2-year recurrence risk was 25.3% in HSIL and 45.7% in dVIN ($p=0.014$), with a median time to recurrence of 1.8 years and 1.2 years, respectively ($p=0.024$). The median time between the first and last HG-VIN diagnosis was 7.8 years in HSIL and 3.1 years in dVIN ($p=0.004$). HSIL patients had a higher number of invasive interventions, with a median of 3 compared to 2 in dVIN ($p=0.024$). Clinical data was collected of 314/624 (50.3%) patients and further analyses on risk factors for disease burden are currently ongoing.
Conclusions: dVIN patients experience a high disease burden due to the high risk of recurrence and cancer. Although the cancer risk is relatively limited, HSIL patients experience a significant disease burden characterized by multiple interventions over a prolonged period.

Biomarkers

Cell-Free DNA Genomic and Fragmentomic Features for Early Outcome Prediction in Large B-Cell Lymphoma

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and early risk stratification strategy as prompt therapy escalation may improve survival.

Methods: We evaluated cell free DNA (cfDNA) genomic and fragmentomic features from 188 DLBCL patients enrolled in the HOVON-902 and HOVON-152 across > 40 hospitals in the Benelux using Whole Genome Sequencing (WGS). Then combining all cfDNA-derived features after one treatment cycle (T1), we trained a Random Forest model to create the ACT (Aberration, Contribution of short fragments, and fragmenT) score, and validated it through leave-one-out cross-validation (LOOCV). Finally, we evaluated the performance of ACT score for predicting end-of-treatment (EOT) response and survival.

Results: Individual cfDNA features and ACT score were altered in non-responders compared to responders at T1. The ACT score at T1 outperformed all individual cfDNA features to predict EOT response (AUC = 0.76). Patients with a positive ACT score had inferior outcomes compared to ACT score negative patients [progression-free survival (PFS): HR 4.5, 95% CI 2.8-7.2, log-rank test, $p < 0.0001$) and overall survival (OS): HR 6.8, 95% CI 3.6-12.6, log-rank test, $p < 0.001$]. The 2-

year PFS in ACT score positive and negative patients was 32.7% and 74.6%, respectively. The prognostic value of the ACT score is independent of the IPI and interim imaging.

Conclusion: The ACT score, derived from a single blood sample collected after one cycle of treatment can predict clinical outcomes. This low-cost and easy-to-interpret test does not require tissue biopsies or a priori knowledge of mutations, and have the potential to guide interventional clinical trials and risk-adapted treatment strategies.

Biomarkers

Standardizing TILs and PD-L1 Assessment in gastroesophageal adenocarcinoma: Reducing interpathologist variability (STAR)

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With the rise of novel immunotherapy regimens for gastroesophageal adenocarcinoma (GEA), the need for accurate biomarker evaluation and patient stratification methods becomes more apparent. The histological interpretation of prognostic biomarkers like tumor infiltrating lymphocytes (TILs) and programmed death ligand 1 (PD-L1) is characterized by high interobserver variability. The frequent occurrence of resident inflammation in mucosal-based tumors poses a significant challenge for quantifying TILs and PD-L1, particularly in the absence of standardized guidelines. This study aims to develop tailored decision-support tools (DSTs) for TILs and PD-L1 assessment and investigates their potential to reduce interpathologist variability. Digitized whole slide images of 83 pretreatment GEA biopsies stained for hematoxylin and eosin (H&E) and PD-L1 were collected. DSTs incorporating visual reference feedback were developed using the Slide Score platform. TILs will be scored according to modified International Immuno-oncology Working Group guidelines, while PD-L1 expression will be quantified using the Combined Positive Score (CPS). Fifteen gastrointestinal pathologists will evaluate TILs% and PD-L1 CPS in each slide with and without DSTs, with a four-week washout period between assessments. The intra-class correlation coefficient (ICC) will be computed to analyze the associated interobserver variability. We anticipate that the DSTs will enhance pathological interpretation of TILs% and PDL1 CPS in GEA, drawing from previous evidence demonstrating their efficacy in assessing immunotherapy biomarkers in breast cancer. The findings of this study also lay the groundwork for future research, aimed at elucidating the potential added value of deep learning algorithms in facilitating automatic detection and scoring of TILs and PD-L1.

Biomarkers

Exploring Cancer Vulnerabilities Linked to the Sister Chromatid Cohesion Regulatory Network

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In order to proliferate, cells must first replicate their DNA, followed by the transfer of duplicated chromosomes to future daughter cells. To ensure accurate segregation, sister chromatids remain paired from their synthesis until their proper separation is initiated at the metaphase-to-anaphase transition. This pairing is facilitated by the sister chromatid cohesin network, which keeps replicated chromosomes together. Defects in the cohesin network are the cause of several rare genetic diseases known as cohesinopathies. Mutations in genes encoding cohesin subunits and regulators have been identified in a significant number of human tumors. This suggests cohesion defects could signify a novel cancer hallmark, potentially targetable as a vulnerability in therapy.

In this project, we are utilizing CRISPR technology, functional genetic screens, models to explore
cancer-related genomic alterations, and atypical gene expression patterns, of key factors within the
larger sister chromatid cohesin-regulatory network;

As such, the aim is to better understand how tumorigenesis may be affected by aberrant regulation of sister chromatid cohesion and its downstream biological processes and how acquired imbalances in the sister chromatid cohesion network could be exploited therapeutically in cancer.

1. Janne J M van Schie, Klaas de Lint, et al. CRISPR screens in sister chromatid cohesion defective cells reveal PAXIP1-PAGR1 as regulator of chromatin association of cohesion (2023) Nucleic Acids
Research 51, pp 9594–9609, https://doi.org/10.1093/nar/gkad756

Cancer Biology

Understanding clonal dynamics in esophageal adenocarcinoma treatment resistance

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The incidence of esophageal adenocarcinoma (EAC) is steadily increasing, and while treatment with neoadjuvant and adjuvant chemoradiotherapy (CRT) improves outcomes it remains a highly lethal disease. This is due in part to the rapid development of resistance to CRT by EAC cells, highlighting the need for a better understanding of EAC treatment resistance mechanisms. This research project aims to quantify the clonal dynamics associated with therapy resistance in EAC. To do so, we will generate lineage-tracing models allowing for the discrimination of individual EAC cell clones through RGB-marking. This technique involves the simultaneous transduction of cell populations with three lentiviral gene ontology (LeGO) vectors encoding either red, green or blue fluorescent proteins and resulting in color combinations unique to individual cells and stable after cell division. We will then analyze clonal dynamics differences in treated and untreated preclinical RGB-marked EAC models in order to determine if resistance to treatment derives from population-wide adaptations or selective pressure favoring therapy-resistant clones. Through this approach, we aim to optimize existing treatment modalities and uncover innovative therapies to bypass existing resistance mechanisms.

Cancer Biology

Tumor-intrinsic subtypes of esophageal adenocarcinoma associate cellular phenotypes with responses to therapy

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Effective treatment of esophageal adenocarcinoma (EAC) is hampered by a high degree of mesenchymal plasticity, contributing to acquired therapy resistance and disease recurrence. Many trials are or have been investigating new treatments, such as additional epidermal growth factor signaling inhibition, to improve patient outcome. However, patient selection methods are limited. The existence of a tissue-level molecular subtype that could be used to stratify patients has remained elusive, in large part due to an abundance of non-tumor cells confounding bulk gene expression data.

We profiled gene expression from 186 esophageal cancer samples and applied non-negative matrix factorization to identify tumor-intrinsic features. Subgroups were discovered using consensus clustering of the tumor-intrinsic gene expression signatures and single-nucleus RNA sequencing analysis.

Two subtypes were identified; intestinal-like (IL) and mesenchymal-like (ML). The latter enriched following neoadjuvant treatment and during disease progression. In a separate cohort metastatic EAC, the ML subtype was found to predict poor response to treatment. Conversely, IL subtype cancer cells were highly dependent on epidermal growth factor signaling, and sensitive to receptor tyrosine kinase inhibition. Mechanisms behind the dependence on epidermal growth factor signaling are currently elucidated using comprehensive analyses.

Targeting of epidermal growth factor signaling is currently only available for HER2-positive patients, about 15% of all cases. Based on our tumor-intrinsic subtypes, a larger fraction of EAC patients seem to benefit from epidermal growth factor inhibition. Therefore, the ability to assign subtype labels in the clinic will be an important step forward to identify patients who may benefit from additional epidermal growth factor inhibition.

Cancer Biology

Exosomal profiles of mesenchymal and epithelial subtypes in colorectal cancer

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Extracellular vesicles (EVs) emerge as messengers involved in the communication system of cancer cells and surrounding cells. EVs carry DNA, RNA, lipids, protein and miRNAs. Prior research has established the significance of EVs in cancer progression, metastasis, and therapy resistance. However, this has not been explored before in the different consensus molecular subtypes (CMS) of colorectal cancer (CRC). This study examined the distinct exosomal profiles between epithelial (CMS2) and mesenchymal (CMS4) subtypes of CRC.

By investigating the cargo of vesicles secreted by the two subtypes, two tetraspanin top hits were differentially expressed in CMS2 and CMS4. Tetraspanin-8 is observed in CMS2 vesicles and Tetraspanin-4, which is highly CMS4 vesicle-specific, underscoring potential subtype-specific markers and pathways. These differences in cargo prompted a deeper investigation into the subtype differences. CMS2 predominantly presents small EVs. Whereas CMS4, exhibits both large and small vesicles, underscoring the complexity of EV heterogeneity and suggesting consequential implications for cancer therapy.

EVs also contain miRNAs, these exosomal miRNAs play a critical role in cancer. In our study, miRNA sequencing showed subtype differences of differentially expressed miRNAs in patient- and cell line exosomes. Both cell line and patient exosome miRNA expression clustered well within their subtype.

The characterization of exosomal landscape in CMS2 and CMS4 subtypes highlights the role of exosome-mediated communication in CRC heterogeneity. Our findings highlight differences in tetraspanin expression, cargo composition, vesicle morphology and miRNA expression between these subtypes. These differences underscore the importance of considering CRC subtype diversity in the development of targeted therapeutic strategies.

Cancer Biology

A CRISPR screen to unravel endoplasmic reticulum stress in B cell malignancies indicates role for translation factor eIF5A

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Impairments in protein folding in the endoplasmic reticulum (ER) lead to a condition called ER-stress. This activates the unfolded protein response (UPR) and can proceed to trigger apoptosis. ER-stress is clinically targeted in plasma cell malignancies, such as Multiple myeloma (MM). However, treatments are not curative and the exact wiring of UPR signaling in B cell malignancies is still unknown. This is underscored by our finding that various B cell malignancy cell lines clearly differed in sensitivity to four ER stressors, indicating distinct underlying signaling pathways.
We designed a CRISPR Cas9 screen to identify key regulators in ER-stress mediated cell death. The first screen in a MM cell line under Thapsigargin selection showed outgrowth of the known ER stress regulators XBP1, ERN1 and CALR. A novel finding was selection for the unique translation factor EIF5A, of which a possible role in the ER is still unclear. EIF5A is the only protein in the cell that contains the post-translational modification hypusine - making this process potentially targetable. Inducible EIF5A KOs in RPMI8226 cells confirmed reduced sensitivity to Thapsigargin, as well as reduced activation of the UPR. Notably, the role of EIF5A appeared unique for Thapsigargin as other ER-stressors had no or even opposing effects. We are currently investigating this exciting new angle between ER-stress and translation using polysome profiling and proteomics.

In conclusion, we found that EIF5A plays a role in ER-stress caused by Thapsigargin. Ongoing experiments will further decipher the underlying mechanism and whether this may be therapeutically targetable.

Cancer Biology

Targeting metabolic pathways in head and neck cancer

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Background

Head and neck squamous cell carcinoma (HNSCC) is the 7th most common type of cancer globally, with an incidence of 890,000 new cases annually. [1] Despite active research in new treatments, the 5-year survival is only 50%. [2] Alterations in cellular metabolism is one of the hallmarks of cancer and could reveal potential diagnostic and/or therapeutic targets. Cancer cells often display the Warburg effect: glucose is converted into lactate, instead of full oxidation in the Kreb's cycle. While one molecule of glucose can maximally yield two molecules of lactate, we found that HNSCC cells switch to 'Super-Warburg': their lactate production was ≥2.5 times the glucose consumption. Clearly, some of the lactate comes from another source.

Aims

This study aims to elucidate the Super-Warburg effect in HNSCC and pinpoint the source(s) of additionally produced lactate. Candidate genes and metabolic pathways involved will be deduced, which ultimately could identify potential drug inhibitors to improve HNSCC treatment.

Results

Preliminary experiments in several cell lines show that HNSCC cells and tumor biopsies produce more lactate than theoretically possible, characterized by a lactate/glucose ratio >2, whilst keratinocytes do not portray this result. Additionally, the cells seemed to depend on pyruvate. Analysis of consumed amino acids suggest that amino acids are not the source of extra produced lactate.

Discussion

Metabolic fluxes will be determined for 4 genetically distinct HNSCC subgroups in varying medium conditions. From HNSCC metabolism models, candidate genes will be identified as potential effector genes of the Super-Warburg effect and investigated by CRISPR-Cas9 knock-out.

Cancer Biology

Increased HPV viral load in cervical cancer patients correlates to disease progression, immune suppression, and poorer clinical outcomes

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Cervical cancer is caused by persistent infection with high-risk human papillomavirus subtypes. The viral load, i.e., the amount of HPV DNA integrated into the genome of a patient's cervical epithelium, has been correlated to disease severity and clinical outcomes. We have explored potential causes of the above by measuring the viral load in biopsies of 44 patients undergoing surgery at the AUMC, and correlated this to clinical outcomes, proliferation markers, and immune status, via multiplex immunostaining of biopsies taken from these patients. Alongside this clinical study, systematic review of all relevant literature was carried out (EMBASE, PubMed, WoScience) to examine evidence on these associations.

The vast majority of cases within our clinical study and systematic review showed that a higher viral load was correlated to higher disease severity or worse clinical outcome, both in overall and recurrence-free survival. However, several studies reported the opposite, frequently due to different categorisation of load levels, or diverse sampling methods. We suggest that viral load may become an important biomarker for improvement of cervical cancer treatment. Higher viral load groups had fewer infiltrating lymphocytes in the tumor microenvironment, indicating immune dysregulation by HPV. These tumors were also less proliferative, and potentially more invasive. From these results we conclude that immunotherapies reversing this dysregulation may prove applicable in relevant populations for effective and safe treatment in the future. Our findings also demonstrate that there is a dire need for consensus in the way in which viral load is measured and reported.

Cancer Biology

Targeting Tumor-Specific Genomic Sequences in Cancer Cells Using CRISPR-Kill

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Targeting Tumor-Specific Genomic Sequences in Cancer Cells Using CRISPR-Kill

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Recently it was shown that CRISPR reagents can be delivered in vivo for gene therapy purposes (Tan et al. (2023)). CRISPR-Cas9, when programmed with pre-designed gRNAs, will induce double stranded DNA breaks (DSBs) at chosen genomic sequences. Here, we propose to use CRISPR-Cas9 as a flexible method to target the cancer genome directly. We aim to induce gRNA-directed DSBs in tumor-specific DNA sequences and exploit their associated toxicity. A method coined CRISPR-Kill was successfully used to eliminate specific plant tissues by the DSB toxicity of targeting tandem repeats(Schindele et al. (2022)). In this project, we are exploring CRISPR-Kill by lentiviral delivery of a tumor-specific mini-gRNA library, on DNA repair-deficient breast cancer and neuroblastoma cell models. To develop the specificity and efficacy of CRISPR-Kill further, we aim to use co-cultures with non-cancerous human cells, combined with cell surface markers for rapid cellular isolation, as a novel in vitro approach. As such, we are investigating whether CRISPR-Kill can be used to eliminate cancer cells from these co-cultures. By optimizing CRISPR-Kill, therapy resistance and intra-tumor heterogeneity might be tackled using multiple rounds of adapted clinical gRNAs, to target different cancer cell populations and tackle tumor evolution.

Cancer Biology

Unraveling the connections between the the prognostic implications of β-catenin and CDX2 and squamous morules in endometrioid ovarian cancer

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Endometrioid ovarian carcinoma (EOC) is a subtype of ovarian cancer that represents approximately 10% of all epithelial ovarian cancers. The Wnt/β-Catenin signalling pathway is frequently aberrantly activated in endometrioid endometrial and ovarian carcinomas. Additionally, focal squamous differentiation, known as squamous morules, is a common feature of these carcinomas and are also associated with expression of CDX2 and nuclear β-catenin. The prognostic value of β-catenin is a topic of ongoing debate. This study aims to assess the prognostic significance of a mutation in the CTNNB1 gene, encoding for β-catenin, and its association with β-catenin expression. Additionally, this study will evaluate if the prognostic value of CDX2 and β-Catenin expression is dependent on the presence of squamous differentiation.

A retrospective cohort study was conducted on 198 patients with EOC between 1991 and 2021 at four hospitals. Immunohistochemistry for β-catenin and CDX2 was performed on all cases. Molecular exome sequencing of a 70-gene next-generation sequencing panel, including the CTNNB1 gene, was performed for 167 patients.

Pathological mutations were identified in the CTNNB1 gene in 20 of these cases. CDX2 expression was observed in 69 cases, and 54 tumors showed nuclear β-catenin expression. Further analysis will be conducted to determine if β-catenin expression is present in the squamous morules. We hypothesize that nuclear β-catenin and CDX2 expression in squamous morules have a better prognotistic implication compared to cases with either a CTNNB1 mutation or nuclear β-catenin without squamous differentiation. This study's findings will provide new insights into the role of β-catenin in EOC.

Cancer Biology

The role of small leucine-rich proteoglycan in restoring multiple myelomainduced osteolytic lesions

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Multiple myeloma (MM) is the second most common hematological malignancy in adults,
characterized by clonal proliferation of plasma cells. The use of novel therapeutic drugs has led to a substantial
improvement in the overall survival of MM patients during the last 15 years. This extension of life expectancy
has revealed the impact of secondary cancer-induced pathologies. More specifically, up to 90% of MM patients
develop cancer-induced bone lesions due to excessive bone resorption and suppression of bone formation,
which is a major cause of morbidity and mortality. Most of the current therapeutic options for MM focus mainly
on the elimination of the malignant plasma cells. However, even when reaching complete remission of the
disease, bone lesions do not repair. Although bone resorption can be pharmacologically inhibited, until now no
drugs are available to promote osteoblastic bone formation. Moreover, MM cannot be cured. Therefore, there is
an unmet need to identify therapeutic options to reverse the MM-induced bone disease.The overall aim of this proposal is to reverse MM-induced changes in the BM niche by
abolishing the MM cell-induced block on osteogenic differentiation. To this end, we intend to unravel how the
absence of the SLRPs ASPN, OGN and OMD contributes to MM bone disease and whether restoring their
expression can normalize MM-induced osteolytic lesions. Secondly, we will investigate the effect of restoring
bone formation on MM cell growth and responses toconventional MM therapy.Finally, we aim to identify
regulators of the SLRPs ASPN, OGN and OMD expression,which couldserve

astherapeutictargets and
explore how to deliverbone inducing biologicals (e.g. BMP6) locally to the MM microenvironment in a clinically
relevant setting

Cancer Biology

Accuracy of diagnostic NGS mutation panels to test clonality of multiple lung tumors

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Lung cancer patients frequently present with multiple tumors. NGS mutation panels are broadly implemented to guide treatment and therefore often used to test the clonal relationship between two tumors. However, genes and coverage vary between panels and their accuracy to differentiate clonal from non-clonal tumors is unknown.

A gold-standard dataset for clonality assessment was established from whole-exome sequencing (WES) data of 95 patients with metastases enrolled in the TRACERx421 cohort (95 clonal and non-clonal pairs). In silico simulation using (I) a panel with only targetable mutations (12 genes), (II) a specialized lung cancer panel (22 genes) and (III) two comprehensive pan-cancer panels (259-485 genes) was performed for adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC). The relationship between clonal and non-clonal pairs was determined according to guidelines of the International Association for the Study of Lung Cancer.

For the panel with only targetable mutations, ~5% of LUAD tumor pairs were misclassified and 32% were inconclusive. In the specialized lung cancer panel, ~5% of LUAD pairs were misclassified and ~8% were inconclusive. For the comprehensive panels, ~3% of LUAD pairs were misclassified and 4% were inconclusive. In LUSC, the panel with only targetable mutations correctly classified all pairs, however ~56% were inconclusive. The other panels had identical performance in LUSC, with ~2% misclassified and ~2% inconclusive.

Diagnostic mutation panels with only targetable mutations have unacceptable accuracy in assessing clonality. We observed higher accuracy with larger mutation panels. This objective evaluation highlights the need for complementary tests for clonality assessment in clinical practice.

Cancer Biology

Cellular dynamics in hereditary cancer syndromes

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Hereditary cancer syndromes account for about 5 percent of all cancer cases, resulting in tumour formation across a wide range of tissues. How identical mutations however result in different levels of tumour formation across tissues remains poorly understood. Active elimination of wild-type cells is used by APC mutants in familial adenomatous polyposis to acquire a selective advantage. However, the competitive interactions of mutant cells in other cancer syndromes remain largely unexplored. Here, we use image-based lineage tracing of wild-type and mutant cells using an oncogene (KRAS, Red2Onco) or tumour suppressor (PTEN, Red2Flp) associated multicolour reporter mouse model across a wide array of tissues (e.g. oesophagus, bile duct, pancreas, bladder). Understanding why certain mutant clones preferentially expand in particular tissues is essential to identify novel barriers against clonal outgrowth. Insights gained from this research may pave the way for the development of targeted therapies targeting cellular competition in currently untreatable cancer syndromes, thereby interfering with clonal expansion and limiting tumour growth.

Cancer Biology

Construction of disease-mimicking cellular models to study cohesin (mal)functioning in cohesinopathies

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The cohesin complex mediates chromatin interactions in cis, to contribute to 3D chromatin organization and gene expression regulation and in trans, to facilitate sister chromatid cohesion and homology directed DNA repair. Furthermore, cohesin contributes to nucleolar organization, required for ribosome biogenesis and protein translation. Mutation in cohesin genes and defects in cohesin functions are commonly observed in cancer. I addition, impaired cohesin functioning may hamper cellular proliferation during early stages of embryonic development. Cohesinopathies comprise a spectrum of syndromes caused by mutations that directly affect cohesin biology. These include Cornelia de Lange Syndrome (CdLS), Roberts Syndrome (RBS), Warsaw breakage Syndrome (WABS), Chronic Atrial and Intestinal Dysrhythmia (CAID), and syndromes related to defects in the cohesin associated gene STAG2 and the mitotic regulator BUB1. Patients display a heterogeneous spectrum of clinical symptoms such as growth and mental retardation, microcephaly, facial anomalies and cardiovascular abnormalities. The extent to which deregulation of the diverse cohesin-regulated processes contribute to the etiology of the different diseases remains unclear. The aim of this project is the construction of isogenic cell panels carrying patient-derived mutations, and a subsequent comparative interrogation of multiple key cohesin functions. We will use RPE1, a well-characterized, untransformed and diploid cell line that is often used in our lab, but we also intend to generate these mutations in induced pluripotent stem cells (iPSCs) and to differentiate those into several disease-affected tissues, which will allow additional investigations in the differentiated cells as well as in the differentiation process.

Cancer Biology

Novel Roles for Dedicated Integrator Complex Subunits in the Context of BRCA1 Deficiency

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The Integrator Complex components play crucial roles in gene expression, the biogenesis of non-coding RNAs and regulating RNA-Polymerase II levels on chromatin. Integrator Complex elements regulate RNA-Polymerase II pause-release and transcription termination events which is vital for avoiding transcription-replication collisions. In addition, the regulation of RNA polymerase II by Integrator Complex is essential for telomerase RNA biogenesis (Rubtsova et al., 2019), making it crucial for telomere length maintenance.
We identified synthetic lethality/sickness of Integrator loss under DNA damage from Interstrand Crosslinks (van der Weegen et al., 2021) and BRCA1 deficiency (van de Kooij et al., 2024) from our genome-wide CRISPR screens.
Our aim is to investigate the molecular mechanisms that could connect a putative, dedicated role for sub-complex of Integrators to DNA damage repair and cellular fitness in the absence of BRCA1. In accordance with the DEPMAP data, we identified this to be an important phenotype in Osteosarcoma cell lines. In Osteosarcoma cell lines, BRCA1 deficiency leads to elevated telomere fusions and accelerated telomere shortening (Kargaran et al., 2016). Discovering the mechanism behind this synthetic lethality can offer insights in tumorigenesis and targeted therapeutic strategies.

Cancer Biology

Investigating the role of aberrant expression of meiosis-specific cohesin factor Rec8 in neuroblastoma cell lines

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The multi-protein cohesin complex is an essential regulator of diverse chromosomal processes during mitotic and meiotic cell divisions, such as sister chromatid cohesion, genome organization and DNA repair. The meiosis-specific cohesin complex is composed of the two structural maintenance proteins Smc1β and Smc3, the Kleisin proteins Rec8 or Rad21L, and stromal antigen proteins Stag1 or Stag3. Mutations and functional impairments of cohesin are common in several types of cancer and may result in alterations of DNA repair, chromosome biology and increased genomic instability. Publicly available gene expression data show that the meiosis-specific cohesin factor Rec8 is frequently overexpressed in neuroblastoma, a pediatric cancer that develops from neuroblasts. However, there have been no studies which investigate the function of Rec8 in neuroblastoma. Therefore, the aim of this project is to examine what the consequences are of Rec8 presence in neuroblastoma cell lines in which Rec8 overexpression is confirmed. In order to investigate, Rec8 expression will be validated in 4 neuroblastoma cell lines on RNA and protein levels. Next, the goal is to functionally assess re-expression in untransformed cells and look into the composition of the cohesin complex to see if Rec8 is incorporated. In addition, sister chromatid cohesion and growth rate will be analyzed. Subsequently, interference of Rec8 will be assessed, using siRNA transfection and CRISPR-Cas9. Cell viability and cohesion defects will be analyzed. Altogether, this study will assess to what extent Rec8 affects genomic stability and cellular viability in neuroblastoma, potentially opening new avenues for cancer research and therapeutic interventions.

Cancer Biology

LIFESTYLE, MICROBIOME AND POLYPOSIS IN PATIENTS WITH FAMILIAL ADENOMATOUS POLYPOSIS

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Familial adenomatous polyposis (FAP), is a hereditary polyposis syndrome associated with colorectal cancer (CRC) if untreated. Recent studies propose a potential role of the gut microbiome in polyp development among FAP patients, yet the specific association with polyp severity remains unexplored. This pilot study aimed to investigate the relationship between lifestyle, microbiome composition and the severity of colorectal and ileal polyposis in FAP patients. In this cross-sectional cohort study, we collected stool samples from pre- and post colectomy patients and used 16S rRNA sequencing for taxonomic profiling. Patients were categorized based on polyp burden during their last endoscopic surveillance. Dietary habits were evaluated using a dietary screener.
Fecal microbiome analysis from 34 participants revealed no significant difference in alpha diversity between patients based on polyp burden. However, distinct bacterial taxa were identified among patients based on colon status. Additionally, patients with an intact colon and high polyp burden had a higher BMI of 28(IQR 24-33) than patients with low polyp burden, BMI of 23(IQR 19-27), ((p=0.076). The gut microbiota composition is not significantly different in patients based on polyposis severity in patients with FAP. However, these results confirm post-colectomy changes in microbiome diversity. Additionally, individuals with high polyp burden seem to have a higher BMI, suggesting a potential association between polyp burden and BMI. These findings highlight the need for further research with a larger cohort to better understand the role of microbiome and lifestyle in the severity of polyposis in FAP, which could improve screening and treatment strategies.

Cancer Biology

Identification of lipid kinase PI4KIIIβ as a modulator of extracellular vesicle biogenesis and release upon lysosomal inhibition, using a luminescence-based screening approach

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Intercellular communication via extracellular vesicles (EV) is crucial to maintain homeostasis, and can be dysregulated in several pathologies. Despite clear therapeutic potential, our understanding on the mechanisms of EV biogenesis is far from complete due to technicial difficulties of studying EV release.

To overcome this, we engineered a HEK293 cell line with CRISPR-Cas9 in which endogenous CD63 is HA-NanoLuciferace-(NL)-tagged. Our results indicate that under basal conditions, CD63-containing EV release from HEK293 cells is mainly dependent on microvesicle budding (SNAP23-independent). However, inhibition of the vATPase with e.g. bafilomycin leads to enhanced exosome release from multivesicular bodies and amphisomes (SNAP23-dependent).

Using our HA-NL-CD63 cells, we performed a broad-spectrum kinase inhibitor screen of 400 small molecule inhibitors in the presence or absence of bafilomycin. We identified the lipid kinase PI4KIIIβ, which phosphorylates phosphatidylinositol (PI) to generate phosphatidylinositol-4-phosphate (PI4P). The signaling lipid PI4P is mainly localized in the golgi, regulating golgi function and architechture, golgi-to-plasma-membrane protein trafficking and transport of ceramide and cholesterol at the ER-Golgi membrane contact side.

With immunofluorescence, confocal microscopy, western blot and the use of KO cell lines and/or inhibitors of PI4KIIIβ and its interacting proteins (e.g. OSBP, ACBD3, C10orf76), we aim to further investigate via which mechanism PI4KIIIβ modulates bafilomycin-induced exosome secretion and secretory autophagy.

This research shows that HA-NL-CD63 tagging is a reporter strategy allowing for high throughput screening for EV biogenesis and release modulators. Additionally, the identification of PI4KIIIβ as a regulator of exosome release stresses the importance of lipid metabolism and signaling processes in EV research.

Cancer Biology

Enhancing the Efficacy of Hyperthermia in Cancer Treatment through the Combination with Carfilzomib

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Background: Hyperthermia (HT), involving tumor heating between 39°C and 45°C, boosts radiotherapy (RT) and chemotherapy efficacy. However, heat shock proteins (HSPs) induction can impede HT's cytotoxicity in cancer cells.

Methods: A drug repurposing screen examined ~6000 drugs to identify thermosensitizers, with carfilzomib emerging as a potent candidate. In vitro assays on cervical and bladder cancer cell lines assessed HT combined with carfilzomib (1-500 nM), and with chemotherapy and 2 Gy RT. Viability was measured 72 hours post-treatment, with survival assays conducted 7-10 days post-treatment. Assays utilized various in vivo and in vitro methods, including cell culture, viability analysis, microscopy, and cell cycle analysis.

Results: Carfilzomib displayed significant thermosensitizing effects in the screen. Combined HT, carfilzomib, and RT showed synergistic reductions in viability across cervical (SiHa, HeLa) and bladder cancer cells (J82, RT112, T24), notably surpassing individual treatments. Clonogenic survival assays confirmed carfilzomib's thermosensitization, particularly in SiHa and RT112 cells. Combinations with HT, carfilzomib, and cisplatin further enhanced efficacy in HeLa and T24 cells.

Future Directions: Ongoing studies aim to validate synergistic benefits of HT, carfilzomib, and RT, offering promising optimization strategies for cancer treatments.

Cancer Biology

How cancer-associated fibroblasts help cancer cells to invade in colorectal cancer

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Colorectal cancer (CRC) is a highly heterogeneous disease. Four distinct Consensus Molecular Subtypes (CMS) have been defined based on gene expression analysis. Among these, CMS4, the mesenchymal subtype has the worst prognosis and high stromal infiltration.
The stromal infiltrated cells are mainly cancer-associated fibroblasts (CAFs), which play an important role in cancer invasion and metastasis. Our goal is to understand how CAFs help CRC cells to invade. We observed that CMS4 cells co-cultured with fibroblasts were able to invade in vitro, while CMS2 (epithelial subtype) cells were not. Similarly, fibroblasts conditioned medium stimulates the invasion of CMS4 cancer cells only, suggesting that fibroblasts-secreted factor is responsible for invasion. Furthermore, we observed that STAT3 and MEK inhibition blocks invasion. EFEMP2, a factor interacting with EGFR, was among the top hits in the secretome analysis of fibroblasts. In accordance, EGFR inhibition reduced the invasion of CMS4 cells. To understand the clinical relevance of these observations we analyzed the impact of EFEMP2 expression in CMS4 patients and observed a significantly worse overall survival in patients with high levels of EFEMP2. These results help to understand the role of the tumor microenvironment in CRC and to improve therapeutic strategies for the aggressive CMS4 subtype.

Cancer Biology

Estrogen production in pancreatic cancer shapes a tumor suppressive stroma

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Pancreatic cancer stands as a leading cause of death due to the lack of effective therapeutic interventions. The abundance of stroma is thought to contribute to poor outcomes. However, both tumor-promoting and -restraining fibroblast populations have been found in the stroma and it is not fully understood how these are instructed. In this study, we find indications that pancreatic cancer cells have the ability to produce female sex hormones, which direct a tumor-restrictive stroma and, as a result, favorable outcomes. We observed that levels of blood-borne stromal biomarkers were significantly higher in female patients compared to males. This was supported by bioinformatic estimates of stromal abundance across solid cancers. Magnetic resonance elastography confirmed that female PDAC patients had stiffer tumor tissue than those of male counterparts, which could be attributed to high stromal content. Remarkably, we not only found that pancreatic cancer cells express the synthesis machinery for estrogen, but could also detect high levels of estrogens in PDAC tissues. We also determined that estrogen instructs a stromal fibroblast phenotype that is associated with relatively indolent molecular subtypes and a favorable prognosis. This phenotype is maintained by a C-type lectin known as CLEC3B. Our data highlight an unrecognized confounder in cancer biology that might extend beyond sex, and that may explain treatment outcome disparities. The finding that estrogen-

responsive C-type lectins direct tumor-suppressive CAF phenotypes may provide new leads for therapy development.

Cancer Biology

How does loss of the cohesin subunit STAG2 sensitize cancer cells to PARP inhibition?

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The cohesin complex is a conserved ring-shaped protein complex that can physically hold two DNA strands together. Cohesin mediates the cohesion of sister chromatids and organizes the genome into a 3D structure during interphase. Although a complete disruption of cohesin is invariably lethal, components of the cohesin complex or its regulators are frequently mutated in multiple types of cancer. One of the most frequently mutated genes encoding a cohesin subunit is STAG2. Cohesin complexes contain either STAG2 or its paralogue STAG1. While loss of both STAG1 and STAG2 is lethal, loss of either STAG1 or STAG2 is not, indicating that their functions partially overlap. Although we currently do not understand why STAG2 is mutated in cancer, previous studies have shown that inactivation of STAG2 but not STAG1 increases the sensitivity of cancer cells to poly(ADP-ribose) polymerase inhibitors (PARPi). PARP inhibition (PARPi) targets cells with defective DNA damage repair, particularly cells with defects in homologous recombination, and has also been reported to disrupt sister-chromatid cohesion. Here, we aim to understand the molecular mechanism underlying the sensitivity of STAG2 mutant cells to PARPi. We have found that the sensitivity of STAG2-deficient cells to PARPi varies between different types of (cancer) cell lines, and that this differential sensitivity is influenced by oncogene-induced replication stress, PARP1 expression levels, and the ratio between STAG1 and STAG2 expression. Our results suggest that perturbation of cohesin function generally sensitizes cells to PARPi, which has important implications for patient stratification when treating cohesin/STAG2 mutant cancers with PARPi.

Cancer Biology

Unraveling the regulation of cancer-associated fibroblast heterogeneity in pancreatic ductal adenocarcinoma through functional genetic screening

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive and devastating disease with limited treatment options and a poor prognosis, accounting for significant cancer-related mortality. PDAC microenvironment is characterized by a desmoplastic stroma rich in cancer-associated fibroblasts (CAFs), which play a crucial role in tumor progression. However, the heterogeneity of CAF subtypes and their precise regulation remains unclear.

We hypothesized that identifying genes important for the establishment of tumor-promoting and tumor-restraining CAFs could allow for the manipulation of pro-tumor CAFs and overall achieve a more tumor-restraining stroma. To shed light on the regulation of CAF heterogeneity and its impact on PDAC biology, we conducted a functional genetic screen using spontaneously immortalized mouse pancreatic stellate cells as precursors of CAFs.

Our CRISPR screen setting coupled with a FACS-based detection method allowed for the identification of tumor-promoting and tumor-restraining CAFs based on antibody staining of established markers of both phenotypes. Results from the screen will be presented, providing new insights into the regulation of CAF heterogeneity and its impact on PDAC biology. Further investigation of the identified genetic factors may lead to the development of novel therapeutic strategies for PDAC patients.

Cancer Biology

Oncogenic enhancer RNAs (eRNAs) as novel therapeutic targets in CRC

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Colorectal cancer (CRC) is a complex disease that can be stratified into 4 different molecular subtypes (CSM1-4). Importantly, the high molecular heterogeneity of CRC represents a significant therapeutic challenge that impacts patient survival outcomes. Therefore, novel and diverse treatment options are needed to overcome this problem. Interestingly, eRNAs have recently emerged as key transcriptional regulator with high cell and\or tissue specificity. In addition, eRNAs have been reported to be deregulated in various diseases, including cancer. Thus, we hypothesize that targeting oncogenic eRNAs within each CMS subtypes could offer novel targeted therapeutic possibilities for CRC patients. Here, we mapped the expression of eRNAs across a large panel of CMS-classified CRC cell lines using global run-on sequencing (GRO-seq). Moreover, we showed that directing CAS13D to eRNAs can interfere with their stability, which, in turn, can impact the activity of nearby contacted promoters. We are now focusing on generating CAS13D gRNA libraries to target expresed eRNAs in each CRC subtype. We aim to use these libraries to perform dropout screens that will identify possible eRNA-dependent vulnerabilities in CRC. We hope this approach can bring forward novel therapeutic possibilities for the treatment of CRC.

Cancer Biology

Characteristics of peritoneal metastatic disease

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Peritoneal metastases (PM) are a common form of tumor cell dissemination in gastrointestinal malignancies. Although underreported, PM incidence is increasing due to improved diagnostic techniques, registration, awareness, and longer survival after primary tumor diagnosis. Peritoneal metastatic disease (PMD) is associated with severe morbidity and resistance to current therapies. Given the direct dissemination route and unique microenvironment of the peritoneal cavity, specific tumor cell characteristics are required for development of PMD. Previous studies suggest that distinct histopathological, genomic, and transcriptomic features of primary gastrointestinal tumors are associated with PMD. We propose to further investigate these features using an in vivo PM model. By grafting cell lines from different cancer types, we expect to find profiles associated with peritoneal dissemination and look to compare these profiles across cancer types. Identifying a signature associated with PM is an important step in constructing a predictive profile for peritoneal metastases and elucidate actionable biomarkers in future. Additionally, the immunosuppressive nature of the peritoneum will be a focus point for this project, as much is still unknown about its implications in cancer and metastasis. Uncovering its precise role in tumor development will give important insights in treatment effectiveness and resistance.

Cancer Biology

The role of the extracellular matrix and regenerative epithelium in chomosomal instability and transformation in the intestine

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Regeneration of the intestinal epithelium after injury is a distinct process from homeostatic self-renewal. During sustained reparative signalling following tissue injury, the extracellular matrix undergoes remodelling marked by Collagen I synthesis. In response, the nascent epithelium is reprogrammed into a foetal-like state with unique cell populations thought to be crucial for regeneration. Although there is evidence that these foetal-like cells can function as regenerative stem cells, their role in intestinal repair and transformation have not been elucidated. Hereby in this research we successfully induced the foetal-like state in Adenomatous polyposis coli heterozygous models in vitro, by exposing them to Collagen I, interferon gamma and activating oncogenic Kras. Following injury-mimicking treatments, not only the Adenomatous polyposis coli wildtype function was completely lost, but an additional mutant allele was gained, which we confirmed to be via copy neutral loss of heterozygosity. Simultaneously, the cells shifted to a foetal expression profile rather than classical intestinal stem cell. These findings raise the question whether similar changes to the mutant Adenomatous polyposis coli alleles occur in colorectal cancer patients and if those have a stronger effect on the progression to carcinoma.

Cancer Biology

Uncovering Mechanisms of Mitochondrial Decrease in Epithelial-to-Mesenchymal Transition of Gastro-Intestinal Cancer Cells.

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Gastrointestinal cancers have a worldwide high incidence and poor outcome. More insight into the underlying biology is needed to improve treatment options. A shift in glucose metabolism from oxidative phosphorylation to aerobic glycolysis, the Warburg effect, is a well-established phenomenon in cancer. Despite this move away from mitochondrial energy metabolism and the threat of mitochondria as initiators of apoptotic cell death, mitochondria still appear to remain essential for cancer cells. Recent research has shown a reduction of mitochondrial content and oxygen consumption in tumor cells with a mesenchymal morphology compared to epithelial tumor cells.

As mesenchymal tumors are associated with metastasis, it is hypothesized that a reduction in mitochondrial content benefits a more lean make-up, improving motility. Moreover, drugs selectively targeting mitochondria in the mesenchymal state have been identified, suggesting that there are additional vulnerabilities to mitochondria in cells with a mesenchymal cell state.

The goal of this project is to investigate whether the observed reduction of mitochondrial content is crucial for the transition of cancer cells to a mesenchymal state. Secondly, we want to uncover regulatory mechanisms at play that mediate the decrease in mitochondria. Therefore, we will develop a CRISPR-Cas9 screen to identify genes involved in the regulation of mitochondrial content transition to a mesenchymal state.

We hope to identify candidate regulators, which will be validated both phenotypically and mechanistically using inducible systems and preclinical disease models. Furthermore, we want to identify pharmacological interventions that target identified mechanisms.

Cancer Biology

The role of P53 in the protection against keratinocyte cancer in vitiligo patients

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Keratinocyte cancer (KC) represents the most frequent type of cancers among Caucasians. Consisting of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), which both develop in the epidermis of the skin. The incidence of KC is significantly increasing due to more UV exposure, use of immunosuppressive medication and an increasing elderly population. Besides the impact on the quality-of-life of KC patients, it also poses a major burden on the health care system.

Vitiligo is an autoimmune disease characterized by white depigmented skin lesions due to local loss of melanocytes by cytotoxic CD8+ T-cells. Here we show in a meta-analysis that vitiligo patients have a decreased risk of developing KC compared to healthy controls. Non-immunological pathways, such as the p53 pathway of which increased expression has been found in vitiligo skin, could be involved in the protection against KC. Additionally, data shows that the increased P53 specifically increases DNA repair in vitiligo compared to healthy donor skin, possibly a reason for the lower risk of KC in vitiligo

The aim of this study is to explore the p53 overexpression in vitiligo skin and its relation to KC. P53 deregulation in keratinocytes and infiltrating immune cells will be explored using two independent scRNAseq vitiligo datasets. We also validate the increased P53 expression in vitiligo skin using lesional and nonlesional biopsies compared to healthy skin. Additionally, the P53 activity by phosphorylated P53 staining in vitiligo biopsies and the mechanism behind the overexpressed P53 is explored.

Cancer Biology

Molecular subtypes of small intestinal adenocarcinomas

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Background: Small intestinal adenocarcinoma (SIA) is a rare malignancy with globally increasing incidence rates. SIA is treated mostly according to guidelines for colorectal cancer. Nevertheless SIA shows a clearly worse 5-years overall survival (CRC: 70% vs SIA: 31%, respectively), potentially indicating a different tumor biology.
Methods: This retrospective cohort study retrieved clinical patient data and 138 formalin-fixed, paraffin-embedded samples of patients with SIAs, diagnosed between 2000-2020 in four Dutch hospitals. Pathological review to confirm the origin of the tumor took place before inclusion. Clinical data was retrospectively collected from the electronic patient files. RNA-isolation from all samples was performed using an newly optimized protocol and followed by RNA-sequencing. Transcriptome-based subtypes were identified using consensus based clustering and characterized by gene set enrichment analyses.
Results: 138 resection specimens of 135 unique patients were studied. Patients were predominantly male (55%) with a median age of 66.5 (IQR 58.3-72.0) years at diagnosis. Tumors were mostly located in the duodenum (70%), followed by the jejunum (21%) and the ileum (9%). Following transcriptome analysis, four SIA subtypes are defined: SIA1: immune enriched; SIA2: epithelial subtype with metabolic dysregulation; SIA3: epithelial canonical subtype; and SIA4: mesenchymal subtype. Multivariable COX regression analysis showed the described subtypes, age and TNM stage to be independently associated with OS, as tumor location and MMR-status were not.
Conclusion: This study presents the first transcriptome-based taxonomy of SIA. It shows SIA to be an unique gastrointestinal cancer, with four molecular distinct subtypes with significant differences in OS.

Cancer Biology

Explaining the differences in overall survival between male and female MSI gastric cancer patients based on NGS data

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Females with resectable microsatellite instable (MSI) gastric cancer (GC) have a better overall survival (OS) than male resectable MSI GC patients, however the cause of this difference remains unknown. Prior research has shown that females have a stronger innate and adaptive immune response. This research aims to elucidate possible differences on a mutational, copy number and microenvironment level that could explain the discrepancy in OS.
Whole Exome Sequencing (WES) from the TCGA_STAD cohort (n=77) was used to determine differentially mutated genes and copy number aberrations (CNAs) between male (n=37) and female patients (n=40). The results from the differentially mutated genes and CNAs were validated using WES from the in the house CRITICS (n=19) and D1/D2 (n=18) clinical trials. To study the TME, we created a single cell atlas from 4 cohorts with together 420.481 cells and 11 cell types. On this we applied OncoBLADE, informed by DNA-based tumor fraction estimates, to deconvolve Bulk RNA-sequencing (RNA-seq) from 72 patients (m=35, f=37) . This resulted in a reconstruction of both 1) cell fractions and 2) cell-type specific gene expression profiles for all cell types in a total of 72 patients.
Differential mutation analysis showed no specific genes that get more often mutated in either sex, furthermore, no gene had a differential CNA in either sex. Initial deconvolution results showed no difference in estimated cell fractions between both sexes. Further analysis of estimated cell type-specific expression profiles will be done to elucidate any differing cell activity.

Cancer Biology

Characterizing the role of galectin-1 in esophageal adinocarcinoma

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Background: Esophageal cancer is a malignant disease which is currently ranked as the eighth most common cancer globally. Esophageal adenocarcinoma (EAC) patients have a poor prognosis while their pathological response rate to standard treatment remains disappointingly low. The tumor microenvironment has been recognized for its influential role in EAC pathogenesis, with galectins emerging as key components therein. Based on current literature and our preliminary work, we hypothesize that galectin-1 is essential for the growth and immunosuppressive activity of EAC cells. Here, we aim to explore the role of galectin-1 on tumor cell behavior in EAC.
Methods: Functional analyses were performed using EAC wild-type (WT) cells and galetin-1 knock out (KO) cells. The galectin-1 KO cells have been previously generated using CRISPR-cas technology. Functional assays include in vitro cell growth and clonogenicity as well as in vivo tumor growth using the chick chorioallantoic membrane (CAM) assay.
Results: Successful loss of galectin-1 protein expression was confirmed by sequencing and by Western blot. Findings thus far indicate a reduced growth rate of galectin-1 KO cells and an increased radiosensitivity (reduced clonogenicity). WT cells were successfully grafted in the CAM assay while evaluation of the galectin-1 KO cells tumor graft rate and tumor growth rate is still underway.
Conclusion: Results suggest that galectin-1 plays a role in EAC tumor progression. Nevertheless, further research is needed to contribute to a better understanding of mechanisms underlying the role of galectin-1 in EAC.

Cancer Biology

Lymph node stromal cells promote B-cell lymphoma survival and migration

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Diffuse large B-cell lymphoma (DLBCL) preferentially spreads to distant lymph nodes (LNs) upon dissemination, indicating that LNs provide the ideal tumor microenvironment. LN stromal cells (LNSCs), including fibroblastic reticular (FRCs) and lymphatic endothelial (LECs) cells, form a scaffold for immune cell activation and migration, but their role in DLBCL survival and dissemination is unclear.

In FRC/DLBCL co-cultures, we observe increased expression of the cancer-associated fibroblast (CAF) markers podoplanin, FAP and vimentin, which resemble FRC phenotype in tumor tissue from DLBCL patients. Furthermore, FRCs co-cultured with DLBCL cell lines increase production of soluble factors BAFF/CXCL12/CXCL13/CCL19 indicating that LNSCs adapt their functions to support lymphoma B-cell survival and migration. In addition, 2-out-of-4 DLBCL cell lines migrated towards LNSCs in a transwell migration assay, suggesting that LNSCs promote tumor cell migration in specific DLBCL subtypes.

Next, we generated a human lymphoma-on-chip as innovative model to investigate the role of LNSCs in the lymphoma microenvironment. We use a microfluidic chip with a microneedle to cast a lumen in a collagen hydrogel, which is subsequently seeded with LECs forming a lymphatic vessel. In the hydrogel, we culture lymphoma B-cells with(out) FRCs. In presence of FRCs, we detect elevated levels of the survival factor IL-6 and lymphoma B-cells show directed migration. Indeed, we observe interaction of lymphoma B-cells with the 3D FRC network formed within the hydrogel, and interaction with and intravasation into the lymphatic vessel. Thus, LNSCs promote lymphoma B-cell survival and migration in innovative 3D models, indicating a pivotal role in the lymphoma microenvironment.

Cancer Immunology

Investigating the impact of checkpoint blockade on human dendritic cells in melanoma.

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Despite the success of immune checkpoint blockade (ICB) in cancer therapy, many patients still do not respond. Current research has largely focused on direct effects of ICB to T cells, however, emerging evidence has shown direct and indirect mechanisms of actions on other cells, particularly dendritic cells (DCs). DCs are key antigen-presenting cells that are crucial in shaping immune system by governing T cell responses. Here, we investigated the effect of treatment with ICB (nivolumab or ipilimumab) in melanoma patients, focusing on various blood DC subsets using a 27-color spectral flow cytometry panel. At baseline, all DC subsets, DC1, DC2, DC3, Axl+ DC, and pDC, could be found in the blood of melanoma patients. We did not observe clear alterations in the frequencies of blood DC subsets compared to healthy individuals. Interestingly, preliminary analysis showed decreased frequencies of circulating DC1 and DC3 subsets in melanoma patients 4 weeks after treatment with ICB. Ongoing analysis is currently being performed to investigate the expression of co-stimulatory markers and checkpoint molecules of DCs in these patients. Additionally, we recently generated nanobody targeting CD169 molecule that is highly expressed on Axl+ DC, as a DC-selective nanovaccine carrier. Indeed, the CD169-specific nanobody could bind to Axl+ DC efficiently. Future research will investigate the functional analysis of CD169-directed nanobody-vaccine on stimulating anti-tumor T cell responses.

Cancer Immunology

CAR T cell therapy against solid tumors by targeting the tumor vasculature

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Chimeric antigen receptor (CAR) T-cell therapy has demonstrated remarkable efficacy in hematological malignancies. However, its application in solid tumors encounters significant challenges. We propose a novel therapeutic approach, using CAR T cells targeting the tumor vasculature to overcome hurdles associated with targeting solid tumor masses, such as antigenic/mutation driven heterogeneity, limited T cell infiltration, and an immunosuppressive tumor microenvironment (TME). Direct targeting of genetically stable tumor endothelial cells (TEC) with CAR T cells provides direct anti-angiogenic action, resulting in tumor growth inhibition, and bypasses immune suppressive actions of the TME. We developed CAR T cells targeting extracellular vimentin (eVim), which is overexpressed in TEC. eVim is a previously proven safe and relevant target for cancer therapy.

We designed 2nd generation CAR T cells with an anti-eVim scFv, that are functionally activated in the presence of antigen. We demonstrated effector-target cell ratio dependent, and selective killing of target expressing endothelial cells. Antigen exposure of the CAR T cells was associated with enhanced proliferation, yet depending on the co-stimulatory moieties (4-1BB vs CD28), differences in differentiation status over time were observed.

These data support our hypothesis that CAR T cells targeting specific markers of TEC are effective, and are currently being substantiated by development of 3D (microfluidics, organoid, TME dynamics) based angiogenesis assays, and in vivo proof of concept in mice. Concluding, by targeting TECs, an important component of the immunosuppressive TME, we envision to develop an effective and innovative novel treatment modality for patients with solid tumors.

Cancer Immunology

Study protocol: Metformin as a metabolic intervention in oesophageal adenocarcinomas to improve response to neoadjuvant chemoradiotherapy (MEMENTO).

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Background: Worldwide oesophageal adenocarcinoma (OAC) is one of most deadly cancers. In case of nonmetastatic disease patients are treated with neoadjuvant chemoradiotherapy (nCRT) and a surgical resection. Only 23% of OACs have a complete histopathological response after nCRT, this is associated with an active tumour immune microenvironment(TIME) characterized by high T cell levels and a higher CD3:CD163 ratio. Using spatial transcriptomics to identify differences between OACs with a T cell-dominant and a T cell-excluded microenvironment, we identified fatty acid oxidation as central feature of immune suppressed OACs (unpublished). Lipid metabolism is associated with an immunosuppressive microenvironment. Metabolic re-programming drugs, like metformin, have shown to be a promising strategy to reactivate the anti-tumour immune response in other cancer types and cause a more pro-inflammatory TIME.
Methods: MEMENTO is a single arm, single centre, proof-of-concept study to determine whether two week metformin treatment, prior to nCRT, activates the TIME in patients with stage II and III OACs. Tumour biopsies will be taken before and after metformin treatment to study the primary outcome (activation of TIME by single cell RNA sequencing) as well as clinical outcomes (pathological response, toxicity, and progression-free and overall survival) and translational research purposes (SCENITH analysis and immunohistochemistry).
Expected results: We expect a shift in macrophage polarization from M2 to M1 macrophages, an increase in CD8 intratumoral T-cell infiltration and an increase of the CD3:CD163 ratio by metformin, which is expected to lead to an improved pathological response to nCRT, which is associated with a better patient outcomes.

Cancer Immunology

T cells from chronic lymphocytic leukemia patients show signs of senescence

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Chronic lymphocytic leukemia (CLL) is currently an incurable disease with an unmet need for novel therapeutic approaches. Despite the promise of autologous T cell therapies in other leukemia's, their efficacy in CLL is considerably lower, largely due to the underlying T-cell dysfunction. This study is dedicated to unraveling the molecular mechanisms of T-cell dysfunction in CLL, aiming to identify potential therapeutic targets. Through RNAseq analysis of the CLL T-cell transcriptome, distinct molecular signatures indicative of inflammation and disrupted cell cycle regulation were identified. Flow cytometry analysis revealed an increased abundance of senescent T cells (CD27-CD28-¬CD57+KLRG1¬+) in patients compared to age-matched healthy donors. In line with our transcriptional findings, these cells showed heightened production of pro-inflammatory cytokines and disruptions in the cell cycle, with larger proportion of CLL T cells residing in S and G2/M phases after stimulation. Importantly, these defects were reversed upon in vitro culture in the absence of their autologous CLL cells. These findings strongly suggest that CLL T cells undergo senescence, likely triggered by CLL-mediated cellular stressors. Additionally, the dysfunctional mitochondrial metabolism observed in CLL T cells suggests a role for oxidative stress and DNA damage in CLL-associated senescence. Understanding the nature of this dysfunction is highly relevant for development of effective T-cell therapies for CLL.

Cancer Immunology

Cytokine requirements for efficient expansion of Mucosal associated invariant T (MAIT) cells for ACT of cancer.

Beatriz Costa[1], Hans van der Vliet[1], Tanja de Gruijl[1]

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For the past decades, the potential of leveraging the immune system for the treatment of cancer has been exploited but, despite all the efforts, a large proportion of cancer patients suffer from disease relapse or treatment resistance. Adoptive cell therapy (ACT) is a form of immunotherapy in which autologous immune cells are extracted, engineered, expanded and reinfused back into the patient. The genetic modification of these cells help enhancing tumor cell recognition and to prompt a more specific anti-tumor response by the immune system. Mucosal-associated invariant T (MAIT) cells are a subset of innate T cells, abundant in diverse human tissues. Initially, MAIT cells were classified as components of the immune defense against infectious diseases due to their ability to identify processed microbial vitamin B metabolites presented by the non polymorphic MHC class I–like molecule MR1. They are known to bridge the innate and adaptive immunity, and indeed they undergo TCR rearrangement and positive selection like classical T cells. However, MAIT cells feature semi-invariant TCRs that are programmed to recognize ligands without the need for an extensive expansion. In this way, MAIT cells gain effector capacity prior to exiting the thymus, having an inherent capacity to respond to infection. MAIT cells also appear to have anti-tumor functions which makes them attractive effector cells. Despite this breakthrough, more research needs to be conducted to assess MAITs' therapeutic potential and to translate their functional activity into clinical use.

Cancer Immunology

A novel 3D Bone Marrow Model to study tumor-induced immune alterations in bone metastasis

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Background: Bone metastases are common in most tumors and determine increased risk of death and loss of quality of life. Studies into the dynamics underlying bone metastasis formation are challenging due to the lack of mouse models or 3D systems that can recapitulate the complexity of the bone marrow (BM) environment. We generated a 3D model mimicking the BM macro- and microstructure and investigated the role for cancer cell-secreted extracellular vesicles (EVs) in tumor-induced immune suppression.

Methods: The 3D BM system was assembled by combining a collagen-gelatine sponge with a plasma-derived hydrogel. Murine BM was obtained from immunocompetent mice. We stably knocked-down EV biogenesis components (Rab11b, Rab35 and syntenin) in murine breast cancer or osteosarcoma cells (4T1, 4T1.2, K7M2). The impact of the knockdowns (KD) on EVs secretion was assessed by NanoLuc-CD63 reporter assay. Alterations of BM immune cells was assessed by high-dimensional spectral flow cytometry.

Results: Our 3D BM system retained cancer and BM cells and enabled intercellular interactions. NanoLuc-CD63 reporter assay confirmed that Rab11b, Rab35 and syntenin knockdowns determined a decrease of CD63-positive EVs secretion. Deep immune profiling revealed tumor-induced EV-dependent immune alterations, in particular in the myeloid cell compartment, which differ from those observed in conventional 2D cultures.

Conclusions: We developed a robust and easily reproducible 3D BM system for the study of tumor-induced immune alterations. Our results support a role for EVs in BM immunosuppression, and lay the basis for the use of the model as a drug screening platform for cancer therapy development.

Cancer Immunology

Mapping the diversity of tertiary lymphoid structures in primary and peritoneal metastatic gastric cancer

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Tertiary lymphoid structures (TLS) are thought to stimulate anti-tumor immunity and positively impact the response to immune checkpoint blockade (ICB). In gastric cancers (GC) however, TLS are predominantly found in patients with limited treatment response. We hypothesized that TLS immune cell composition and function depends on tumor location and the tumor immune environment.

Spatial transcriptomics was used to characterize immune cells inside and outside of TLS within GC primary tumors (PT) and peritoneal metastases (PM).

We identified significant interpatient diversity of the cellular composition and maturation status of GC-TLS. Tumor location (PT vs PM) accounted for most differences in TLS maturity; PM-TLS were predominantly immature. This was associated with higher levels of tumor-infiltrating macrophages and Tregs and less plasma cells compared to tumors with mature TLS. Furthermore, mature TLS were characterized by overexpression of anti-tumor immune pathways, such as B cell-related pathways and MHC class II antigen presentation, while immature TLS associated with pro-tumor pathways including T cell exhaustion and enhancement of DNA repair pathways in the corresponding cancer.

The observation that PM often contain immature TLS, which are associated with immune suppressive tumor-infiltrating leukocytes, might explain the limited response to ICB and should be considered when optimizing immunomodulatory strategies.

Cancer Immunology

chronic inflammation-induced immune adaptations revealed by spatial transcriptomics in patients with colitis-associated colorectal cancer

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Chronic colonic inflammation in patients with inflammatory bowel disease (IBD) increases the risk of colorectal cancer. The biological processes underlying increased risk of cancer in IBD are incompletely understood. Here, we aimed to decipher the molecular and immunological alterations that lead to malignant transformation in IBD-associated cancer (CAC).

We collected FFPE tissue of 13 IBD patients with either dysplasia, cancer, or both. 6 patients without IBD with sporadic dysplasia or cancer were used as a control (sCRC). A dedicated IBD pathologist scored the slides to define 3 regions of interest: normal (non-dysplastic, non-inflamed) tissue, dysplasia or cancer. We used a novel spatial profiling platform, Nanostring digital spatial profiling, to determine the transcriptome in these regions specifically in epithelial cells and immune cells.

Differential gene expression analysis in the epithelial compartment identified normal areas as the regions with bigger differences in transcriptome between CAC and sCRC. Interestingly, biological pathways related to T cell immune responses and cytokine production were downregulated in these areas in CAC as compared to sCRC, suggesting a possible decrease in epithelial immunosurveillance in CAC. Conversely, pathways related to increased cell metabolism and oxidative stress were upregulated, indicating a stress condition of epithelial cells in CAC even in non-dysplastic, non-inflamed areas. Summarising, spatial profiling exposed differential expressed genes as well as biological pathways that stand out in the sequence from normal tissue to cancer in CAC. Specifically, chronic inflammation may induce immune adaptations in non-dysplastic areas that might compromise immunosurveillance in CAC.

Cancer Immunology

Multiplex immunohistochemistry to study the role of JAK3 and TEC family kinases in vitiligo and skin cancer.

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Vitiligo is an autoimmune disease in which the skin melanocytes are destroyed, leading to depigmented skin lesions. A protective effect of vitiligo against skin cancer has been found, caused by the autoimmune reactions underlying vitiligo. In the field of vitiligo therapies, current research is being done on broad JAK/TEC inhibitors. These have been shown effective in mice and patients by showing almost complete repigmentation, but a rapid relapse is often reported after treatment cessation. However, little is known about the exact cell types targeted by JAK/TEC inhibitors and in which cells JAK or TEC family kinases are expressed. Exploring this will not only reveal the contribution of the different immune cells in the vitiligo pathogenesis, but may also tailor future immunotherapies as these specific immune cell population(s) may also effectively target skin cancer cells. Therefore, the goal of this project is to analyze the expression of JAK and TEC family kinases both on RNA and protein level. RNA expression was analyzed using the R2 platform and two publically available vitiligo scRNAseq datasets. Multiplex immunohistochemistry is used to further analyze these expression patterns in both healthy and vitiligo skin. Additionally, a human vitiligo skin explant model will be used to analyze the effect of ritlecitinib treatment; a more selective JAK3/TEC inhibitor. The cells that contribute to vitiligo progression and are targeted by ritlecitinib could lead to new ideas on which cell types potentially play a role in the protection against skin cancer and how these can be targeted for novel therapies.

Cancer Immunology

Vaccination against NOTUM and TIMP1 for treatment of colorectal cancer

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Background:
Colorectal cancer (CRC) is the third most prevalent cancer type and the second-leading cause of cancer deaths worldwide. It is expected that incidence and mortality will almost double in the next 20 years. A better understanding of the disease and new treatment options are needed to prevent this scenario. With our conjugate vaccine strategy, named iBoost, it is possible to target self-antigens that are overexpressed in cancer by fusing a high immunogenic sequence of a bacteria to a target protein, leading to antibody production against the self-antigens. This project focuses on developing a vaccine targeting two self-antigens, NOTUM and TIMP1, using the iBoost technology.

Material and Methods:
The vaccine was produced by transforming pET21-CDP-NOTUM and pET21-CDP-TIMP1 in E.coli bacteria (BL21). After protein production, the bacterial suspensions were sonicated and centrifuged, and the supernatants were used for purification of the proteins. After purification, the protein was dialyzed and diluted to the proper concentration.

Future experiments:
The vaccines will be used to vaccinate mice with an acquired, sporadic APC mutation to determine the preventative effect of vaccination against NOTUM and TIMP1. The mice will be vaccinated every two weeks, with a total of four vaccinations. One week after each vaccination, antibody titers will be determined. A week after the last vaccination, the mice will get injected with Tamoxifen, which will generate an APC mutation in the crypts of the mice. The mice will be monitored for tumor growth and antibody titers will be determined after 4 weeks.

Cancer Immunology

Vaccination against extracellular vimentin enhances the sensitivity of B16F10 melanoma to immune checkpoint blockade

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Introduction:
The immunosuppressive character of the tumor microenvironment is a limiting factor in the response to immunotherapy. Endothelial cell anergy is an important player in immunosuppression, which is characterized by a reduced expression of adhesion molecules, limiting immune cell infiltration into the tumor. We previously showed that vaccination against extracellular vimentin, a specific marker of the tumor vasculature, reversed endothelial cell anergy, turning the tumor microenvironment into a state that is likely more permissive for immune checkpoint blockade (ICB)
Aim:
In current study, we aimed to investigate the potential synergy between vaccination against extracellular vimentin and ICB in B16F10 melanoma.
Results:
Our findings reveal that vaccination against vimentin yields the most substantial suppression of tumor growth when combined with both anti-PD1 and anti-CTLA4 ICB. This treatment strategy also resulted in the highest intratumoral T cell infiltration. Treatment with both anti-PD1 and anti-CTLA4 resulted in lung inflammation in mice, but was not related to vaccination against vimentin, since this pathology was also observed in control mice that were treated with ICB.
Conclusion:
In summary, this study points towards the potential synergy between vimentin vaccination and anti-PD1 and anti-CTLA4 therapy.
Future:
Future studies in different murine tumor models, such as CT26 colon carcinoma or 4T1 breast cancer, are warranted to verify these initial findings.

Cancer Immunology

Immune-modulating effects of various doses of (chemo)radiotherapy (in combination with immunotherapy) on tumor draining lymph nodes in T3-4N0-1 NSCLC patients.

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Tumor-draining lymph nodes (TDLN) play a significant role in the immunological response to immune checkpoint inhibitors (ICIs). It is the core area where antigen-presenting cells take up tumor antigens and present to the tumor-microenvironment (TME), leading to T cell attack responses and therefore elimination of tumor cells. In case of chemoradiation, these TDLN are most often within the area exposed to radiation and the impact of this radiation on their crucial immunogenic role remains unclear.
Using spatial transcriptomics and duplex IHC to analyze TDLNs resected six weeks after CRT(+ICI), we show that a high radiation dose to the TDLNs has few immune-modulating effects. Nanostring GeoMX analysis did suggest that there is radiation-induced fibrosis, though potentially less severe in TDLNs of patients that received CRT+ICI. Also, ICI addition induced a strong Type I immune response (upregulation of Th1 and IFNgamma gene signatures, increase in Ki67+/CD8+ T cells), which was not inhibited by TDLNs receiving a higher radiation dose.

Cancer Immunology

Improving CAR T cell targeting of Multiple Myeloma by investigating CAR T cells and tumor cells in their native bone marrow micro environment

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The role of the tumor microenvironment (TME) has also recently emerged as a significant player affecting the outcomes of adoptive T cell therapy. Tumor cells can shape the microenvironment via the secretion of molecular mediators, which can cause T cell suppression directly or indirectly by recruitment of immunosuppressive cellular mediators. In addition, therapeutic CAR T cells in the TME have to face hypoxic and metabolic reprogramming conditions. On the other hand, the tumor stroma can hinder tumor cell lysis by supporting tumor cell survival and resistance. Although several individual mechanisms of T cell suppression have been studied most of the studies have been performed in fully murine models. Overall, little is known about the major molecular events that simultaneously take place in the interplay between the tumor, the stroma and the CAR T cells and drive the shift from remission to tumor progression.
In this project, we propose to investigate mechanisms of resistance to CAR T cell therapy. To this end, we will: i) evaluate the molecular changes occurring in both CAR T cells and MM tumor cells using the huBMsc xenograft model; ii) identify genes that are synthetically lethal with BCMA or CD38 loss; iii) investigate the use of CAR T cells to locally deliver proteins that neutralize and revert the tumor supporting microenvironment.

Cancer Immunology

High-dimensional immune-metabolic profiling for cancer patients' personalized medicine

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Immunotherapy has revolutionized the landscape of cancer treatment, however the quest for reliable biomarkers that can predict therapy response remains ongoing. Growing evidence indicates that pre-treatment biomarkers, such as protein expression in tumor tissues, are insufficient for patient stratification. Indeed, antitumor responses are shaped not only by inherent tumor traits but also by the evolving immune status of the host, underscoring the importance of gaining insights into early on-treatment immune changes.
So far, in depth understanding of local and systemic immune dynamics with conventional flow cytometry has been restricted by the limited number of markers that can be detected simultaneously. High dimensional immune profiling methodology including spectral flow and mass cytometry are gaining increasing interest, however they do not address the actual immune cell functional status. We developed a highly parametric spectral flow cytometry-based methodology that addresses not only the phenotype but also the metabolic status of immune cells, an increasingly recognized indicator of effector function. The methodology consists of two customizable 40-marker panels to thoroughly characterize T and NK cells (Panel A), and B, dendritic and myeloid cells (Panel B). The two panels share a metabolic core including critical components of key metabolic processes (glycolysis, OXPHOS, PPP, fatty-acid and amino acid metabolism).
Apart from addressing the need for improved therapy response prediction, our spectral flow methodology may facilitate the identification of new therapeutic targets for combination immunotherapy, and is likely useful for a wide range of cancer types including hematological malignancies, where dynamic risk profiling has demonstrated clinical potential.

Cancer Immunology

Investigating T-cell Metabolism in Multiple Myeloma

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Introduction:
Bispecific antibodies (BsAbs) are efficacious for the treatment of relapsed/refractory multiple myeloma (RRMM) patients. However, continuous exposure of T-cells to BsAbs induces features of T-cell exhaustion. Effective T-cell metabolism is crucial for the function, proliferation and differentiation of T-cells, however little is known about T-cell metabolism in different stages of disease.

Methods:
We used PBMC from newly-diagnosed multiple myeloma (NDMM), daratumumab-naïve RRMM, daratumumab-refractory RRMM and BsAb-treated patients and HD in a 48 hour assay with CD3/CD28 stimulation beads. Metabolic and phenotypic readouts were performed by FACS. ELISA and CBA assays were used for cytokine analysis.

Results:
MM patients have a reduced percentage of naïve CD8+ and CD4+ T-cells and an increased percentage of EM and TEMRA CD8+ and EM CD4+ T-cells over time, compared to HD. MM patients have reduced granzyme B and cytokine production compared to HD upon activation, with the lowest production in BsAb patients. MM patients have increased expression of exhaustion markers in comparison to HD, with the highest levels seen in BsAb patients. BsAb patients demonstrate reduced glucose uptake in CD8+ and CD4+ T-cells compared to all other groups. MM patients had a higher percentage of depolarized mitochondria in their CD4+ T-cells compared to HD and had higher ROS production in CD8+ and CD4+ T-cells in their unstimulated samples compared to HD. This may indicate dysfunctional glycolytic and mitochondrial metabolism in BsAb-treated patients.

Conclusion:
MM patients experience changes in their T-cell subset composition overtime which is accompanied by reduced cytokine production, increased expression of exhaustion markers and changes in T-cell metabolism.

Cancer Immunology

Multimodal, single-cell characterization of T-cell dysfunction in chronic lymphocytic leukemia

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Autologous T-cell-based therapy such as chimeric antigen receptor (CAR) T-cell treatment is successful against several B-cell malignancies, however efficacy in chronic lymphocytic leukemia is low; complete remissions range between 20-30%. This has been attributed to the acquired T-cell dysfunction CLL patients develop during their disease course. In vitro observations point to interactions of CLL cells with T cells as underlying cause of this T-cell dysfunction, however these interactions and the molecular consequences on T cells are not yet fully understood.

In this study we aim to uncover multimodal networks regulating T-cell dysfunction by studying single-cell gene expression patterns and chromatin profiles of T, B and CLL cells. Combined analysis of transcription and chromatin modalities allows the study of fine-grained gene-regulatory networks underlying T-cell phenotypes. Currently, we have collected data on peripheral-blood-derived T and B/CLL cells from 2 CLL patients and 2 age-matched healthy controls. Clusters were generated based on integrated RNA and ATAC data using a weighted nearest neighbor analysis and cell type annotation is performed based on RNA. Preprocessing resulted in 9500 cells for analysis, from which 26% B cells, 26% CD8T cells and 39% CD4T cells based on an Azimuth annotation on unfiltered cells.
Further analyses of these multiomic data are ongoing and we expect to gain insight on the differential abundance of dysfunctional T-cell subsets such as exhausted and senescence subtypes, between CLL patients and healthy controls. In addition, key gene-regulatory networks underlying these transcriptional programs potentially present molecular targets to improve future autologous T-cell-based therapies.

Cancer Immunology

Exploring the bivalent LoopCAR strategy to enhance low-affinity targeting of CD38

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Chimeric antigen receptor (CAR)-T cell therapy has brought a paradigm shift in cancer treatment via targeting tumor-specific or tumor-associated antigens (TAA) on cell surfaces and leading to impressive clinical results in the treatment of B-cell malignancies and multiple myeloma (MM). CD38, a profoundly expressed cell surface antigen on MM cells has emerged as a promising TAA target, as evidenced by the success of CD38-specific monoclonal antibodies. In this study, we employed a phage library screen which resulted in the selection of nine distinct single-chain variable fragments recognizing CD38. Subsequently, these domains were integrated into CAR construct designs for evaluation of their cytotoxic potential against well-established MM cell line models. Initial findings revealed that these CAR-T cells exhibited low affinity for CD38, exemplified by low lytic capacity. To address this limitation, we explored a bivalent LoopCAR design, wherein two single-chain variable fragments (scFv) were incorporated into a single CAR construct, aiming to enhance the anti-tumor efficacy of these low-affinity scFvs. To investigate this LoopCAR model, we selected the three best performing low-affinity scFvs and used them to form six LoopCAR constructs. Notably, one specific combination of two scFvs within the LoopCAR framework exhibited enhanced cytotoxicity against MM cell line models, as well as increased cytokine secretion compared to the single scFv harboring CAR-T cells. In summary, our findings highlight the potential of combining two low-affinity scFvs targeting CD38 within a single CAR construct to augment the affinity of the CAR towards its target.

Cancer Immunology

Identification of the immune suppressive tumor-derived factors in esophageal adenocarcinoma

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Introduction:
Response to treatment in esophageal adenocarcinoma (EAC) is associated with an inflamed tumor microenvironment, characterized by high levels of infiltrating CD8+ T-cells and a higher CD8+:CD163+ ratio. Resistance to chemoradiotherapy is associated with higher mitochondrial metabolism and an infiltrate characterized by oxidative phosphorylation (OXPHOS) dependent suppressive macrophage-like cells. In order to improve response to treatment we want to characterize the influence of cancer-cell metabolism on the polarization of infiltrating monocytes.
Methods:
We have co-cultured monocyte-derived macrophages with 2 EAC cell lines (OE19, OE33) and 2 patient-derived cell lines (081R, 289B) of which the metabolic dependencies (OCR:ECAR ratio) are known. After co-culturing, we determined the macrophage phenotype using flow cytometry. Additionally, we will generate an EAC-conditioned medium (CM) with different metabolic inhibitors to determine the influence of specific metabolic pathways.
Results:
Culture of M0-macrophages with OE19-CM showed increased expression of CD163 and CD206, while HLA-DR decreased, in line with a more M2-like phenotype. A pilot co-culture of M0 macrophages with cell lines showed more M1 marker expression (CD80, CD86) in the glycolytic-dependent cell lines OE19 and OE33 compared to the OXPHOS-dependent cell lines 081R and 289B which showed more M2 marker expression (CD163, CD206).
Conclusion:
Preliminary results point toward more M2 skewing in co-culture and that this effect seems to be metabolism-dependent. Further experiments consisting of repetition with more monocyte donors and the implication of metabolic inhibitors will further elucidate the effect of EAC cell metabolism on monocyte polarization.

Cancer Immunology

Spatial transcriptomics identifies metabolic dysregulation as a key driver of T cell exclusion in esophageal adenocarcinoma

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Introduction:Success of neoadjuvant chemoradiotherapy (nCRT) in esophageal adenocarcinoma (EAC) is dependent on the density of intratumoral CD8 T-cells and the CD8:CD163 ratio. Thus, to improve response to nCRT we have to identify and target the mechanisms EACs use to keep CD8 T-cells out.

Methods: We analyzed tissues of patients with high and low intratumoral CD8 T-cell density with spatial whole transcriptomics (Nanostring GeoMx DSP) to separately characterize the transcriptome of cancer- and adjacent immune-cells. Findings were validated using the TCGA and Single Cell ENergetIc metabolism (SCENITH) using fresh resection material.

Results: Whole transcriptome analyses of CK+ cells identified clustering based on T-cell infiltration, indicative of distinct transcriptomes in inflamed and non-inflamed EACs. Differential gene analysis showed less antigen presentation in CD8-low tumors and higher expression of myeloid chemoattractants. Furthermore, they show higher expression of the HDL and chylomicron remodeling pathways. In the CD45+ compartment the PGC1a pathway is upregulated in CD8-low tumors, which is associated with mitochondrial biogenesis and suppressive myeloid cells. The association between CD8 infiltration and mitochondrial metabolism was confirmed using the TCGA where OXPHOS associates with low cytolytic scores. Using single cell metabolic analysis (SCENITH) on fresh tumors we confirmed the presence of OXPHOS dependent myeloid cells in CRT resistant EACs.

Conclusion: We identified distinct transcriptomes in CD8 T-cell rich and poor tumors and found myeloid chemoattactants to associate with low CD8 T-cell infiltration. Moreover, we found dysregulation of lipid metabolism in tumor and immune cell compartments as a potential driver of immune suppression in EAC.

Cancer Immunology

Variation in peripheral blood monocytes (PBMCs) between metastatic gastro-esophageal cancer patients with and without liver metastases

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Background:
Liver metastases (LM) are the second most common site of synchronous metastatic disease in both esophageal and gastric cancer (49 and 40% respectively). LM are associated with poor prognosis and unfavorable responses to immune checkpoint inhibition in several tumor types, including advanced gastric cancer.

The tumor microenvironment (TME) of gastroesophageal cancer (GEC) and specifically liver metastases can be immunosuppressive due to the upregulation of inhibitory checkpoints. There is a paucity of data regarding the interaction between LM and PBMCs. The aim of this study was to investigate whether there is a difference in PBMC composition between metastatic GEC patients with and without LM.

Methods:
We conducted a multicolor panel, flow-cytometry analysis of PBMCs from 33 patients with Her2 negative metastatic GEC before start of systemic treatment. Activation and co-inhibitory marker levels were determined on T-cell subsets. We performed unpaired Mann-Whitney U tests to compare medians between patients with and without LM.

Results:
The number of patients with esophageal, junction and gastric carcinoma was 22, 2 and 9, respectively. 10 patients presented with recurrent disease. Metastatic sites included lymph nodes, bones, local recurrence and 14 patients had LM. We observed no statistically significant differences in T-cell subset frequencies in patients with and without LM.

Conclusion:
In this small population of 33 patients we did not find statistically significant differences when comparing gastro-esophageal cancer patients with and without liver metastases. Further research with a bigger patient population should be performed to investigate differences in PBMC composition in this patient population.

Cancer Immunology

Malignant cell fraction-informed RNA deconvolution to address the heterogeneity within tumor intrinsic molecular subtypes of Pancreatic Ductal AdenoCarcinoma in large cohorts

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Recently we established BLADE, an RNA deconvolution technique which can accurately estimate both cell fractions and cell type-specific gene expression. This allows transferring findings from small cohorts of single cell RNA sequencing (scRNA-seq) to commonly available bulk RNA-seq data. For instance, numerous scRNA-seq studies in Pancreatic Ductal AdenoCarcinoma (PDAC) revealed profound intra-tumoral heterogeneity, where basal-like and classical molecular subtypes co-exist within one tumor. Additionally, some cells showed intermediate expression of markers of both subtypes. Identification of these characteristics in large cohorts of PDAC by applying BLADE would enable us to evaluate their prognostic value. However, RNA deconvolution is generally hindered by large computational complexity and highly heterogeneous malignant cells.
Therefore we developed OncoBLADE, a computationally efficient and cancer specific improvement of BLADE. For a tailored application to PDAC, we derived PDAC dedicated cell signatures by establishing a single cell atlas of 54,928 cells, in which we recapitulated the basal-like, classical and intermediate malignant cell types. Using these signatures and informed by DNA-based malignant cell fraction estimates, OncoBLADE addressed cellular heterogeneity in two large cohorts: one European (SPACIOUS; n=221) and one Asian cohort (SNU; n=196).
Relative fractions of basal-like and classical cells estimated by OncoBLADE were significantly higher in their respective subtypes classified using bulk RNA-seq in both cohorts, confirming our framework. Furthermore, we also found that intermediate malignant cells were related to bad overall survival with a hazard ratio of 1.17 (1.02-1.34) in SPACIOUS and 1.18 (1.00-1.39) in SNU, which may indicate a transformation to the basal-like subtype.

Cancer Immunology

SONATA: Integration of Whole-Genome Sequencing PrOfiles and ImmuNe StATus in CAncer - view on drug targets and immune contexture in melanoma and pancreatic cancer

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Immune checkpoint inhibitors (ICI) have marked a therapeutic renaissance for patients with advanced melanoma and other types of cancer. However, not all patients experience (long term) benefit from ICI therapy. Acquired and intrinsic ICI resistance is a major problem, yet its underlying mechanisms are complex and incompletely understood. Genetic aberrations may (in)directly contribute to resistance. Beside tumour mutational load, whole-genome sequencing (WGS) may potentially inform immunotherapy response or resistance as certain oncogenic driver mutations, such as KRAS in pancreatic ductal adenocarcinoma (PDAC), are linked to immune suppressive mechanisms that shape the tumour immune microenvironment and immune response. Linking WGS profiles with immune

status may widen the scope of therapeutic targets. It is of interest to explore this in an immunogenic (melanoma) vs. poorly immunogenic tumour type (pancreatic cancer).

In the SONATA multi-omics profiling study, patients with (advanced) melanoma and pancreatic cancer who have displayed tumour progression upon treatment with, anti-PD1-based ICI therapy and first-line chemotherapy, respectively, are eligible. Upon inclusion, patients will undergo tumour biopsy (WGS, RNA sequencing, proteomics, immunohistochemistry) and venepuncture (whole-blood, plasma and PBMCs) once.

Since July 2023, 22 patients have been included (7 melanoma and 15 PDAC); WGS was successfully performed in 14 (86% of pts with melanoma, 53% of pts with PDAC).
42,9% and 14,3% of patients with melanoma harboured NRAS and BRAF mutations, respectively; KRAS mutations were identified in 33,3% of pts with PDAC.
Following WGS, thus far two patients were eligible for study treatment.

This ongoing study will proceed to include 180 patients.

Cancer Immunology

Combination of vaccination and CAR T cell therapy for solid tumor treatment

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The development of an effective cancer therapy has been the aim of the scientific community for decades. As a result, different immunotherapies have been developed as Chimeric antigen receptor (CAR) T cell therapy that presents significant results, especially for the treatment of hematologic malignancies. However, there are limitations and challenges to be overcome to achieve similar success for the treatment of solid tumors. These challenges involve selection of the target, infiltration into the tumor microenvironment (TME) and maintenance of functionality. The efficiency of CAR T cell therapy is closely related to the access of the TME where the CAR T cells can have direct contact with the target antigen and the tumoral cells. The tumor vasculature is a major barrier for leukocytes to enter the tumor parenchyma. Due to the exposure of the vasculature to angiogenic growth factors during tumor progression, the endothelial cells become anergic to inflammatory cytokines, resulting in reduced leukocyte adhesion molecule expression. As such adhesion molecules are a prerequisite for leukocyte extravasation, endothelial cell anergy allows tumors to escape from endogenous immunity, as well as from cellular immunotherapies such as CAR T cells. Thereby, the response of CAR T cells therapy can be potentially improved by the combination with a vaccine targeting a protein that plays an important role at the tumoral vasculature such as Vimentin. We, therefore, hypothesize that the combined therapy is the best approach to enhance the success of CAR T cell therapy also for solid tumor treatment.

Cancer Immunology

The GARP-TGFβ axis in immune suppression of head and neck cancer

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Background: Recurrent/metastatic head and neck squamous cell carcinoma (r/m HNSCC) is treated with anti-PD-1 immune checkpoint inhibitors, but the durable response rate is only 15-20%. Elucidation of immune suppression mechanisms is crucial to improve patient outcomes. A potential relevant pathway is the GARP-TGFβ axis, in which glycoprotein-A repetitions predominant (GARP) facilitates activation of latent TGFβ.

Aim: This project aims to investigate the contribution of the GARP-TGFβ axis to the immune suppressive environment, which may hamper response to anti-PD-1 therapy. Ultimately we aim to improve anti-PD-1 response rates.

Results: Immunostaining of oral SCC (OSCC) revealed various patterns of GARP expression. Marginal expression on tumor cells at the invasive border of tumor islands was associated with reduced overall survival. Bulk transcriptional profiling revealed gene signatures characteristic for tumor-promoting inflammation and epithelial-to-mesenchymal-transition in GARP-marginal tumors. Moreover, GARP-marginal tumor islands had fewer infiltrated immune cells compared to GARP-negative tumor islands in OSCC.

Discussion and future plans: The GARP-TGFβ axis seems to play an important role in immune suppression of HNSCC. Spatial transcriptomics of GARP-marginal tumors will be performed to reveal the suppressive profile of GARP-marginal tumor cells, as well as tumor-field infiltrating and surrounding immune cell populations. GARP-marginal localization will be evaluated as prognostic biomarker for HNSCC at various anatomical sites, and in association with response to anti-PD-1 therapy in retrospective cohorts. In addition, inhibition of GARP will be functionally studied in relation to immune cell activation and anti-PD-1 efficacy.

Cancer Immunology

A novel cancer vaccine targeting HER2/3 positive cancers

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Breast cancer is the most common cancer in women. Human epidermal growth factor receptor 2 (HER2) is overexpressed in 20-25% of all breast cancers. Targeted therapy against the HER family is currently well-established in the clinical management of several different tumor types, including breast cancer. Many of HER2-targeting therapeutics have been approved. However, clinical benefits are limited due to the development of therapy resistance caused by receptor dimerization with amongst others HER3. The vaccine is an effective treatment because it can stimulate a long-lasting immune response that will eliminate the tumor cells. This project aims to develop a HER2/3 targeting vaccine. Vaccine constructs will be based on the immune Boost (iBoost) technology, in which the HER self-antigen will be fused to a bacterial antigen (CDP2.2). By this means the immune tolerance can be broken and high circulating antibody titers against the self-antigen can be generated. Various vaccine constructs were designed, encompassing the integral extracellular regions of murine HER family members or solely the HER2-HER3 dimerization domains. The selected self-antigen sequences were cloned in-frame with CDP2.2 into a pET21a expression vector. Plasmids were transformed in BL21 for protein expression. Proteins were purified by Ni-agarose and purity and yield checked by SDS-PAGE. Proteins were dialyzed and protein concentration was assessed by BCA assay.
Now, HER and fusion protein vaccine constructs were successfully produced and purified. In addition, these vaccines are on their way for testing in mice.

Cancer Immunology

Development of an in vitro co-culture system for the evaluation of immunotherapy responses in GEA

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Background:
Gastric Esophageal Adenocarcinoma (GEA) ranks amongst the most lethal malignancies, characterized by its late diagnosis and poor survival outcomes. Moreover, the heterogeneity seen within the GEA tumor microenvironment (TME) along with the usual presence of immunosuppressive features further complicates its therapy. While immune checkpoint inhibition has shown efficacy in inflamed subtypes, its benefits appear limited in tumors characterized by low immune cell densities, which constitute the majority. Currently, addressing this unmet clinical need is hampered by the scarcity of suitable mouse and in vitro models that could recapitulate the heterogeneity of the TME.

Approach:
Initially, we established a live-cell imaging model within a 96-well plate setup that allows the quantification of immune-mediated killing of cancer cells, using a Caspase3/7 staining. By pre-labelling immune cells, we were also able to track and evaluate individual T cell interactions and motility patterns. We now plan to use the knowledge obtained from our initial experiments to design a physiologically more relevant 3D microfluidic system, which will better recapitulate the TME. We plan to optimize this system to be able culture primary tumor tissues, including patient biopsies and resection specimens, alongside homologous T cells to test novel immunotherapeutic approaches in GEAs.

Expected outcome:
Utilizing our patient derived-model system, we plan to expand our knowledge on the immunosuppressive mechanisms within GEA. By doing so we hope to be able to predict patient responses to immunotherapy and improve its efficacy by treating GEA in a more personalized manner.

Imaging

Quantitative MRI in the pre-operative evaluation of liver function for hepatectomy – a scoping review

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Background: Accurate pre-operative evaluation of the liver is crucial before hepatectomy to achieve optimal tissue removal while preserving sufficient healthy liver. This scoping review explores the potential of quantitative MRI (qMRI) in the preoperative evaluation for major liver surgery, aiming to provide valuable insights into liver function and pathology.

Methods: In compliance with PRISMA-ScR guidelines, systematic searches of Embase, Web of Science and Medline databases were conducted from 9 October 2013 until 9 October 2023.

Results: 206 articles were included and classified into nine groups: T1-relaxometry, dynamic contrast enhanced (DCE)-MRI, T2-relaxometry, T2*relaxometry, diffusion-weighted MRI, susceptibility-weighted MRI, proton density fat fraction (PDFF), magnetic resonance elastography (MRE) and possible ultimately multiparametric combinations. The study explored the practical applicability, restrictions, and added diagnostic value in the quantitative preoperative liver assessment.

Conclusion: The potential of qMRI sequences may improve the predictive potentional of preopetative liver assessment in the risk evaluation of posthepatectomy liver failure

Imaging

The predicitve

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Introduction
We evaluated whether in patients with primary PCa, the SUVmax on preoperative PSMA PET/CT was associated with the development of biochemical recurrence (BCR) after robot-assisted radical prostatectomy (RARP).
Methods
We retrospectively analysed 446 PCa patients who underwent a 68Ga-PSMA or 18F-DCFPyL PSMA PET/CT scan prior to RARP. PET/CT scan images were visually and semi-quantitatively analysed by measuring SUVmax in the clinically suspicious prostate cancer lesion. BCR was defined as two consecutive PSA values ≥0.2 ng/mL after RARP. The predictive value of SUVmax for BCR was evaluated using uni- and multivariable Cox regression analyses, adjusting for preoperative variables: radiologic tumor stage (mT), biopsy International Society of Urological Pathology grade group (bISUP), and positive lymph nodes on PSMA PET/CT (miN1), or postoperative variables: pathologic ISUP group (pISUP), pathologic T-stage (pT), and positive surgical margins (R1). Based on the SUVmax distribution among the patients, SUVmax was classified into two groups (high: SUVmax >10 and low: SUVmax ≤10).
Results
SUVmax >10 was a significant predictor for BCR ($p<0.001$). Negative lymph node status (miN0) was associated with developing BCR ($p=0.01$) as opposed to miN1 ($p=0.11$). In multivariable analysis, adjusting for mT, bISUP, and miN1, SUVmax was an independent preoperative predictor for the development of BCR ($p=0.03$).
Conclusion
PSMA tracer expression of the dominant prostate cancer lesion on PSMA PET/CT, defined as SUVmax, was an independent predictor for BCR after RARP in patients with primary PCa. SUVmax >10 can be used as a prognostic factor for an unfavourable outcome after RARP.

Imaging

“Prostate cancer diagnosis by multparametric ultrasound (mp-US) – whole mount correlation for optimization of mp-US”

Bram van Bruggen[1]

[1] Amsterdam UMC, urology

“Prostate cancer diagnosis by multparametric ultrasound (mp-US) – whole mount correlation for optimization of mp-US”

B.W. van Bruggen

Background:
This study is the second phase of a three-part NIH-funded study, aimed at advancing the diagnostic capabilities for prostate cancer through multiparametric ultrasound (mpUS), This second phase focuses on collecting mpUS data from 50 patients immediately before radical prostatectomy and correlating this data with whole mount prostatectomy specimens. The study aims to optimize mpUS using a machine learning approach.

Methods:
The study will enroll 50 adult males scheduled for radical prostatectomy, with participants evenly distributed between Thomas Jefferson University (TJU) and the Amsterdam University Medical Centers (Amsterdam UMC). Utilizing 3D mp-US with contrast-enhanced ultrasound (CEUS), the study will involve transrectal ultrasound with infusion of a microbubble contrast agent (Luminity). Machine learning techniques will be applied to analyze the mpUS data and predict the presence of significant prostate cancer based on pathological evaluation criteria.

Results:
The study anticipates completion within one year, with a scanning duration of up to 30 minutes per participant. Subject recruitment is projected to span 12 months, from June 2023 to June 2024.

Conclusion:
This multicenter study seeks to refine the diagnostic accuracy of mpUS for prostate cancer through an analysis of imaging data and pathological specimens. By integrating machine learning techniques, the study aims to optimize the utility

of mpUS in predicting prostate cancer characteristics. Ultimately, this research holds the potential to enhance early detection and treatment decision-making for patients with prostate cancer.

Keywords:
Prostate cancer; artificial intelligence; transrectal ultrasound,

Imaging

PSMA intensity (SUVmax) as a predictor for castrationresistant prostate cancer in patients with biochemical recurrence after radical prostatectomy

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Introduction:
PSMA PET/CT stands as the golden standard for patients experiencing biochemical recurrence (BCR) after radical prostatectomy (RP). A high maximum standard uptake value (SUVmax) is associated with features such as a high Gleason score or an increased likelyhood of BCR after RP during primary staging. However, in patients experiencing BCR following curative therapy, no established correlation exists between SUVmax and oncological outcomes.The aim of this study is to investigate whether the maximum SUVmax on PSMA PET/CT at time of BCR can predict progression to castrationresistant prostate cancer (CRPC), potentially identifying candidates for intensified treatment regimens.

Methods:
A total of 142 patients with BCR after RP who underwent a PSMA PET/CT were enrolled from April 2015 - December 2018. The highest SUVmax, PSA levels and EAU-BCR-risk profiles were analysed. The primary outcome measure was CRPC.

Results:
The median follow up was 6.5 years (interquartile range (IQR) 5.3-7.6). The median PSA at time of PSMA PET/CT was 0.6 (IQR 0.3-1.8). A positive PSMA PET/CT was significantly correlated with CRPC development (HR 6.8, 95% CI 1.6-28.6, $p<0.001$). Multivariable analysis reveales a 29% increased risk of CRPC per doubling of the SUVmax (HR 1.29, 95% CI 1.11-1.50, $p=0.0016$).

Conclusion
Our findings suggest that, in addition to clinical characteristics, PSMA PET/CT outcomes may have value in predicting long-term oncological outcomes in patients experiencing BCR after RP. This could potentially guide treatment strategies.

Imaging

Predicting Overall Survival of Glioblastoma Patients with Machine Learning

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Glioblastoma, a highly aggressive brain tumor, presents a challenging prognosis with limited survival outcomes. Our study delves into the application of statistical, machine and deep learning techniques to improve the accuracy of overall survival prediction, focusing on survival regression for both censored and uncensored glioblastoma patient data. We introduce a novel approach that incorporates multiple timepoints throughout the patient's treatment journey, including pre- and post-operative stages, to offer a dynamic and comprehensive analysis of treatment efficacy and patient trajectories. Utilizing a multi-center dataset of MR imaging and patient clinical variables, we employ key survival analysis methods, namely Cox Proportional Hazards, Random Survival Forests, and Deep Survival, chosen for their robustness in handling the complexities of survival data. These methods are evaluated using the Concordance Index (C-index) and Brier score over time to assess predictive accuracy and reliability across different treatment stages. The results highlight the importance of incorporating multiple timepoints and advanced analysis techniques, significantly enhancing our understanding of treatment outcomes in glioblastoma patients. This multifaceted approach not only aids in assessing treatment efficacy but also provides insights into the impact of comprehensive information availability at various treatment stages on patient prognosis.

Imaging

Predictive Modeling of 18F-DCFPyL Tissue Distribution in Prostate Cancer: A Physiologically-Based Population Approach for Imaging and Future Therapy Optimization.

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Introduction: Prostate cancer is a prevalent disease affecting middle to older aged men. The diagnostic imaging agent 18F-DCFPyL, with high affinity for prostate-specific membrane antigen (PSMA) expressed on prostate cancer cells, also substantially distributes to other tissues, including organs-at-risk (OAR), thereby increasing the risk of toxicity and, when extrapolated to therapeutic ligands, potentially hampering treatment benefit. Pharmacokinetic modeling has shown promise in predicting individual exposures in all tissues, including tumor, by considering patient-specific, drug-related, and tissue-specific characteristics. This study aimed to develop a physiologically based pharmacokinetic (PBPK) population model to predict individual 18F-DCFPyL (tumor) tissue distribution.

Method: A PBPK model was developed, modifying a previously published model to simulate the distribution of 18F-DCFPyL throughout the body. Relevant physiological and pharmacokinetic parameters, along with estimated PSMA receptor density, were incorporated. Internal validation involved assessing predicted and actual concentrations in various tissues of eight prostate cancer patients, while external validation utilized a prospectively enrolled patient cohort undergoing total-body 18F-DCFPyL PET/CT scans.

Results: The PBPK model accurately predicted concentrations of 18F-DCFPyL in tumor, blood, and other relevant organs. The mean prediction error for tumor tissue was 6.68% (CI 2.64 to 10.72%) and 4.83% (CI -5.25 to 14.91%), in the internal and external validation cohorts, respectively.

Conclusion: PBPK modeling of 18F-DCFPyL provides an accurate approach to predict individual tissue distribution in prostate cancer patients. Combining PBPK-modeling with data from individual diagnostic PET/CT scans showed accurate

predictions of 18F-DCFPyL concentrations in various tissues, indicating its potential clinical application to guide personalized dosing strategies.

Imaging

Dynamic fibroblast activation protein inhibitor (FAPI) PET/CT scans with blood sampling to determine the optimal simplified scan protocol and quantitative measurements, PANSCAN-1 part A.

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Radiolabelled quinoline-based fibroblast activation protein inhibitors (FAPI) are promising novel PET tracers that target cancer associated fibroblasts (CAFs) through specific binding to fibroblast activation protein (FAP). Primary hepato-pancreato-biliary (HPB) tumours are histopathologically characterised by a high stromal content and recruitment of FAP expressing CAFs. Previous studies with FAPI tracers in HPB tumours showed promising results, however optimal timing of the scan after injection is unknown. The aim of this study was to determine the optimal scan protocol and simplified quantitative measurements for [68Ga]Ga-FAPI-46.

Methods: A prospective observational study was performed in patients with pancreatic ductal adenocarcinoma (PDAC) or cholangiocarcinoma (intra- or extrahepatic) with a minimum tumour size of 2 cm. All participants underwent a dynamic (90 minutes) long axial field-of-view PET/CT scan with arterial and venous blood sampling. [68Ga]Ga-FAPI-46 was intravenously administered in a dosage of 200-300 MBq. A comparison of the image-derived and plasma input functions were performed.

Results: In total, 10 patients were included, with an average age of 70 years. The following tumours type were included: 5 intra-hepatic cholangiocarcinoma (CCA), 2 perihilar CCA, 3 pancreatic ductal adenocarcinoma (PDAC) and 1 ampullary carcinoma. The optimal model to describe the pharmacokinetics of [68Ga]Ga-FAPI-46 was the two tissue compartment model (2T4K). The optimal scan timing post-injection is between …. (The last patient was included last week, final analysis is currently being performed.)

Conclusion: (To be concluded after final analysis.)

Imaging

PET-Guided Physiologically-Based Pharmacokinetic Modeling of tyrosine kinase inhibitors: focus on NSCLC with brain metastases

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Osimertinib, a tyrosine kinase inhibitor (TKI), treats non-small cell lung cancer (NSCLC) with epidermal growth factor receptor (EGFR) mutations, including patients with NSCLC-derived brain metastases. However, its efficacy may vary due to heterogeneous drug distribution and limited blood-brain barrier passage. To predict treatment effectiveness, modelling of radiolabeled positron emission tomography (PET) is a potential tool. This study aims to predict osimertinib's tissue concentration-time profiles using a whole-body physiologically-based pharmacokinetic (PBPK) model, accommodating non-linear PK processes in (brain)

tumors.
The fully mechanistic PBPK-model included absorption, distribution, metabolism, and elimination (ADME), tailored to the tumors' immune microenvironment, perfusion, acidity, and EGFR binding characteristics. Passive and active transport mechanisms in the brain were integrated. Model accuracy was assessed by comparing quantitative PET measurements of radiolabeled [11C]-osimertinib concentrations in various (tumor) tissues and plasma with model predictions.
The PBPK-model adequately predicted the ADME of osimertinib in blood, lung (tumor), liver and renal tissues. Predicted and mean observed microdose concentrations after 5 and 60 minutes were 0.85/0.69±0.29 and 0.21/0.61±0.32 nM in tumor tissue and 0.95/0.87±0.32 and 0.28/0.61±0.29 nM in lung tissue. The model effectively described drug accumulation within brain tissue and metastases, currently exceeding three-fold differences compared to observed concentrations. Incorporating target binding, NSCLC hallmarks and brain penetration kinetics improved tissue PK predictions, providing insights into distribution mechanisms. Although further mechanistical insight is needed, this PET-based PBPK-model holds promise for enhancing TKI treatment efficacy and minimizing side effects in precision medicine, particularly in predicting brain distribution for compounds with diverse characteristics and efficacy profiles.

Imaging

Prognostic and Predictive Value of Total Tumor Volume in Patients With Colorectal Liver Metastases

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Aim

This study aimed to assess the prognostic value of total tumour volume (TTV) for early recurrence (within 6 months) and overall survival (OS) in patients with colorectal liver metastases (CRLM), treated with induction systemic therapy followed by complete local treatment.

Methods

Patients with initially unresectable CRLM from the multicenter randomized CAIRO5 trial (NCT02162563) who received induction systemic therapy followed by local treatment were included. Baseline TTV and change in TTV as response to systemic therapy were calculated using CT scan before and after the first systemic treatment, and were assessed for their added prognostic value. The findings were validated in an external cohort of patients treated at a tertiary center.

Results

In total, 215 CAIRO5 patients were included. Baseline TTV and absolute change in TTV were significantly associated with early recurrence (P=0.005 and P=0.040, respectively) and OS in multivariable analyses (P=0.024 and P=0.006, respectively), whereas RECIST1.1 was not prognostic for either early recurrence (P=0.88) nor OS (P=0.35). In the validation cohort (n=85), baseline TTV and absolute change in TTV remained prognostic for early recurrence (P=0.041 and P=0.021, respectively) and OS in multivariable analyses (P<0.0001 and P=0.012, respectively), and showed added prognostic value over conventional clinicopathological variables (increase C-statistic, 0.06; 95% CI, 0.02 to 0.14; P=0.008).

Conclusion

TTV is strongly prognostic for early recurrence (<6 months) and OS in patients who underwent complete local treatment of initially unresectable CRLM, both in the CAIRO5 trial and the validation cohort. In contrast, RECIST1.1 did not show independent prognostic value for neither early recurrence nor OS.

Imaging

Uptake of [89Zr]Zr-ipilimumab-labeled ipilimumab in ipilimumab treated patients with metastatic melanoma

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Introduction

Ipilimumab, a cytotoxic T-lymphocyte associated protein-4 (CTLA-4) blocking antibody, can be very effective in the treatment of late-stage malignant melanoma. However, not all patients benefit and immune related adverse events can be severe. Identifying biomarkers predictive for clinical activity is crucial regarding potential severe toxicity and high costs. ImmunoPET (with [89Zr]Zr-ipilimumab) might be used as such a biomarker. Therefore, tumoruptake of [89Zr]Zr-ipilimumab is investigated in relation with clinical outcome. We hypothesize that a higher [89Zr]Zr-ipilimumab uptake corresponds with a better clinical outcome, since ¬¬ipilimumab can cause enhanced T-cell priming and thereby increased effector Tcells in tumor lesions.

Methods

37MBq, 10mg [89Zr]Zr-labeled ipilimumab was injected to localize ipilimumab in vivo in 18 metastatic melanoma patients. Whole body PET/CT-scans were acquired 144 hours post administration. After visual assessment, quantification of lesions >2cm with uptake above background was performed.

Results and conclusion

Correlation between uptake and clinical outcome was assessed using Response Evaluation Criteria In Solid Tumors (RECIST). Patients were subdivided in groups of complete response, partial response, stable disease and progressive disease using RECIST version 1.1 criteria and iRECIST. The uptake of [89Zr]Zr-ipilimumab per subgroup (including but not limited to SUVpeak) will be presented and the value

as a biomarker predictive for clinical activity will be evaluated.

Imaging

Towards a new standard for staging: a comparative study of [18F]FES-PET vs [18F]FDG-PET in patients with stage II/III and locoregional recurrent ER+ breast cancer

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Background: According to current guidelines, staging of patients with locally advanced breast cancer (BC) is preferably done with positron emission tomography (PET) using 2-[18F]fluoro-2-deoxy-D-glucose ([18F]FDG). However, [18F]FDG-PET can underperform in patients with low-grade estrogen receptor positive (ER+) BC. 16α-[18F]-fluoro-17β-estradiol ([18F]FES) has emerged as a tracer for in vivo visualization of ER+ lesions. This study aimed to assess whether [18F]FES-PET improves staging of grade 1-2, ER+ BC compared to [18F]FDG-PET.

Methods: Patients with clinical stage II/III or locally recurrent, grade 1-2, ER+ BC were included in this prospective multicentre clinical trial (NCT03726931). Patients underwent an [18F]FES-PET scan in addition to staging with conventional imaging and [18F]FDG-PET. Both PET-scans were independently assessed and lesions suspect for malignancy were verified pathologically. The disease stage was determined independently based on conventional imaging and pathology, and [18F]FDG-PET or [18F]FES-PET, respectively.

Results: 41 patients with 44 breast tumours were included. [18F]FDG-PET correctly staged 31/44 (70%), compared to 37/44 (84%) with [18F]FES-PET. The largest difference in diagnostic accuracy between [18F]FES- and [18F]FDG-PET was seen in lobular (90% vs 70%) and grade 1 tumours (93% vs 58%). [18F]FES-PET correctly staged the regional lymph nodes in all cases, whereas [18F]FDG-PET staged 18% incorrectly. Both imaging methods had a sensitivity of 1.0, and a specificity of 0.91 to detect metastatic disease.

Conclusion: In this study, [18F]FES-PET had a higher diagnostic accuracy for staging grade 1-2, ER+ BC than [18F]FDG-PET. In future studies, [18F]FES-PET could be assessed as the primary staging modality, especially in lobular and grade 1 tumours.

Imaging

A correction for modeling of radial, spiral, and PROPELLER DCE data: time-averaged extended Tofts

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Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is a technique used for characterizing tissue perfusion, a promising biomarker in oncological research. Achieving high temporal resolution in the acquisition of image series is crucial for accurate quantitative pharmacokinetic modeling, often achieved through signal undersampling. These undersampled signals differ from conventionally acquired signals by representing an average of the acquisition time. While conventional pharmacokinetic models like the Extended Tofts (ET) model show good results with conventional signal sampling, they fail to account for time-averaged undersampled signals. To address this limitation, we propose a modified ET model tailored to accommodate time-averaged signals, aiming to assess its impact on DCE modeling accuracy and feasibility in both simulation and in-vivo scenarios.

Our modification involves analytically convolving the pharmacokinetic model with a rectangular function, mimicking the time-averaged signal acquisition. We compared proposed and conventional models in simulations and in-vivo. Simulations revealed that the modified model yielded more precise predictions, particularly at acquisition times of 8 seconds-per-frame or higher. In-vivo results echoed these findings, with discrepancies between the models more pronounced at higher acquisition times. Notably, the modified model exhibited greater robustness.

Our study presents a promising modification to the ET model, addressing the challenge of time-averaged signals in DCE-MRI. Enhanced accuracy and precision were observed, particularly at higher temporal resolutions. However, further investigation is warranted to validate these outcomes, considering the non-linear relation between the contrast agent and signal domains.

Imaging

Retrospective validation of a computer aided diagnosis system based on multiparametric transrectal ultrasound for the localization of clinically significant prostate cancer

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Introduction
Ultrasound (US) is widely available and cost-effective but lacks diagnostic accuracy for prostate cancer detection. Computer-aided diagnosis (CAD) is a promising method to increase this accuracy. The aim of this study is to assess the diagnostic performance of CAD based on 3D multiparametric transrectal prostate ultrasound (3D mpUS) for the detection of clinically significant prostate cancer (csPCa).
Materials & Methods
The evaluated CAD system was developed using artificial intelligence and trained using 3D mpUS images from 'positive' patients undergoing radical prostatectomy (RP) and 'negative' patients (PIRADS≤2 on MRI or negative systematic biopsies). 3D mpUS consisted of 3D B-mode, 4D contrast-enhanced US, and 3D shear wave elastography. Histopathology of RP specimen was used as ground truth, csPCa was defined as Gleason Group (GG)≥2. Performance was assessed by comparing the CAD prediction with the ground truth on the US images. Visual assessment determined if targeted biopsies (TBx) would have resulted in detection of csPCa.
Results
Based on retrospective validation of 252 patients, the sensitivity and specificity were 82.9% (76.4–88.1) and 37.7% (26.9–49.4) for csPCa detection. The PPV and NPV were 75.6% (72.0–78.9) and 48.5% (37.9–59.3). For PCa GG≥3, the sensitivity was 90.3% (83.3–95.0). This study is limited as the cohort holds a significantly higher prevalence of csPCa than a general population and performance was assessed by simulating TBx and not by taking actual TBx.
Conclusion
The diagnostic accuracy of CAD based on mpUS shows robust detection of PCa and is similar to MRI. Prospective clinical validation is needed to further investigate the non-inferiority compared to MRI

Imaging

Quantification of fluorescence angiography for visceral perfusion assessment: measuring agreement between two software algorithms

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Background: Indocyanine green fluorescence angiography (ICG-FA) potentially mitigates perfusion-related complications in gastrointestinal anastomosis. Emerging quantification software aims to objectify ICG-FA interpretation, necessitating comparison between algorithms to assess the external validity assessment.
Methods: A retrospective cohort analysis involved standardized ICG-FA recordings from patients with esophageal cancer undergoing esophagectomy with gastric conduit reconstruction (August 2020-February 2022). Recordings were analysed by two quantification software implementations: AMS and CPH. The quantitative parameter used to measure visceral perfusion was the normalized maximum slope derived from fluorescence time curves. Agreement was assessed via Bland-Altman analysis. The relation between intraoperative perfusion measurement and anastomotic leakage incidence was explored for both software.
Results: Seventy pre-anastomosis ICG-FA recordings were analyzed. Bland-Altman analysis revealed a mean relative difference of +58.2% in normalized maximum slope measurement between AMS and CPH. Agreement deteriorated with increasing values, indicating a proportional bias ($R2 = 0.512$, $p < .001$). Neither AMS nor CPH measurements significantly correlated with anastomotic leakage occurrence (median 0.081 vs. 0.074, $p = 0.32$ and 0.041 vs. 0.042, $p = 0.51$, respectively).
Conclusion: Technical differences in software implementations can lead to discrepancies in ICG-FA quantification. The possible variation among software-based quantification methods should be considered when interpreting studies that report quantitative ICG-FA parameters and derived thresholds, as there may be a limited external validity.

Imaging

Comparing background organ uptake of ^{89}Zr-DFO*-trastuzumab with ^{89}Zr-DFO-trastuzumab in patients with HER2-positive mammacarcinoma

Hylke Sebus[1], Jelijn Knip[1], Jessica Wijngaarden[2], Gerben Zwezerijnen[2], Daniëlle Vugts[2], Marc Huisman[2], Willemien Menke - van der Houven van Oordt[1]

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As I am awaiting data (and sponsor approval) to come back, I do not yet have the final abastract. This is discussed with Gitta, I will hand over an abstract by the end of April.

Imaging

Ultra-low foetal radiation exposure in 18F-FDG PET/CT imaging with a long axial field-of-view PET/CT system

Charlotte L. C. Smith[1], Maqsood Yaqub[1], Ruud H. H. Wellenberg[1], Jelijn J. Knip[2], Ronald Boellaard[1], Gerben J. C. Zwezerijnen[1]

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Purpose: The use of 18F-FDG PET/CT scans can cause a dilemma in pregnant patients due to radiation exposure to the foetus. Fortunately, the new long axial field-of-view (LAFOV) PET/CT systems enable PET/CT scans with reduced injected activities because of their ultra-high sensitivity. This study aims to report the foetal radiation dose when performing an 18F-FDG PET/CT scan on a LAFOV PET/CT system with reduced injected activity.
Methods: Two pregnant women were retrospectively included and received an 18F-FDG PET/CT scan on a LAFOV PET/CT system with an intravenous bolus injection of 0.30 MBq/kg. Foetal radiation exposure from the PET was estimated using dose conversion factors from three published papers (Stabin, JNM, 2018; Takalkar et al., JNM, 2011; Zanottti-Fregonara et al., JNM, 2015). Radiation exposure from the low-dose CT scans was calculated based on the computed tomography dose index (CTDI) and estimated using CT-Expo.
Results: The fetal radiation dose conversion factors from the PET from the three studies ranged between 0.004 - 0.014 mGy/MBq. The radiation dose on the foetus from the LAFOV PET scans ranged between 0.11 - 0.44 mGy. Foetal radiation exposure from the low-dose CT scan ranged between <0.10 – 0.90 mGy depending if the foetus was included in the field-of-view.
Conclusion: Foetal radiation dose could be reduced to <1.5 mGy when scanning pregnant patients on a LAFOV PET/CT system. Based on these findings, we can conclude that the ultra-high sensitivity of LAFOV PET/CT systems significantly reduced the radiation dose to the foetus.

Imaging

[89Zr]Zr-ipilimumab PET imaging does not show accumulation of ipilimumab in benign lymph nodes

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Introduction

Ipilimumab, a cytotoxic T-lymphocyte associated protein-4 (CTLA-4) blocking antibody, combined with nivolumab, has improved overall survival in metastatic melanoma patients. However, why only 40-50% benefits, remains uncertain. To evaluate possible mechanisms of action (MoA), whole body CTLA-4 expression was investigated by 89Zr-immuno-PET with [89Zr]Zr-ipilimumab. Hypothesized was that, as supposed primary MoA, [89Zr]Zr-ipilimumab would accumulate in lymph nodes to block CD80/CD86-mediated Tcell-inhibition. High tumor uptake would support presence of activated (i.e. CTLA-4-expressing) tumor-infiltrating lymphocytes, Treg binding, and possibly, depletion through antibody-dependent phagocytosis.

Methods

144 hours post-injection of 10mg 37MBq [89Zr]Zr-ipilimumab, 5 melanoma patients (planned for ipilimumab/nivolumab treatment) underwent a PET/CT-scan. A long axial field-of-view scanner was used to optimize signal-to-noise ratio for visual examination of lymph nodes, of both benign (<1cm in axilla/groin) and malignant imposing origin. Lymph nodes were ‘positive’ if uptake was visually above background, ‘negative’ for uptake below background and ‘intermediate’ if background was equaled. Binding and internalization of [89Zr]Zr-ipilimumab would result in increasing uptake over time, due to residualizing properties of [89Zr].

Results

Of 34 benign lymph nodes, 22 were intermediate and 12 negative, whereas of 16 malignant lymph nodes (1-7/patient), 15 were positive and 1 negative (Kruskal-

Wallistest: $p<0.001$ for difference in score between ‘benign’ and ‘malignant’).

Conclusion

[89Zr]Zr-ipilimumab exceeded background in malignant, but not in benign lymph nodes, which underlined lower CTLA-4-expression in benign lymph nodes (within 144 hours). Comparison with visual- and quantitative data of tumor uptake, related to clinical outcome, will be presented. Future flow cytometry analyses will indicate actual CTLA-4-expressing cell types.

Imaging

Association Between Clinicopathological Tumor Characteristics and Semiquantitative [18F]FDG-PET and [18F]FES-PET Imaging Parameters in ER+ Breast Cancer

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Background: Positron Emission Tomography (PET) with 2-[18F]fluoro-2-deoxy-D-glucose ([18F]FDG)-PET is commonly used for the staging of locally advanced and locoregional recurrent (LRR) breast cancer. However, the emergence of 16α-[18F]-fluoro-17β-estradiol ([18F]FES)-PET presents a promising alternative staging method, particularly for estrogen receptor positive (ER+) breast cancer. To determine the optimal staging procedures for each breast cancer subtype, the association between clinicopathological tumor characteristics and [18F]FDG- and [18F]FES-uptake needs to be investigated.
Methods: We will analyze data from the FORESIGHT pilot-study (NCT03726931), where patients with clinical stage II/III or LRR ER+ breast cancer underwent both [18F]FES-PET and [18F]FDG-PET imaging for staging purposes. Semi-quantitative PET parameters, standardized uptake values (SUVs) and tumor-to-blood ratios (TBRs), were obtained by defining volumes of interest in the aorta ascendens and in suspect tumor lesions. These PET parameters will be correlated with various pathological features, such as histological subtype, grade and ER, progesterone receptor (PR) and GLUT1 expression. The results of these analyses will be presented on the poster.
Discussion: It is hypothesized that a positive association exists between [18F]FES-uptake and ER and PR expression level. [18F]FDG-uptake is expected to be positively correlated to higher tumor grade and GLUT1 expression level. Furthermore, [18F]FDG-uptake is expected to be higher in the histological subtype invasive ductal carcinoma, whereas [18F]FES uptake is expected to be higher in invasive lobular carcinoma. Incorporating the status of these clinicopathological parameters into decisions regarding PET imaging could improve diagnostic

accuracy and optimize treatment strategies for ER+ breast cancer patients.

Imaging

Opportunities for CT-free Total-Body PET Imaging

Konstantinos Drymas Vrakidis[1], Charlotte Smith[1], Marijke den Hollander[1], Maqsood Yaqub[1], Ronald Boellaard[1]

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Purpose: Achieving quantitative accuracy in Positron Emission Tomography (PET) images necessitates the use of Computed Tomography (CT) imaging, contributing to the radiation burden in PET/CT examinations. In total-body (TB) PET/CT systems where a fraction of the standard injected dose is adequate for PET imaging, the CT radiation contribution becomes relatively significant. Aiming to eradicate this radiation contribution, this study assesses the performance of two CT-free methods for PET imaging in TB-PET/CT systems.
Methods: Two CT-free methods were applied and compared against the clinical CT-based one. The first one exploits the intrinsic background radiation of the lutetium oxyorthosilicate (LSO) scintillators present in modern PET systems. In the second method, synthetic CTs are generated using artificial intelligence (AI). The performance of these methods was evaluated based on reconstructed PET images, by comparing the mean standardized uptake values (SUV-mean) extracted from several organ tissues.
Results: In total, twelve 18F-FDG PET/CT with LSO-based scans and one hundred 18F-FGD PET/CT scans were collected from oncology patients using a commercial TB-PET/CT scanner, respectively for the first and the second methods. The LSO method yielded reconstructed images with a mean relative SUV-mean error (Δ-SUV) of 28±7%, while with the AI one, we obtained a mean Δ-SUV of 7±4 %. The performance difference between the two methods was measured as significant.
Conclusions: Of the two methods we tested, only the AI-based one managed to approach the quantitative accuracy of the clinical-grade PET/CT. Further improvement of this method can ultimately enable safer CT-free PET imaging protocols using TB-PET/CT systems.

Imaging

DEVELOPMENT AND EVALUATION OF A MEDICAL-BASED EXPLAINABLE ARTIFICIAL INTELLIGENCE APPROACH TO PREDICT PROGRESSION FREE SURVIVAL IN PATIENTS WITH METASTATIC COLORECTAL CANCER USING PRE-TREATMENT 18F-FDG PET

Bart de Vries[1]

[1] Mr.

Introduction: 18F-fluorodeoxyglucose (18F-FDG) PET is crucial in diagnosing and managing metastatic colorectal cancer (mCRC) and may aid in disease stratification. Utilizing computer-aided pattern recognition, we aimed to develop and validate an explainable CNN for predicting progression-free survival (PFS) in mCRC patients undergoing anti-EGFR antibody treatment.
Methods: Pre-treatment 18F-FDG PET images from 78 patients with mCRC eligible for anti-EGFR antibody treatment were acquired. A 2.5D-CNN was built to capture PET features, and a custom made eXplainable Artificial Intelligence (XAI) algorithm was used to generate attribution maps to estimate what PET features the CNN uses for prediction. Performance of the CNN was evaluated based on average area under curve (AUC), accuracy, sensitivity and specificity from a 10-fold cross-validation. Results: The 2.5D-CNN was able to classify dichotomous PFS (152 days) with an average AUC of 0.95 ± 0.11 (SD), accuracy of 94% ± 12, sensitivity of 91% ± 21 and specificity of 94% ± 21%. Inspection of the attribution maps showed that especially the liver has high attribution importance. Interestingly, also non-lesion SUV in the liver holds prognostic value to predict dichotomous PFS in patients with mCRC. Conclusion: The coronal 2.5D-CNN showed potential to predict dichotomous PFS from pre-treatment 18F-FDG PET images in patients with mCRC undergoing anti-EGFR antibody treatment. In addition, both lesion as non-lesion specific SUV seems to be prognostic to predict dichotomous PFS.

Imaging

Acquisition and reconstruction optimization for abdominal quantitative Dynamic Contrast Enhanced MRI

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The tumor microenvironment significantly influences the effectiveness of local radiotherapy and systemic chemotherapy. Accurate quantification of tissue characteristics is crucial for monitoring treatment response and predicting outcomes. Dynamic contrast enhanced MRI (DCE-MRI) is a valuable technique for assessing the vascular microenvironment of tumors by tracking the distribution of a contrast agent over time. By applying the extended Tofts model to signal-time curves, DCE-MRI enables quantification of pharmacokinetic parameters: vessel permeability, extravascular extracellular space (EES), and blood plasma volumes. Achieving reliable DCE-MRI requires high temporal resolution, but this often compromises image quality due to reduced time per frame for data acquisition. In this study, the acquisition parameters (pseudo-spiral k-space) and reconstruction parameters (compressed sensing) for DCE-MRI were optimized. The image quality and accuracy of pharmacokinetic parameters of a total of 704 different strategies were assessed in a digital phantom mimicking the abdomen. The best and the worst strategies were also applied in vivo in healthy volunteers and a pancreatic ductal adenocarcinoma (PDAC) patient.
The best acquisition and reconstruction strategy, demonstrated superior accuracy and precision of the estimated pharmacokinetic parameters. Evaluation in healthy

volunteers confirmed sharper images and fewer artifacts with the optimized strategy compared to the worst. Application of this strategy in a PDAC patient yielded pharmacokinetic parameters consistent with literature values.
This study shows that pseudo-spiral acquisition of DCE-MRI data is feasible and results in accurate parameter maps. Improvement of DCE-MRI quality is needed for this technique to be used as imaging biomarker in oncological setting.

Imaging

Pharmacokinetic analysis and simplified uptake measures for tumor lesion [18F]F-AraG PET imaging in patients with non-small cell lung cancer

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Introduction: The novel positron emission tomography (PET) imaging tracer, [18F]F-AraG, targets activated T-cells, offering a potential means to improve our understanding of immune-oncological processes. The aim of this study was to determine the optimal pharmacokinetic model to quantify tumor [18F]F-AraG uptake in patients with non-small cell lung cancer (NSCLC), and to validate simplified measures against the pharmacokinetic uptake parameter.

Methods: Ten patients with early-stage NSCLC and three patients with advanced NSCLC underwent a dynamic PET scan of minimal 60 minutes, using either an Ingenuity TF PET/CT scanner or a Biograph Vision Quadra PET/CT scanner. Six patients underwent a second scan within median=2.5 (range=1-4) days. Venous and/or arterial blood sampling was obtained at maximal seven time points. Tumor time activity curves and metabolite-corrected input functions were analyzed using single-tissue reversible, two-tissue reversible and two-tissue irreversible (2T3k) plasma input models. Simplified uptake measures, i.e. standardized uptake value (SUV), tumor-to-blood (TBR) or tumor-to-plasma ratio (TPR), were evaluated for different time intervals.

Results: Whole-blood and plasma radioactivity concentrations showed rapid clearance of [18F]F-AraG. Metabolite analysis revealed a low rate of metabolism,

at 70 min p.i. 79% of the total blood radioactivity corresponded to intact [18F]F-AraG. Tumor time activity curves were best fitted by the 2T3k model. TBR at 60-70 min p.i. correlated stronger with 2T3K-derived Ki ($r(20) = 0.87$, $p<0.01$), than SUVBW ($r(20) = 0.78$, $p<0.01$).

Conclusion: Tumor [18F]F-AraG uptake in patients with NSCLC is characterized by a 2T3k model. TBR and TPR show most potential for simplified quantification of tumor [18F]F-AraG uptake.

Supportive Care

Baseline factors relating to depressive symptoms at one year postoperative in patients with diffuse glioma

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Purpose:
Depressive symptoms are common in patients with diffuse glioma, potentially reducing their quality of life. Which baseline factors determine the development of depressive symptoms so far remains elusive. In this study, we investigate the associations of baseline patient- and tumor-related characteristics and depressive symptoms one year post-surgery.
Methods:
Retrospective longitudinal datasets from Amsterdam UMC and Rigshospitalet Copenhagen were combined. Several characteristics of patients and tumors were retrieved, in particular items of their mood and functioning status. Depression instruments and functioning items were harmonized through previously developed item response theory and linking methods to the PROMIS Depression scale. We analyzed the associations of 25 factors including International Classification of Functioning, Disability and Health (ICF) domains with depressive symptoms one year post-surgery by multivariable stepwise backward linear regression models and verified model robustness using best subset selection.
Results:
We included 118 patients with diffuse glioma with a mean age of 48 years and a glioblastoma in 29%. Baseline depressive symptoms, lower ICF energy , and impaired ICF language functioning were associated with more depressive symptoms at follow-up in the multivariable model (R-squared: 0.379).
Conclusion:
We identified three important baseline factors associated with depressive symptoms one year post-surgery. Our findings contribute to advancing the comprehension of predictive factors associated with depressive symptoms, possibly allowing early intervention.

Supportive Care

Enriching Perspectives: Patient perspectives regarding the implementation of risk prediction using an intraoperative ML (IPML) models in colorectal surgery.

Sara Ben Hmido[1], Freek Daams[1]

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The rapid advancement of predictive machine learning (ML) models in healthcare, particularly in surgical decision-making, lacks literature on Integrated Intra-operative Predictive ML (IPML). The views of patients regarding the utilization of IPML in colorectal surgery particularly in critical areas like the prediction of colorectal anastomotic leakage,remain largely unexplored.
This study investigates patients' views on implementing IPML models in colorectal surgery, focusing on trust, expectations, and the models' role in decision-making. Employing focus groups and interviews with colorectal surgery patients, the study analyzed data using grounded theory. The associations in the code tree were established based on a co-occurrence table. The patient sample size was determined using a saturation analysis.
Findings from n = 19 participants revealed a generally positive outlook on IPML models, recognizing their potential to enhance decision-making. Patients emphasized surgeons' primary role in decisions but advocated for IPML as advisory tools. Surgeons should be able to override recommendations. Some participants wanted the ability to override to correlate with the amount of experience a surgeon has. Personalized patient communication and considerations for quality of life were deemed crucial. The study emphasizes the need for a balanced integration of IPML to support clinical judgment and address patient preferences. It underscores IPML's potential to enhance decision-making while preserving healthcare professionals' expertise.
Patients are of the opinion that IPML developments should prioritize transparency, patient-centered communication, and human judgment integration to align with patient expectations and improve healthcare outcomes. Further research is warranted to refine these models and explore patient perspectives in other medical specialties.

Supportive Care

The effects of clinician responses to cancer patients' online health information seeking behavior

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Background
Cancer patients increasingly rely on online information about their disease. Yet, we don't know the effects of clinicians' various communication approaches when patients discuss such online information. This study systematically tested the impact of clinicians' communication approaches on cancer patients' trust, satisfaction, information seeking and information discussion. Additionally, we explored moderating effects of patients' personality characteristics.
Methods
In an online vignette experiment portraying an oncological consultation, we manipulated: (1) clinicians' communication approaches, i.e., patient-centered vs clinician-centered and (2) modality of the vignette, i.e., video vs text. (Former) cancer patients (N = 270, 62 ± 13 years old, 55% female) were randomly assigned to one group as analogue patients. 1-way ANOVA's, independent samples t-test and multiple regression were conducted.
Findings
Participants exposed to the patient-centered approach reported higher satisfaction ($d = 0.62$, $p < .001$), increased trust ($d = 0.49$, $p < .001$), and stronger intentions to seek and discuss online information ($d = 0.40$, $p < .001$ and $d = 0.69$, $p < .001$, respectively) compared to the clinician-centered approach. Moderation analyses indicated that the effect of communication approach on intention to discuss online information depended on participants' trait anxiety ($\beta = -0.43$, $p < .05$). Participants' monitoring coping style moderated the effect of communication approach on online information seeking ($\beta = 0.23$, $p < .05$).
Discussion and conclusion
Clinicians' patient-centered responses to online information seeking may positively affect patient satisfaction, trust and online information seeking behavior, partly depending on patients' personality characteristics. Our recommendations for clinicians include emphasizing collaborative information exchange and referring patients to trustworthy online sources.

Supportive Care

FeelFit study protocol: high-intensity interval training to improve self-reported physical fitness in brain tumor patients.

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Background. Brain tumor patients may experience decreased physical fitness during stable disease. This can affect daily functioning and quality of life. Exercise programs are an effective way to improve physical fitness. A relatively new type of training is high-intensity interval training (HIIT) and shows promising effects in different oncology patient groups. The primary objective of this study is to investigate the efficacy of high-intensity interval training in brain tumor patients in terms of self-reported physical fitness.

Methods. A monocenter randomized controlled trial will be conducted in a sample of 36 patients with a primary brain tumor during stable disease and a decreased self-reported physical fitness. The intervention will consist of a HIIT program for 12 weeks with two supervised training sessions per week on a stationary bike. In addition, patients from the intervention and control group will receive physical activity advice and will be asked to keep an exercise diary. The control group patients will be placed on a waiting-list and can still do the HIIT program after the study. The primary outcome is self-reported physical fitness and will be measured with the International Fitness Scale. Measurements will take place at baseline, post-intervention and at 6 weeks follow-up. Other measurements include a cardiopulmonary exercise test, MRI-scan, MEG, neuropsychological assessment and various questionnaires by which we measure multiple secondary and explorative outcomes.

Discussion. This study will provide insight into the effects of HIIT and the effects of receiving physical activity advice, in brain tumor patients, and may help develop exercise guidelines.

Supportive Care

Elucidation of older adults values in clinical practice – a scoping review on available instruments

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Background: In older adults, both disease oriented overtreatment and palliative oriented under treatment are present. To ensure appropriate treatment for the individual patient, treatments should align with patients values. However, in daily practice, clinicians often struggle with how to elucidate patient values effectively. This scoping review maps the field on available instruments which were scientifically developed to elucidate values of older adults in clinical practice.

Methods: A systematic search was conducted up to October 2023 in the electronic databases of PubMed, Psychinfo, CINAHL, Medline and Cochrane Library. Articles on instruments elucidating older adults values in a clinical setting were included. Articles on instruments elucidating patient's wishes, preferences or goals were excluded.

Results: After screening of 7759 eligible studies, we included 49 studies containing 38 instruments. Instruments were subdivided in the following categories based on the setting in which the instruments were used: 'health record based interventions', 'advance directives', 'advance care planning programs', 'decision support tools' and 'remaining instruments'. Values were elucidated in different ways, ranging from dichotomous to open questions. Further, instruments promoted different approaches. Some focused on establishing a process of

deliberation, whereas other instruments put focus on reaching a final treatment decision.

Conclusion: We found and categorized a range of instruments promoting different ways to elucidate older adults values. We show how these instruments are used in different settings with various aims. Our overview of available instruments serves as a solid introduction for clinicians to select an instrument for clarifying their individual patients values.

Supportive Care

Fear of cancer recurrence in childhood cancer survivors and their parents

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Background: Primary bone tumours in the lower extremity mostly affect children and adolescents. After diagnosis, several arduous decisions must be made regarding surgical treatment. During follow-up, fear of cancer recurrence (FCR) may cause psychological distress and decrease quality of life (QoL). More information about FCR may allow for the development of strategies that optimize rehabilitation, increase QoL and decrease FCR associated burdens.

Objectives: (1)To determine the prevalence of FCR in childhood cancer survivors (CCS) and their parents, (2)to determine FCR severity and (3)to determine the impact and effects of FCR.

Methods:A systematic literature review was conducted according to the PRISMA statements.Four databases were searched for studies reporting FCR data in CCS or their parents. Quantitative and qualitative syntheses were included.

Results: Thirty-seven studies were included (CCS=6909, parents=1493). The pooled mean prevalence was 64.5% (11.5-88.9%) in CCS and 82.1% (54.0-91.4%) in parents. FCR was more prevalent in adult CCS (M=68.6%,66.0-88.3%) compared to youth CCS (M=36.8%,29.0-55.2%). Severe FCR in CCS and parents was present in 12.1% (0-20.1%) and 9.0% (9.0-10.0%), respectively. The pooled mean FCR transformed to a 0-4 scale was 1.7±0.7 (0.4–2.3) in CCS and 1.9±0.8 (1.8–2.2) in parents. Physical sSymptoms, social cues and medical exams triggered FCR and increased severity in CCS and parents. A high FCR was associated with lower QoL. FCR caused a decrease in self-examinationspatients examining themselves in some patients.

Conclusion: FCR prevalence was high, is relatively often severe and presents as lower QoL among others. This suggests the importance of early recognition and discussion of FCR during follow-up.

Supportive Care

Drawing Cancer Chronicles: Exploring the Visual Narratives of People with Advanced Incurable Cancer Using Rich Pictures Over Time

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Introduction

Stories occupy an important place in social life. They help us to understand the world around us, to find meaning in our experiences, and to establish a sense of who we are. However, when diagnosed with incurable cancer, patients may need to create new stories about their lives to replace the ones disrupted by their diagnosis. Yet, telling verbal stories about life with cancer may also be hard for patients. Therefore, we investigate patients' visual stories about illness over time through so-called Rich Pictures (RPs) - hand-drawn pictures about complex experiences.

Methods

RPs about life with cancer were drawn by Dutch advanced incurable cancer patients (n=26) at the start (T1, n=26) and end (T2, n=11) of an arts-based, narrative intervention. RPs were analyzed using an inductive methodology working from icon-level coding towards generating theory grounded in the data – so-called 'grounded theory'.

Results

Through RPs, patients tell multi-dimensional stories about their experiences. These included their disrupted sense of self; shifts in sources of meaning; and the dynamics of their relationships with others. Comparing RPs T1 and T2 showed changes in patients' stories. For example, stories once focused on disruption, barriers, suspension and uncertainty, evolved into natural and progressive flows, connectedness and completion.

Conclusion

RPs are useful in exploring the multi-dimensional stories patients tell about their illness. Repeated use of RPs enables 'chronicling' illness experiences, revealing

how patients' stories and meaning making of their illness evolve over time. We therefore forward RPs as novel qualitative research tools.

Supportive Care

Work resumption following lower grade glioma surgery: a multicenter cohort study

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Patients with newly-diagnosed lower grade glioma are typically of working age. However, work resumption after surgical resection is uncertain, possibly due to loss of capacity from resection of tumor-infiltrated brain regions. Therefore, we explore the association between work resumption and the locations of surgical cavities in addition to other patient, tumor and treatment characteristics.
This retrospective cohort consisted of adults undergoing first-time resection for lower-grade glioma between 2011 and 2016 in three hospitals. Employment was evaluated at baseline and within one year following surgery to determine work resumption. The association between work resumption and patient, tumor and treatment characteristics was analyzed using logistic regression. Resection cavities were segmented from post-operative MRI scans, registered to standard brain space and related to grey nuclei, cortical networks and white matter tracts using atlas parcellations. To identify brain regions potentially involved with work resumption, the association between work resumption and surgical cavities was analyzed using Bayesian hurdle regression. The identified regions and characteristics were jointly analyzed in their association with work resumption using multiple logistic regression.
Of 207 patients, 181 (87%) were employed at baseline. Of these employed

patients, 111 (61%) had resumed work at follow-up. Males of younger age with smaller tumor volumes, and larger extent of resection were significantly more likely to resume work. Surgical cavity locations were not associated with work resumption.

Two thirds of patients resumed work one year after surgery. Work resumption was associated with patient characteristics, smaller pre-operative tumor volume, and extent of resection, but not with surgical cavity location.

Supportive Care

Experiencing Art-Based Learning in Palliative Care: Promoting and hindering factors of organizing an exhibition for online and on-site Art-Based Learning for patients with incurable cancer.

Marike Geurts[1]

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Art has proven to be an effective medium in complementary therapies aimed at improving quality of life of cancer patients. This research builds on a pilot study by Russel et al. (2023) that argues for the feasibility of the method of Art-Based Learning to facilitate meaning-making processes for cancer patients in palliative care. The aim of this study is to gain insight in the promoting and hindering factors of organizing the ABL-intervention for patients with incurable cancer through an online and on-site art exhibition using the Amsterdam UMC art collection. The research is part of a project on Art-Based Learning in palliative care with partners such as ArtEZ, University of Twente, Amsterdam Museum and Museum Jan Cunen, among others.

In order to do is, we chose a qualitative research method using semi-structured interviews. Patients are included if they are diagnosed with incurable cancer, in a good health condition (WHO 0 or 1), above 18 years old, able to speak Dutch and able to independently come to the hospital. In addition, for the online sessions, patients need access to the internet, a device and a microphone. After the ABL-session, an interview will be conducted using a questionnaire with open questions. The interviews will be recorded, transcribed and thematically analyzed using MAXQDA.

The inclusion of patients starts in April 2024. Since this abstract is from March, no research has yet been conducted. This also means that at the CCA conference, preliminary results will be presented.

Supportive Care

Reducing cognitive impairment in glioma with personalized repetitive transcranial magnetic stimulation and cognitive strategy training – TRUE GRIT study protocol

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Introduction: Glioma patients often experience cognitive problems, which are associated with decreased functional independence and health-related quality of life. Current interventions for cognitive impairment, such as cognitive strategy training, are based on the principle of brain plasticity. However, the effects of these interventions are hampered by small effect sizes. Repetitive transcranial magnetic stimulation (rTMS) has the potential to induce plasticity of brain networks that are related to cognitive processes. Identifying treatment targets based on individual resting-state brain networks is promising in creating a more effective rTMS treatment. Adding such personalized rTMS intervention to cognitive strategy training might enhance plasticity and possibly creates greater, more beneficial effects on cognition.

Aim: To investigate the feasibility of a personalized rTMS in combination with a cognitive strategy training to improve cognition in glioma patients during stable disease.

Methods: In this feasibility study 16 diffuse glioma patients during stable disease stage, i.e. without clinical or radiological progression, will be included. Patients will be randomized to an active or sham rTMS condition. Both groups receive 24 rTMS sessions over 12 weeks. Simultaneously with the rTMS, all patients receive a cognitive strategy training ("Niet Rennen Maar Plannen"). The primary endpoint is the feasibility of the combined intervention and study measurements. Feasibility will be concluded by a completed study rate of 80%.

Conclusion: A feasibility study for a combination intervention consisting of rTMS and cognitive strategy training to improve cognition in glioma patients.

Supportive Care

A transatlantic evaluation of ideal outcome after distal pancreatectomy

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Introduction
Assessment of surgical outcomes is important to improve quality of care. Recently, the Global Audits on Pancreatic Surgery Group (GAPASURG) introduced 'ideal outcome' (IO) as a new international composite measure. In pancreatoduodenectomy the rate of IO was 54%. The rate of IO has not been studied yet in distal pancreatectomy, the second largest group in pancreatic surgery.
Methods:
This transatlantic analysis included patients after distal pancreatectomy from the audits from North-America (NSQIP), Germany (STUDOQ), the Netherlands (DPCA), and Sweden (2018 – 2020). The IO is defined by the absence of (1) in-hospital mortality, (2) severe complications (Clavien Dindo ≥3), (3) postoperative pancreatic fistula (ISGPS grade B/C), (4) reoperation, (5) hospital stay exceeding the 75th percentile, and (6) readmission.
Results:
In total, 20,158 patients after distal pancreatectomy were included, of whom 46% underwent a minimally invasive distal pancreatectomy (MIDP). The median length of stay was 6 days (IQR 5-10) and in-hospital-mortality 1%. Overall, 11,907 (59%) patients achieved IO. The IO rate varied across audits: 58.5% in North-America, 53.6% in Germany, 56.9% in the Netherlands, and 61.9% in Sweden (P<0.001). Prolonged length of stay (22.0%; 21.0-24.2%) and severe complications (20.2%; 11.0-22.8%) were the most common reasons for failure to reach IO. The use of MIDP was associated with IO (65.3% vs. 53.3%, p<0.001), compared to open surgery
Conclusion:
In this transatlantic analysis, the new composite measure IO was reached in 59% of over 20,000 patients after distal pancreatectomy and can be used for evaluating, comparing, and improving patient outcome.

Supportive Care

Severe Thrombocytopenia Results in Disproportional Healthcare Utilisation in Female Patients Treated for Glioblastoma

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Thrombocytopenia is a chemotherapy-induced adverse event during the standard treatment of glioblastoma and necessitates, generally, treatment modifications. This cohort study aims to assess the influence of thrombocytopenia on healthcare utilisation among patients diagnosed with glioblastoma, with particular emphasis on potential sex-based disparities.

We retrospectively collected patient demographics, treatment details, healthcare resource utilisation data, and thrombocytopenia occurrences during both the chemoradiotherapy (CRT) and maintenance phases of standard glioblastoma treatment. The nadir thrombocyte values per week during CRT and per month during the adjuvant phase were categorized based on the Common Terminology Criteria for Adverse Events (CTCAE). The association between the severity of thrombocytopenia and healthcare utilisation was analysed using Log-Linked Generalized Linear Mixed Models.

We included 206 patients with a median age of 60.3 (IQR: 51.6–67.7) years, 35.4% were women, and thrombocytopenia occurred in 60.0% of patients. The occurrence of thrombocytopenia during CRT was associated with increased utilisation of healthcare resources and was largest in women who developed grade 4 thrombocytopenia (OR=30.144, $p<0.001$ in women and OR=11.11, $p<0.001$ in men). Comparatively, the onset of thrombocytopenia was equally linked to heightened utilisation of healthcare resources during the maintenance phase, but no discernible differences were observed between males and females (OR=17,78, $p<0.001$).

Severe thrombocytopenia during the treatment of glioblastoma results in a

considerable increase in healthcare utilisation disproportionally impacting female patients. These data suggest that prevention and early management of thrombocytopenia, especially in women, can reduce healthcare utilisation in patients with glioblastoma and may prevent early treatment cessation.

Supportive Care

Family involvement in clinical care: an approach to change hospital culture

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Even though family caregivers (FC) play an essential role in the delivery of simple fundamental care activities to oncology patients, this is inadequately applied. FC mention feeling unprepared and lacking knowledge.

Active involvement of family caregivers in fundamental care activities may improve quality of health care during and after hospitalization, and may result in higher confidence in family caregivers to deliver fundamental care to the patient.

Using mixed methods research, this PhD trajectory concerns the active involvement of family in clinical care.

Supportive Care

Prevention of opioid-induced constipation in cancer patients: a systematic review

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Background: Cancer-related pain often requires opioid treatment, with opioid-induced constipation (OIC) as a prevalent gastrointestinal side-effect. For prevention of this side-effect osmotic (e.g. polyethylene glycol) and stimulant (e.g. bisacodyl) laxatives are widely available and used. Newer drugs such as naloxone in a fixed combination with oxycodone and the peripherally acting μ-opioid receptor antagonist naldemedine also have become available for the prevention of OIC. This systematic review aims to summarize the scientific evidence on pharmacological strategies for the prevention of OIC in cancer patients.
Methods: A systematic search in PubMed, Embase, Web of Science and the Cochrane Library was completed up to 22 October 2022 for both randomized and non-randomized studies with OIC and adverse events as an outcome.
Results: Eight trials were included in the review. Three RCTs compared laxatives with other laxatives, finding no clear differences in effectivity of the laxatives used. One cohort study showed a significant benefit of magnesium oxide compared to no laxative. One RCT found a significant benefit for naldemedine compared with magnesium oxide. Preventive use of oxycodone/naloxone did not show a significant difference in two out of three studies compared to oxycodone or fentanyl. A meta-analysis was not possible.
Conclusions: Magnesium oxide and naldemedine are most likely effective for prevention of OIC in cancer patients. However, due to the heterogeneity of the studies no hard conclusions can be drawn. More studies comparing laxatives with each other and with opioid antagonists are necessary before evidence-based recommendations for clinical practice can be made.

Supportive Care

Research Project RbNeuroQol Study

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Retinoblastoma (Rb) is the most common form of ocular cancer in children, with high survival rates in developing countries (>90%). Children are usually diagnosed at a young age (<5 years) and are subjected to intensive treatment such as laser, cryo-, chemotherapy (CH) and/or radiotherapy (RT), or enucleation in case the eye appears to much affected. Brothers and sisters or offspring of heredity Rb survivors could be at risk to develop Rb themselves (so called 'Rb risk carriers') and will be screened according to the Dutch Rb screening protocol. The medical treatment and follow-up or screening takes place under general anesthesia (GA) up to four or five years of age, while the brain is still developing and therefore extra vulnerable to iatrogenic damage, including neuropsychological complications that impact cognitive functioning. Over the past years awareness for the emotional aspects and health related quality of life (HRQoL) of Rb expanded enormously. However, immediate effects of the oncological treatment, as well as secondary effects due to multiple GA on cognitive development and emotional wellbeing of the Rb population is still understudied. Therefore the RbNeuroQoL Study was designed to gain insight in neurocognitive development and psychosocial functioning from childhood into young adulthood of Rb survivors and Rb risk carriers. It is hypothesized that extensive treatment and multiple GA is negatively associated with cognitive functioning, psychosocial functioning and HRQoL in Rb survivors. Moreover, that extensive treatment and multiple GA increases psychosocial struggles including trauma, that puts additional pressure on cognition and HRQoL

Supportive Care

TIRELESS: Making cognitive behavioral therapy for cancer-related fatigue fit for implementation in patients with cancer receiving palliative systemic treatment

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Background:
Fatigue is a prevalent and highly disturbing symptom for patients with cancer receiving systemic treatment with palliative intent. Cognitive behavioral therapy (CBT) has been shown to effectively reduce cancer-related fatigue in this patient group. However, its implementation faces challenges as the intervention is time-intensive and the availability of trained psychologists is limited. The primary aim of this study is to determine the non-inferiority of interdisciplinary web-based CBT provided by nurses, compared to a benchmark study where CBT was provided by psychologists in its effect on reduction in cancer-related fatigue.

Methods:
The TIRELESS study is a pragmatic prospective non-randomized trial. The participants will receive a 12-14 week CBT intervention. Primary and secondary outcome measures will be assessed at baseline, post-intervention (14 weeks), and follow-up (26 weeks). The primary outcome measure is fatigue severity (Checklist Individual Strength subscale fatigue severity). Secondary outcome measures are fatigue, functional impairment, quality of life and health care costs. Outcomes of the TIRELESS study and the benchmark study will be compared to determine non-inferiority. In addition, interviews will be conducted to gain insight in nurses' and patients' experiences with the intervention.

Results:
Recruitment will start in April 2024. Results are expected in August 2026.

Discussion:
Especially for this patient group, integration of CBT for fatigue in routine medical care is essential. If our approach is non-inferior to the original evidence-based (benchmark) intervention, this will enable implementation of cancer-related fatigue interventions for the growing group of patients with cancer receiving palliative treatment with minimal burden and good scalability.

Supportive Care

Interpretable Survival Analysis using Explainable Artificial Intelligence

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Survival analysis is a commonly used method for time-to-event analysis in medicine because it can handle censored data. However, interpreting these survival algorithms can be challenging due to their opaque methodology, and these algorithms are often unable to account for variable interactions.

This study explores the implementation of Explainable Artificial Intelligence (XAI) into a survival analysis model that can take advantage of non-linearities between the variables. The Genetic Programming for Gene-pool Optimal Mixing Evolutionary Algorithm (GP-GOMEA) uses a gray-box principle to create new variables, resulting in symbolic expressions as non-linear combinations of initial variables. A survival tree will be created using both the initial and new variables.

To ensure that this survival tree remains interpretable and provides good accuracy, a multi-objective approach is used. This approach generates multiple solutions with competing trade-offs between prediction accuracy, symbolic expression complexity, and survival tree complexity. Furthermore, two variables are created simultaneously to uncover relationships between non-linear variables.

The feasibility of using XAI for survival analysis is demonstrated using a dataset containing numerical data of cervical cancer patients treated with radiotherapy. Nonlinear variables were created to reduce the size of the survival tree while maintaining predictive ability comparable to, or better than, a (traditional) survival tree using single linear variables.

Given the increasing importance of model explainability in real-world clinical applications, our approach has demonstrated the ability to identify interpretable non-linear features. Furthermore, it results in a smaller decision tree which can be used to explain the survival risks in a stepwise fashion.

Supportive Care

Expert Nursing Views on Barriers Related to Improving Health-Related QOL Post Oesophagogastric Surgery and Use of Patient Reported Outcome Measures in the Clinical Setting-

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Introduction: Nurses as healthcare providers play an integral role in identifying and addressing social needs, decreasing distress and promoting the quality of life of cancer patients and their family caregivers. Patient-reported outcome measurements (PROMs) may help nurses achieve these aims. There have been few studies to help understand nurses' views on barriers related to improving health-related QOL post-oesophagogastric surgery and the use of PROMs. This study aims to explore the perspectives of specialist nurses regarding health-related quality of life post OG cancer surgery, understand the barriers, and facilitate interventions to improve HRQOL post-surgery.
Materials and methods: A qualitative semi-structured interview study and thematic analysis were applied. Data was audiotaped and transcribed from nine specialist nurses in OG cancer surgery in the UK. Multi-centre study across the UK where every participant was an upper GI specialist nurse with at least one year of working experience in OG cancer surgery.
Results: Four themes were deduced from the data: psychological, emotional, and social issues that affect postoperative recovery, challenges in using PROMS in clinical practice, obstacles to implementing patient-centred care and improving HRQOL, and post-surgery patients encountering difficulties accessing healthcare. These were mentioned by nurses as barriers to improving patients' QOL post-OG cancer surgery.
Conclusion: Awareness of barriers to improving health-related QOL post-oesophagogastric surgery will help future healthcare providers enhance PROM use and improve patient-centred care in a clinical setting. Improved patient-centred care will enable healthcare providers to address issues affecting QOL post-oesophagogastric surgery patients.

Supportive Care

Sustainable implementation of tailored self-help speech and swallowing exercise programs for patients treated for head and neck cancer

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Introduction

Treatment of speech and swallowing problems in head and neck cancer (HNC) care is of major importance as it has a large impact on a patient's daily life and health-related quality of life. Exercise programs are found to be effective in reducing these problems. In AmsterdamUMC two tailored self-help exercise programs have been developed targeting the prevention or reduction of speech and swallowing problems among HNC patients: Head Matters (HM) and In Tune without Cords (ITwC). We aim to make these two programs available for all HNC patients in the Netherlands. The aim of this implementation-study is therefore i) to investigate barriers and facilitators of implementation of HM and ITwC among healthcare professionals, and ii) to investigate the uptake and usage of HM and ITwC among HNC patients.

Methods

To optimize the implementation and upscaling strategy, we specifically aim to adapt and renew the self-help exercise programs with integration in the EPD. A mixed methods design, combining qualitative interviews and quantitative research methods will be used to investigate barriers and facilitators on the level of the healthcare professionals and patient.

Results

So far, 12 out of 14 hospitals involved in HNC care showed interest in implementing HM. Insights on barriers and facilitators will immediately be used to facilitate implementation of the exercise programs.

Conclusion

This project is highly relevant for the implementation of self-help exercise programs in The Netherlands. Offering self-help programs will improve and maintain the accessibility and quality of supportive care for HNC patients.

Supportive Care

The use of Patient-Reported Outcome Measures within Gynaecologic Oncology for Patients with Limited Health Literacy: a mixed-methods study

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Gynecological cancer profoundly affects the well-being of women, highlighting the importance of thorough follow-up care. Patient-Reported Outcome Measures (PROMs) seem to benefit follow-up care by providing valuable insights into patient experiences. For populations with low health literacy levels it remains uncertain if PROMs are efficient. This study seeks to address this gap by assessing the prevalence of low health literacy levels, and by delving into perceived challenges and their perspectives regarding PROMs.

This study entailed a mixed-methods approach. Gynaecological cancer patients with limited health literacy were identified using the Single-Item Literacy Screener (SILS). Descriptive statistical analyses were performed. With a subset of the identified patients cognitive and semi-structured interviews were performed. Interviews were transcribed verbatim and analyzed using an iterative thematic approach.

Statistical analysis on 84 patients revealed that 26.2% of the patients had low health literacy based on the SILS. Cognitive interviews showed that patients interpret the questions differently and sometimes not as intended by physicians. Participants universally criticized the extensiveness of PROMs, citing feelings of frustration and weariness. Despite challenges, most participants were willing to complete them. Personal contact with their physician throughout follow-up care was important.

A significant proportion of patients with gynecological cancer, who visit the outpatient clinic, face limited health literacy. Patients with limited health literacy have little understanding of the purpose of PROMs, which may influence their approach to completing them. Improving communication with physicians, adapting the questionnaire and the way it is embedded in healthcare may promote its use for these patients.

Supportive Care

Late toxicity and health-related quality of life following definitive chemoradiotherapy for esophageal cancer: a systematic review and meta-analysis

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Background: Definitive chemoradiotherapy (dCRT) is a treatment option with curative intent for patients with esophageal cancer that could result in late toxicities and impact health-related quality of life (HRQoL). This study aimed to review the literature and perform a meta-analysis to investigate the effect of dCRT on late toxicities and HRQoL in esophageal cancer.
Methods: A systematic search was performed in MEDLINE, EMBASE and PsychINFO. Prospective phase II and III clinical trials, population-based studies and retrospective chart reviews investigating late toxicity and/or HRQoL after dCRT (≥50 Gy) were included. HRQoL outcomes were analyzed using linear mixed effect models with restricted cubic spline transformation. HRQoL changes of ≥10 points were considered clinically relevant. Risk of toxicities was calculated using number of events and the total study population.
Results: Among 41 included studies 10 assessed HRQoL and 31 late toxicity. Global health status remained stable over time and improved after 36 months compared to baseline (mean change +10.5). Several tumor-specific symptoms, including dysphagia, eating restrictions and pain, improved after 6 months compared to baseline. Dyspnea worsened after 6 months (mean change +16 points) compared to baseline. Risk of any late toxicity was 48% (95% confidence interval [CI] 33-64%). Late toxicity risk of any grade for the esophagus was 17% (95% CI 12-21%), pulmonary 21% (95% CI 11-31%), cardiac 12% (95% CI 6-17%) and any other organ 24% (95% CI 2-45%).
Conclusion: Global health status and tumor-specific symptoms improved within 6

months after dCRT, but substantial risks of late toxicity were observed.

Supportive Care

Listening to the Patients' Voice: What Can We Learn From Patients About Gynaecologic Oncology Follow-up Care During the COVID-19 Pandemic?

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OBJECTIVES
This study aimed to explore how patients with gynaecological cancers experienced follow-up care during the COVID-19 pandemic and their view on general aspects of follow-up care practices in order to identify key elements of follow-up care.

METHODS
Five focus group (FG) discussions and two individual interviews were organised in the Netherlands to explore the perspectives of patients with ovarian (n=5), cervical (n=6), endometrial (n=5), or vulvar cancer (n=4) who received follow-up care during the COVID-19 pandemic. Subsequent transcripts were analysed using a deductive approach to thematic analysis using the existing framework of the eight Picker's Principles of Person-Centred Care (PPC).
RESULTS
Eighth themes were generated from data analysis 1) patient experience of the COVID-19 pandemic, 2) accessibility, 3) individualised care, 4) healthcare coordination, 5) information, 6) guidance in healthcare 7) patient-provider relationship, and 8) emotions and feelings. The main changes experienced during the COVID-19 pandemic were the introduction to remote healthcare and the absence of family members. An interaction between themes was found highlighting that a well-defined point of contact acts as a catalyst, improving the provision of clear information and healthcare guidance, which in turn empowers patients and improves overall well-being.
CONCLUSION
In conclusion, this study highlights the need for flexible and personalised follow-up care to promote patient-empowerment. The findings highlight the importance of tailored support, involvement of family members, addressing information gaps, and overcoming barriers to self-management and how to approach during future periods of scarcity.

Supportive Care

A qualitative evaluation of a brief intervention on meaning-making in the re-entry phase

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Introduction

Patients in the re-entry phase (that is, the first 18 months after curative cancer treatment) may use meaning-making to deal with existential concerns imposed by the cancer. Meaning-making involves the use of existing sources of meaning, and a search for or use of new or adapted sources of meaning. We developed an intervention to support patients with this meaning-making process, with a focus on setting meaningful goal. The present pilot-study used a qualitative design to evaluate this intervention.

Methods

Patients were included after finishing systemic treatment for breast cancer stage I-III or melanoma stage III. The intervention comprised a one-hour conversation guided by a spiritual counsellor who explored patients sources of meaning, in order to help patients to set meaningful goals for picking up life. We used a semi-structured interview post-intervention to evaluate the experience, appreciation and benefits of the intervention. Thematic analysis was used to analyse the data.

Results

Fifteen patients were included (8 melanoma and 7 breast cancer). Qualitative interviews demonstrated an overall positive experience and appreciation of the intervention. Patients reported the following benefits: reflection on existential concerns and sources of meaning, validation of existing sources of meaning, insights regarding new sources of meaning and motivation to pick up life; and to a lesser extent goal-setting or undertaking specific action post-intervention.

Conclusions

This pilot-study shows that an intervention for patients with cancer to set meaningful goals for the re-entry phase was positively experienced and well appreciated. It suggests that the intervention supported patients meaning-making process.

Target & Therapy Discovery

Modulating CRC Stem Cell Dynamics with Combination Therapy

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Colorectal cancer (CRC) progression depends on the survival mechanisms orchestrated by BCL-XL, a key regulator in the disease evolution. While targeting BCL-XL with BH3 mimetics holds therapeutic promise for CRC, its clinical use is limited by associated platelet toxicity. This highlights the need to identify sensitizers for BCL-XL inhibitors at non-toxic doses. To overcome this problem, we performed a drug screen where GSK3 inhibitors were identified as potent sensitizers to low doses of the BCL-XL inhibitor A-1155463. This combination treatment synergistically induced apoptosis in CRC cells and mechanistic insights further revealed that simultaneous targeting of GSK3 and BCL-XL efficiently triggered apoptosis by facilitating BAX translocation to the mitochondria. Notably, this synergistic effect disrupted the dominance of APC mutant stem cells over their wild-type counterparts, offering a promising therapeutic strategy to block tumor progression. Our findings underscore the potential of using GSK3 and BCL-XL inhibitors as combination therapy, providing new options for developing effective treatments for CRC.

Target & Therapy Discovery

Finding and targeting TEAD-regulated long non-coding RNAs in colorectal cancer

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TEAD is a seminal protein in a plethora of important pathways in the intestine. Upregulation of TEAD is known to have oncogenic potential in the initiation and progression of colorectal cancer. However, the role that long non-coding RNAs (lncRNAs) play in this oncogenic process remains unexplored. We successfully silenced TEAD expression in 10 colorectal cancer cell lines, and profiled changes in the lncRNA landscape using Global Run-On Sequencing (GROSeq). To assess the function of all TEAD-regulated lncRNAs, we then made use of CRISPR-interference (CRISPRi). Large-scale CRISPRi screens have been performed in 2 colorectal cancer cell lines constituting 2 different Consensus Molecular Subtypes (CMS). Further analysis has revealed a promising oncogenic candidate, which is being investigated for its therapeutic potential in colorectal cancer.

Target & Therapy Discovery

Unveiling pathway compensation and target-independent resistance mechanisms to EGFR kinase inhibitors in glioblastoma via phosphoproteomics, and CRISPR-mediated knockdowns for identifying effective therapeutic strategies.

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Glioblastoma poses significant therapeutic challenges, including resistance to epidermal growth factor receptor (EGFR) kinase inhibitors. Resistance to kinase inhibitors can be classified as innate- or acquired resistance. Furthermore, resistance mechanisms can be grouped as target independent mechanisms or pathway compensation. Our study integrates phosphoproteomics and CRISPR-mediated knockouts to uncover resistance mechanisms and identify potential drug targets for combination therapy.

Using phosphoproteomics, we evaluated pan-kinase activity and EGFR-site specific phosphorylation after treating with EGFR kinase inhibitors, revealing altered kinase activity, potentially associated with EGFR inhibitor resistance. CDK1 and CAMK2A emerged as promising targets due to their elevated activity.

Drug combination experiments targeting CDK1 or CAMK2A alongside EGFR inhibitors demonstrated somewhat synergistic effects on Glioblastoma cell viability in a short-term assay, suggesting a potent therapeutic strategy. Our approach, informed by phosphoproteomics and functional genomics, elucidates novel targets and treatment strategies for overcoming EGFR inhibitor resistance in GBM. Newly identified drug synergies are currently being tested in long-term assays.

This study highlights the potential of precision medicine in GBM treatment and underscores the importance of further clinical validation to advance therapeutic outcomes for patients facing this challenging disease.

Target & Therapy Discovery

Enhancing the targeting ability of RNA therapeutics-loaded extracellular vesicles to pancreatic cancer by glycoengineering

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Introduction: In vivo studies have shown that extracellular vesicles (EV) from fibroblasts loaded with siRNAs can target pancreatic ductal adenocarconoma (PDAC), but with low specificity and efficiency. Previously, we demonstrated that immune-cell EVs efficiently mediate RNA delivery for targeted gene repression. Glycosylation may enhance EV cell-specific targeting and cargo delivery as shown for many viruses.

We hypothesized that the targeting ability and therapeutic efficiency of RNA-loaded EVs could be further improved by understanding the glycosylation status of the EV surface.
Methods: We designed a KRAS-mutant model to explore the role of active macropinocytosis and glycosylation in EV uptake . To this end, we first measured the uptake of 70 kDa FITC-dextran by cancer cells as a marker of macropinocytosis by fluorescence microscopy and FACS. EIPA is used as an inhibitor of macropinocytosis. Serum starvation are used to increase the level of macropinocytosis. Subsequently, we also measured the uptake of PKH67-stained EVs with different glycosylation status by PDAC cells.
Results: Fluorescence microcopy shows that dextran enters mutant KRAS cells via macropinocytosis conforming previous reports. FACS showed that the uptake of FITC-dextran by recipient cells increased with incubation time, in both KRAS-mutant cancer cells and is enhanced under serum-starvation, a driver of macropinocytosis. Fluorescence microscopy and FACS results showed that PDAC EVs are taken up in a dose-dependent manner.
Conclusions: We developed a cell model the biochemical properties and dynamics of EV uptake by KRAS-mutant cancer cells. Screening strategies will be applied to enhance the drug delivery potential of EVs.

Target & Therapy Discovery

Blood-borne Assessment of Stromal activation in esophageAL adenocarcinoma to guide tocilizumab Therapy: a randomized phase II proof-of-concept study (NCT04554771)

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Background:
Production of IL6 by the tumor stroma of esophageal adenocarcinoma(EAC) causes epithelial-to-mesenchymal transition of tumor cells and resistance to chemoradiation. Serum ADAM12 protein levels measure stroma activity whilst IL6 and IL6R monitor IL6 inhibition. We aim to demonstrate that targeting stroma with tocilizumab in EAC patients increases efficacy of chemoradiation.
Methods:
This was a multicenter, randomized, open label, phase II proof-of-concept trial in 48 patients with resectable EAC. Patients were stratified for serum ADAM12(cutoff of 203 pg/mL) and randomized for chemoradiation(CROSS-regimen) or addition of tocilizumab to chemoradiation, followed by surgery. Tocilizumab(8 mg/kg) with a maximum of 800mg was given intravenously for three cycles. Biopsies and tissue were collected at baseline, during treatment, at resection and three months thereafter. Primary endpoint was histopathological response to chemoradiation according to the Mandard score.
Results:
After inclusion of 36 patients, the trial was discontinued due to safety concerns. Perforation of the esophageal wall was observed in 3/20(15%) of patients in the intervention arm compared to 0/16(0%) in the control arm. There was no significant difference in Mandard score between treatment arms($p = 1.000$). Patients treated with tocilizumab had significantly higher serum levels of IL6 and IL6R during treatment($p < 0.0001$).
Conclusions:
Addition of tocilizumab to chemoradiation in patients with EAC is unsafe and should not be considered for clinical practice. Potentially, concurrent inhibition of IL6 impairs wound healing whilst chemoradiation obliviates the tumor in the

esophageal wall, increasing the risk for perforation. We are currently investigating this hypothesis with RNA sequencing and immunohistochemistry.

Target & Therapy Discovery

Cell competition in the intestine: Msh2-mutant cells lose the battle

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Colorectal cancer (CRC) development is characterized by stepwise accumulation of mutations over time. As it generally takes 10-15 years to develop CRC, it is assumed that development is initiated in the long-lived intestinal stem cells (ISCs). In every crypt bottom, a number of functional ISCs continuously compete for crypt occupancy, a process characterized by stochastic loss-and-replacement events. We previously demonstrated that ISCs carrying oncogenic mutations have a competitive advantage over wildtype ISCs, thereby biasing competition in favour of mutants resulting in permanent fixation of mutants within a crypt. Importantly, modulating cell competition and preventing fixation of mutants can provide a powerful chemoprevention strategy for hereditary CRC syndromes with known germline mutations, such as Lynch Syndrome (LS).

Although LS is the most common heritable CRC syndrome, its pathogenesis remains poorly understood. LS is characterized by mutations in genes encoding proteins functioning in the mismatch repair (MMR) pathway, such as MSH2. To study competition between wildtype and MMR-deficient ISCs, we use in vivo mouse models for conditional Msh2 loss as well as in vitro intestinal organoids. Surprisingly, our preliminary results suggest that Msh2-deficient ISCs have a competitive disadvantage compared to wildtype neighboring ISCs. We are currently characterizing the reciprocal interactions between competing wildtype and mutant ISCs to identify molecular mechanisms driving this disadvantage. Ultimately, we aim to exploit this vulnerability to inhibit further expansion of mutant ISCs and prevent the onset of CRC. Moreover, unraveling the vulnerabilities of MMR-deficient ISCs will provide important cues for novel therapeutic strategies to target established MMR-deficient CRCs.

Target & Therapy Discovery

Identifying the role of Wnt signaling-associated circular RNAs in colorectal cancer

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Colorectal cancer can be characterized by the sequential accumulation of mutations in driver genes involved in cellular proliferation. Early on in the adenoma-to-carcinoma sequence, the majority of colorectal cancer patients acquire mutations in the Wnt signaling-associated APC gene. Even though this aberrant Wnt signaling pathway has been widely characterized, direct therapeutic targeting of its protein intermediates remains challenging. In contrast, non-coding RNAs have recently been shown to be highly tumor specific molecules and to mediate oncogenic signaling through various mechanisms. Their subgroup of circular RNAs, which are formed by an alternative splicing process called back-splicing, are rather undiscovered molecules that have the potential to influence cellular signaling. Due their ability to effectively bind miRNAs, RNA binding proteins and influence transcription, research into the function of circRNAs in aberrant cancer signaling has gained attention. Therefore, here we attempt to identify functional circRNAs associated with the oncogenic Wnt signaling pathway. Using RNA sequencing, we identify multiple circRNAs which expression levels are influenced upon ß-catenin knockdown. Using a cas13D-mediated CRISPR dropout screening approach we specifically knock down circRNAs in a panel of CRC cell lines to evaluate their impact on tumor proliferation. We are currently investigating the mechanism in which the top candidate(s) from this CRISPR screening influence CRC proliferation. Further characterization of the oncogenic role of these circRNAs will ultimately provide insights into how we can use these molecules for novel, tumor-specific therapeutic strategies.

Target & Therapy Discovery

Video versus face-to-face preoperative surgical consultation (VIDEOGO): a multicentre, randomized controlled, non-inferiority trial

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Video consultation (VC) rapidly gained popularity as a standard component of outpatient care during the COVID-19 pandemic. However, high-quality evidence regarding patient satisfaction and information retention using VC is lacking. We aimed to assess whether VC is non-inferior to F2F consultation in the surgical outpatient clinic focusing on satisfaction, information retention and efficacy in the post-pandemic era.

This multicentre randomized controlled non-inferiority trial (NL9108) was conducted in three centres in the Netherlands. Patients scheduled for pre-operative appointment in the surgical outpatient clinic were randomly allocated (1:1) to VC or F2F consultation. Primary outcomes were patient satisfaction, assessed for non-inferiority with a predefined margin of -10% and information retention. Secondary outcomes included surgeon satisfaction, and efficacy analysed for superiority. All outcomes were assessed using online questionnaires.

Between February 2021 and October 2023, 112 patients were randomly assigned to VC (n=57) or F2F (n=55). The patient satisfaction score was 85·4 out of 100 (12·3) in the VC-group versus 85·2 out of 100 (14·2) in the F2F-group (mean difference (MD) 0·2, 95%CI -5·1-4·8), well within the non-inferiority margin (Pnon-inferiority<0·001). The surgeon satisfaction score was 76·3 (10·9) in the VC-group versus 78·5 (8·4) in the F2F-group (MD 1·6, 95%CI -2·1-5·3). Regarding CO2 footprint, the CO2 emission per consultation was 93·0 (0·0) and 895·1 [588·2-

1534·7] grams CO2eq per consultation (P<0·001), respectively.

We found that VC satisfaction scored not worse than F2F in terms of patient and surgeon satisfaction, without impacting information retention, while benefiting from improved efficacy, supporting its implementation as an option alongside F2F consultation.

Target & Therapy Discovery

Identifying the functional role of GPRC5A in AML

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Acute myeloid leukemia (AML) is a hematological malignancy characterized by the accumulation of immature myeloid precursors in the bone marrow. To eliminate the leukemic blasts and achieve complete remission, patients are treated with combination chemotherapy. Despite good responses to initial treatment, the 5-year overall survival rate is 20-40% due to the survival of chemotherapy-resistant leukemia cells, known as minimal residual disease (MRD). Current knowledge gaps on MRD development and ways to target the therapy-resistant cells are hampering progress in development of treatments that successfully prevent relapse and increase AML cure rates. To delineate mechanisms responsible for MRD development and identify vulnerabilities of chemoresistant AML cells, we purified leukemic cells from three AML patients at diagnosis and after treatment by using the leukemia-associated immunophenotypes. Gene expression profiling was performed, and G-protein-coupled receptor family-C, member-5, group (GPRC5a) was identified as a top upregulated gene in MRD. As GPRC5A is dysregulated in various human cancers and associated with poor prognosis, we firstly studied the prognostic value of GPRC5A and its expression in different molecular AML subtypes and checked its membrane expression in a panel of AML cases at diagnosis. We found that AML cases with ASXL1- or p53-mutation have higher GPRC5A expression and medium/high membrane expression relates to poorer treatment outcome. Moreover, we observed that GPRC5A is expressed on both immature and mature leukemic blasts and that daunorubicin induces GPRC5A membrane expression

Therefore, GPRC5A is biomarker associated with poor clinical outcome in AML and its expression can be induced by chemotherapy.

Target & Therapy Discovery

Introducing elesclomol in a novel small molecule drug conjugate to improve it precision in vivo.

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Peritoneal metastases (PM) resulting from colorectal cancer (CRC) are associated with a poor prognosis. When surgical removal of the tumors is possible, current treatment is a combination of cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC). Despite the potential for cure from the combined approach, the effectiveness of the chemotherapy component remains limited. The development of novel drugs with high efficacy against PM could potentially improve clinical outcomes. In drug databases we compared the mesenchymal CMS4 classified cell lines, which are strongly associated with PM, to the CMS2 classified cell lines. Although the identified copper ionophore elesclomol proved effective in vitro, the data was not reflected in vivo due to minimal copper accumulating in the tumor. As a result, it can be assumed that insufficient elesclomol is reaching the tumors specifically. To improve the specificity of elesclomol and enrich the delivery of copper in tumor cells, a method of active delivery, known as a small molecule drug conjugate (SMDC), will be introduced. This type of therapeutic consists of four different components: a cytotoxic drug, a cleavable linker, a spacer, and a targeting ligand. The ligand will trigger endocytosis by binding to a cancer cell-specific receptor, in this case glucose transporter 1, which is highly expressed in PM lesions. Subsequently, in the chemical environment of the endosome the SMDC will release the cytotoxic drug. The combination of these factors is expected to yield a more precise and effective therapy.

Target & Therapy Discovery

CRISPR Loss-of-Adhesion Screen in Multiple Myeloma identifies GRK6 as target in Homing and Retention

Fangxue Ma[1]

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Multiple myeloma (MM) is a B-cell malignancy originating from plasma cells in the bone marrow. Over the decade years, stem cell transplant, proteasome inhibitors, chemotherapy have significantly improved the outcome of MM but still incurable. MM cells strictly depend on the BM niche for their survival and proliferation and CXCL12-CXCR4 signaling plays a prominent role in controlling integrins-mediated adhesion and migration in MM homing and retention. By means of unbiased functional genomic loss-of-adhesion CRISPR/Cas9 screens we conducted in MM cells, G protein-coupled receptor kinase 6 (GRK6) were identified as potential major regulators of chemokine CXCL12-stimulated integrin-mediated adhesion of these cells. GRK6 is a member of GRK (G-protein-coupled recepter kinase) superfamily which expressed ubiquitously. Interestingly, we found target GRK6 could not only efficiently decrease CXCL12-stimulated integrin-mediated adhesion but also significantly reduced adhesion with stroma cells. CXCL12 stimulation could activated CXCR4, thereby initiating internalization, phosphorylation and downstream signals. We found target GRK6 could impact CXCR4 internalization and decrease phosphorylation in the site of ser324/5 and ser339 of C-terminal tail of CXCR4. Furthermore, CXCL12 binding to CXCR4 could active Gα and Gβγ proteins, then produced intracellular calcium and switch small GTPase of Rac1 and Rap1 between GDP(inactive state) and GTP(active state). What we found is that if we target GRK6, it could significantly impair intracellular calcium production and efficiently inhibit the activation of small GTPase Rac1/Rap1. These novel insights provide a strongly rationale for GRK6 could be a target as therapeutic strategy for MM patients.

Target & Therapy Discovery

Novel approach for an Evolution-Proof Influenza Vaccine

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The development of a universal vaccine against influenza is necessary to overcome the limitations of the current seasonal vaccines. Our research group developed a technology, called Immuno-Boost (iBoost) which aims at increasing targeted immune recognition of non- or low-immunogenic epitopes in cancer, more specifically tumor vasculature, and against the spike protein in SARS-COV-2. Therefore, this approach seems perfectly suited for vaccination against the influenza surface glycoproteins which may allow for safe and scalable production while improving vaccine immunogenicity.

Aim: Development of an evolution-proof vaccine that targets both HA and NA using the iBoost technology

Methods: This is achieved by conjugating the HA and/or NA epitopes to an engineered chimeric designer peptide (CDP) sequence of bacterial origin. The selected constructs are then expressed in BL21 bacteria (E.coli) and subsequently purified. After design and development, mice were immunized with 30ug protein and an adjuvant.

Results: Seroconversion was detectable by day 21 for CDP-H1 and CDP-H1N1 and by day 7 for CDP-N1. By day 28, all immunized with the CDP conjugated vaccines elicited a stronger antibody response compared to H1, N1 or H1N1 alone. The differences among the two groups (conjugated versus unconjugated) were largely maintained until day 35 with the unconjugated vaccine still lagging behind with the unconjugated.

Conclusion: Using the iBoost platform for vaccination against HA and/or NA results in not only faster, but also stronger antibody responses compared to the unconjugated counterparts. The iBoost platform allows for specific targeting of critical epitopes within key viral proteins while reducing the limitations involved in production

Target & Therapy Discovery

TARGET ID: Exploring functional communication between colorectal cancer cells and cancer associated fibroblasts driving colorectal cancer cell clonogenicity

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Colorectal cancer (CRC) is the third most prevalent form of cancer in the world. As a heterogeneous disease, its prognosis can vary considerably. The observed growth of CRC tumors is known to be modulated by interactions occurring between cells in the tumor microenvironment (TME) and tumors. Fibroblasts, in particular, have been of interest due to their abundance in stroma and proximity to tumors. Several mechanisms and pathways by which cancer associated fibroblasts (CAFs) promote or suppress tumor growth have been studied but our understanding is not yet exhaustive. In this project, a combinatorial in silico and in vitro approach is taken to further identify and validate interactions occurring between CRC cells and CAFs driving the clonogenicity of CRC cells. CRC cells are first co-cultured with human embryonic fibroblasts (HEFs). Depending on the location of CRC cells and HEFs within the co-cultures, annotations: clonogenic or non-clonogenic (CRC cells) and effective or non-effective (HEFs), are assigned. Following the assignment of annotations, patterned light is used to label cells of interest. The labelled cells from the co-cultures are then sorted and subjected to single cell RNA sequencing (scRNA-Seq). By performing cellular interaction analysis on the generated scRNA-Seq data, significantly enriched interactions present only between clonogenic CRC cells and effective HEFs are identified. Finally, genes enabling the identified interactions are knocked down to validate their function. This combinatorial approach therefore outlines a comprehensive pipeline to further delineate the role of CAFs in promoting CRC cell clonogenicity through cellular interactions.

Target & Therapy Discovery

Rational design of EV-mediated CRISPR-Cas9 delivery for cancer gene therapy

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The possibility to precisely interfere with DNA/RNA sequences with CRISPR-Cas systems opened new avenues for therapeutic gene editing. However, the safe and efficient delivery of the CRISPR components to target cells in vivo remains an unmet challenge. Several delivery methods have been proposed. However, they present significant drawbacks, such as immunogenicity, unfavourable biodistribution and rapid clearance. To tackle this hurdle, our research focused on Extracellular Vesicles (EVs) as natural, non-immunogenic communication devices that can horizontally transfer functional RNA molecules between cells. Based on our knowledge on the mechanisms underlying cell-mediated RNA packaging into EVs, we hypothesized that sorting of the CRISPR guide RNAs (gRNAs) into EVs can be enhanced by rational design of their 3'-end. Thus, we designed a library of gRNA variants with different 3'ends and test their functionality and enrichment in EVs. We found that some 3' U-rich variants are more efficiently sorted into EVs in two independent cell systems. We devised two reporter systems based on positive selection following p53 inactivation and color switch, with which we aim to assess whether EV-associated RNAs can be functionally transferred to recipient cells in vitro.

Target & Therapy Discovery

CAPN2-responsive mesoporous silica nanoparticles: A promising nanocarrier for targeted therapy of pancreatic cancer

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[1] Amsterdam UMC

Background and objective:
Pancreatic adenocarcinoma (PDAC) is highly resistant to conventional chemotherapeutic interventions, resulting in exceptionally low survival rates. The limited efficacy can in part be attributed to dose limitations and treatment cessation urged by toxicity of currently used chemotherapy. The advent of targeted delivery strategies has kindled hope for circumventing off-target toxicity. We have previously reported the synthesis of MSNs responsive to the proteolytic activity of extracellular protease ADAM9, that very efficiently induced PDAC cell death with limited bone marrow and neurotoxicity in vitro. However, no antitumor activity was observed in vivo.

Results:
We propose that an efficient uptake of MSNs by tumor cells might underlie the lack of antitumor efficacy of MSNs functionalized with a linker responsive to extracellular proteases. Harnessing this premise to improve antitumor efficacy, we performed an in silico analysis to identify PDAC-enriched intracellular proteases. We report the identification of BACE2, CAPN2 and DPP3 as PDAC-enriched intracellular proteases, and report the synthesis of BACE2-, CAPN2- and DPP3-responsive MSNs. Extensive preclinical assessments revealed that paclitaxel-loaded CAPN2- and DPP3-MSNs exhibit high PDAC specificity in vitro as opposed to free paclitaxel. The administration of paclitaxel-loaded CAPN2- and DPP3-MSNs in vivo confirmed the reduction of leukopenia and induced no organ damage. Promisingly, in two mouse models CAPN2-MSNs reduced tumor growth at least as efficiently as free paclitaxel.

Conclusion:
This study poses CAPN2-responsive MSNs as a promising nanocarrier for the targeted delivery of chemotherapeutics in PDAC.

Target & Therapy Discovery

Multiple drug combinations affecting HSP90, TRAIL and CDK7 to outsmart cancer chemoresistance

Francesca Terrana[1]

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Non-small cell lung cancer (NSCLC) comprises 85% of lung cancer cases, with a dismal 5-year survival rate below 15%, necessitating innovative therapies. Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) selectively induces tumor cell apoptosis, though resistance limits its efficacy. Heat shock proteins, notably Hsp90, are upregulated in NSCLC, suggesting a role in tumorigenesis. The Hsp90 inhibitor, 17-allylamino-17-demethoxy-geldanamycin (17-AAG), induces apoptosis in NSCLC.

This study examined whether 17-AAG could enhance TRAIL-induced apoptosis in NSCLC cells, sensitive and resistant to TRAIL (H460 and A549). Synergy was assessed using the combination index (CI), and apoptotic events via flow cytometry and Western blotting.

Results demonstrated synergistic effects of 17-AAG and TRAIL in both cell lines, notably reducing CI, particularly in TRAIL-resistant A549 cells. 17-AAG induced cell cycle delay and enhanced TRAIL-induced apoptosis by activating caspases, particularly caspase-8. It also reinstated apoptosis in TRAIL-resistant A549 cells by cleaving RIP1 and suppressing Akt activity.

This study suggests combining Hsp90 inhibitors with TRAIL receptor agonists holds promise for NSCLC treatment. Exploring CDK7 inhibitors alongside TRAIL-based therapies could offer a combinatorial approach. Future research should focus on overcoming TRAIL resistance for effective cancer treatment.

Similar study,on specific CDK7 inhibitors, are ongoing in chemoresistance models of pancreatic cancer.

Target & Therapy Discovery

Uncovering vulnerabilities in the long non-coding landscape of KRAS-mutated PDAC and CRC.

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KRAS is one of the most commonly mutated genes in solid tumors, including pancreatic ductal adenocarcinoma (PDAC) and colorectal cancer (CRC). Indeed, while ~40% of CRC patients harbor a KRAS mutation (KRASmut), more than 90% of PDAC patients present an activating mutation in this oncogene. Despite the substantial dependency of cancer cells on KRAS mutant proteins, their specific inhibition remains a daunting task. Long noncoding RNAs (lncRNAs) recently emerged as potent regulators of key cellular processes often deregulated in cancer. Because of their exceptional tissue- and tumor-specificity, these regulatory RNAs represent promising targets for cancer therapy. The noncoding transcriptome associated with KRAS activation remains mostly unexplored. We hypothesize that KRASmut-regulated lncRNAs represent novel and potent therapeutic targets for patients with CRC and PDAC. Our efforts aim to uncover essential oncogenic lncRNAs regulated by KRASmut and develop strategies to target these RNA molecules in both PDAC and CRC. We selected 8 CRC and 4 PDAC cell lines known to harbor a KRAS mutation and engineered them to silence KRAS expression with doxycycline-dependent shRNAs. We confirmed that all selected cell lines were dependent on KRAS expression. Indeed, KRAS knockdown greatly reduced the proliferation and clonogenic potential of all tested cell lines. We profiled all cell lines upon KRAS knockdown. Next, bulk RNA-sequencing revealed 391 and 545 co-regulated lncRNAs in CRC and PDAC, respectively. Among KRAS-regulated lncRNAs, 138 were shared between both cancer types. Our ongoing efforts aim at assessing the function of all KRAS-regulated lncRNAs in both systems, using functional genetic screens.

Target & Therapy Discovery

Exploring Sensitization to Thermoradiation: Novel Insights into MicroRNA-Mediated Effects Utilizing 3D Cultures

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Chemoradiotherapy is the standard treatment for various locally advanced cancers, but often causes severe side effects. A better-tolerated alternative is thermoradiotherapy (radiotherapy plus hyperthermia), yet it yields suboptimal overall survival of patients, emphasizing the pressing clinical need for improvement. MicroRNAs (miRNAs) are frequently dysregulated in cancer, play pivotal roles in cancer progression, and exhibit significant potential as radiosensitizers by targeting genes associated with DNA damage response.
This study aims to identify miRNAs enhancing the therapeutic effect of thermoradiotherapy using 3D cell cultures, and to assess their mechanism of enhancement through miRNA target prediction and functional validations.
We performed a miRNA screen employing 378 miRNA mimics using spheroid cell cultures across four cervical cancer cell lines, followed by a validation screen on 10 additional independent cancer cell lines (cervical cancer and head and neck cancer). Cell viability was measured as readout for treatment effect and subsequent analysis resulted in 18 potential sensitizing miRNAs. Clonogenic assays revealed similar effects. γH2AX stainings 24h post-thermoradiotherapy showed increased unrepaired double-stranded breaks among miRNA transfected groups. Western blot confirmed substantial downregulation of several proteins essential to DNA damage response such as RAD51 and Ku80.
In conclusion, using a newly developed high-throughput screening method, we identified 18 miRNAs with strong sensitizing potential in cervical and head and neck cancer. Sensitization was associated with miRNA-mediated downregulation of DNA damage repair proteins. Therefore, these miRNAs hold promise as both therapeutic targets and biomarkers for predicting treatment effect of thermoradiotherapy in cervical and head and neck cancer.

Target & Therapy Discovery

Inhibition of casein kinase 2 sensitizes Multiple Myeloma to the BCL-2 inhibitor Venetoclax via MCL-1 downregulation

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Despite the development of novel therapeutic strategies in the management of multiple myeloma (MM), the majority of patients will become resistant to existing therapies and experience relapse. Venetoclax, an inhibitor of the anti-apoptotic protein BCL-2, has been FDA-approved for the treatment of acute myeloid leukemia and chronic lymphocytic leukemia. Several phase III clinical trials are investigating the efficacy of BCL-2 inhibitor venetoclax in patients with relapsed MM; unfortunately, however, only a small subset of MM patients are sensitive to venetoclax. We have recently demonstrated that inhibition of casein kinase 2 (CK2) can sensitizes mantle cell lymphoma to venetoclax (Thus et al., 2023 Haematologica 108:797). In the current study we investigated whether CK2 inhibition could elicit similar venetoclax sensitizing effects in MM. We show that short hairpin RNA (shRNA)-based knockdown of CK2 or treatment with the CK2 inhibitor silmitasertib does not affect cell viability by itself, but confers venetoclax sensitization in several MM cell lines. Mechanistically, CK2 knockdown dramatically reduces the level of the anti-apoptotic BCL-2 family protein MCL-1, without affecting BCL-2 and BCL-XL expression. This results in enhanced BCL-2 dependence of MM cells and thereby venetoclax sensitization. Collectively, our study reveals a critical role for CK2 in venetoclax resistance of MM cells, providing strong support to further explore CK2 as a potential therapeutic target to improve venetoclax sensitivity in MM patients.

Therapy

Risk factors for benign anastomotic stenosis after esophageal cancer surgery

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Background
Benign strictures occur frequently following an esophagectomy, causing dysphagia, problems with eating, and a diminished overall quality of life. The aim of this study is to identify risk factors associated with anastomotic stenosis following esophagectomy for cancer.
Methods
This study included patients with esophageal and gastroesophageal junction cancer who underwent esophagectomy in the Amsterdam UMC between 2012 and 2022. Intrathoracic and cervical anastomoses were analyzed separately. Benign anastomotic stenosis was defined as the occurrence of postoperative dysphagia for which at least 1 endoscopic anastomotic dilation was needed.
Results
This study included 902 patients: 605 with an intrathoracic and 297 with a cervical anastomosis. Anastomotic stenosis was observed in 18.4% and 49.8%($p<0.001$). Patients respectively requiring a median of 4 and 7 dilations($p=0.001$). Median of time to anastomotic stenosis was 99 and 78 days ($p=0.001$). In multivariate analysis of intrathoracic anastomosis, anastomotic leakage was a significant risk factor for stenosis(OR 2.034; 95% CI[1.116-3.708]). In patients without anastomotic leakage, stapler size 29mm was a negative predictor(OR 0.444; 95% CI[1.116-3.708]). Immunosuppressants(OR 3.492; 95% CI[1.186-10.279]) and chronic pulmonary disease were significant risk factors(OR 2.717; 95% CI[1.293-5.707]). In multivariate analysis of cervical anastomosis, circular stapled anastomosis was a negative predictor(OR 0.350 95% CI[0.188-0.652]) for stenosis.
Conclusion
This study identified the most important risk factors for anastomotic stricture after esophagectomy. Anastomotic leakage, the circular stapler diameter, and the circular stapled technique are factors that could be surgical modifiable, while patients taking immunosuppressants and with chronic pulmonary disease should be counseled for higher risk of stenosis.

Therapy

Predicting personalized treatment effects of neoadjuvant therapy vs. upfront surgery for (borderline) resectable pancreatic cancer: a secondary analysis of the PREOPANC trial

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Background

Treatment guidelines for (borderline) resectable pancreatic cancer recommend weighing patient characteristics and biomarker levels to facilitate individualized decision-making between neoadjuvant chemo(radio)therapy and upfront surgery. However, validated decision tools to guide this process are lacking.

Objective

To derive and validate a prediction model (ABCD-score) to predict 5-year mortality risk and estimate personalized treatment effects of neoadjuvant chemoradiotherapy vs. upfront surgery for (borderline) resectable pancreatic cancer, using baseline patient characteristics.

Methods

In total, 2238 patients from the Netherlands Cancer Registry (2016–2021) and 246 patients from the phase 3 randomized PREOPANC trial (2013–2017) were used to develop and validate the ABCD-score using Cox regression analysis and flexible parametric survival models. The score was evaluated for its discrimination, calibration, and clinical utility.

Results

At internal-external cross-validation, the ABCD-score showed helpful discriminative performance for predicting 5-year overall survival (Uno's C-statistic, 0.68 [95% confidence interval, 0.63 to 0.73]). The model was well-calibrated and

there was no evidence that the model systematically over- or underestimated the 5-year mortality risk (calibration slope, 1.03 [0.87 to 1.22]; P=0.71). The treatment benefit of neoadjuvant therapy decreased with higher ABCD-2 score values: 5-year survival benefit was 15% (4 to 25%) at an ABCD-2 score of 20 compared to 1% (–2 to 4%) at an ABCD-2 score of 80.

Conclusion

The ABCD-score can help identify patients who will benefit most from neoadjuvant chemoradiotherapy. The score is available as a simple, web-based calculator. As such, this score has the potential to guide personalized therapeutic decision-making for patients with (borderline) resectable pancreatic cancer.

Therapy

Investigating the role of monosomy 7 in drug resistance in acute myeloid leukaemia

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Acute myeloid leukaemia (AML) is a devastating disease with dismal five-year overall survival rates. Despite the introduction of novel treatments, therapy resistance remains a major problem in achieving cures for AML patients. Venetoclax, a BH3-mimetic that targets the pro-survival protein BCL2, has shown promise for AML treatment, especially when combined with azacitidine, an epigenetic therapeutic or low-dose cytarabine. Nonetheless, resistance to this combination is a major problem, necessitating a deeper understanding of the underlying mechanisms and the development of strategies to overcome it.

Our clinical observation revealed an unrecognized association between the deletion of chromosome 7 (monosomy 7) and a substantial reduction in overall survival among AML patients undergoing venetoclax-based regimens. This connection has not been studied previously and we anticipate pioneering new insights with potential clinical applications. This discovery holds the potential to identify at-risk patients with monosomy 7 and develop innovative approaches to sensitize them to venetoclax.

Our overall hypothesis is that the deletion of chromosome 7 may harbour critical genetic events responsible for driving resistance to venetoclax, and it may also reveal potential therapeutic vulnerabilities. To test our overall hypothesis, I will pursue the following aims:

Aim 1: Elucidate if monosomy 7 contributes to venetoclax resistance.

Aim 2: Examine and validate novel therapeutic vulnerabilities in monosomy 7 AML.

This research project holds great promise in enhancing our understanding of AML

and improving therapeutic outcomes. This work will unravel the complexities of venetoclax-combination therapy resistance, ultimately benefiting AML patients by offering more effective treatment options.

Therapy

A national study of the rate of benign pathology after partial nephrectomy for T1 renal cell carcinoma: should we be satisfied?

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Previous studies report rates of benign pathology after partial nephrectomy ranging from 8-30%. This study aims to determine the rate of benign pathology in pT1 tumors following partial nephrectomy in The Netherlands, thereby evaluating the rate of overtreatment.
Methods and Materials: Data was collected from a nationwide database containing histopathology of resected renal tissue from 2014-2022. Patients who underwent partial nephrectomy for suspected RCC staged pT1a-b were extracted for analysis. Data is shown in percentages and multivariable logistic regression was done to determine predictive factors for benign pathology.
Results: After exclusion, 3409 cases were left for analysis, of which 403 (12%) benign and 3006 (88%) malignant. Overall, 2674 (78%) cases were pT1a tumors and 735 (22%) pT1b. Analysis of subtype showed 2126 (62%) cases of clear-cell RCC, followed by 604 (18%) papillary RCC and 344 (10%) oncocytomas. Mean age was 63 years among patients with malignant pathology versus 65 years for patients with benign lesions ($p<0.001$). Mean tumor size was 3.2 cm for malignant pathology and 2.9 cm for benign ($p<0.001$). The rates of benign and malignant pathology did not change between 2014-2022 ($p=0.377$). Multivariable regression showed age ≥65 years (65-79 years [OR 1.875, $p=0.002$], ≥80 years [OR 3.640, $p<0.001$]) and tumor size (OR 0.799, $p<0.001$) as predictors for benign pathology.
Conclusion: This study reports a low rate of 12% benign pathology after partial nephrectomy in The Netherlands. It remains debatable whether these rates are acceptable, or if renal tumor biopsies should be utilized more frequently to reduce overtreatment.

Therapy

Preoperative partial breast re-irradiation and repeat breast-conserving surgery in patients with recurrent breast cancer: the REPEAT trial – a study protocol

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BACKGROUND: Salvage mastectomy is the standard for patients with an ipsilateral recurrent breast event (IRBE). Previous data have shown that repeat breast-conserving surgery and postoperative multi-fractionated re-irradiation (i.e. 5-25 fractions) can be safe and well tolerated by patients with a low-risk IRBE. Simultaneously, preoperative radiotherapy can result in a pCR in 42%, in patients with primary low-risk breast cancer and results in smaller irradiated volumes. This study will evaluate the feasibility of single-dose preoperative partial breast re-irradiation (PBI) and repeat breast-conserving surgery (BCS) for low-risk IRBE.
METHODS: The REPEAT trial is a multicenter prospective single-arm trial for patients ≥ 50 years with non-lobular invasive breast cancer ≤ 2cm, grade 1 or 2, estrogen receptor-positive, HER2 negative, clinically node-negative and no or mild (≤ grade 2) late toxicity from previous BCT. Enrollment targets 25 patients. BCS will be performed three weeks following preoperative single-dose PBI. The primary endpoint is the rate of grade ≥ 2 acute radiation-induced toxicity and postoperative complications within 30 days assessed by CTCAEv.5.0 and Clavien-Dindo classification. Secondary endpoints are late toxicity, quality of life, cosmetic outcome, radiotherapy-associated immune- and biomarkers, and oncological outcomes.
DISCUSSION: This novel treatment approach has the potential to avoid standard salvage mastectomy or multi-fractionated re-irradiation in future patients with low-risk IRBE. Preoperative single-dose PBI reduces irradiated volumes compared to standard postoperative PBI, potentially lowering the dose to the surrounding healthy breast tissue and thereby reduced toxicity with improved quality of life and reduced treatment burden.

Therapy

Defining the optimal radiation-induced lymphopenia metric to discern its survival impact in esophageal cancer.

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Background: A detrimental association between radiation-induced lymphopenia (RIL) and oncologic outcomes in esophageal cancer patients has been established. However, an optimal metric for RIL remains undefined, but is important for application of this knowledge in clinical decision-making and trial designs. The aim of this study was to find the optimal RIL metric discerning survival.
Methods: Esophageal cancer patients treated with concurrent chemoradiotherapy (CRT; 2004-2022) were selected. Studied metrics included absolute lymphocyte counts (ALCs) and neutrophil counts -and calculated derivatives- at baseline and during CRT. Multivariable Cox regression models for progression-free survival (PFS) and overall survival (OS) were developed for each RIL metric. The optimal RIL metric was defined as the one in the model with the highest c-statistic.
Results: Among 1,339 included patients, 68% received photon-based and 32% proton-based CRT (median follow-up 24.9 months). In multivariable analysis, the best performing models included “ALC in week 3 of CRT” (corrected c-statistic 0.683 for PFS, and 0.662 for OS). At an optimal threshold of <0.5 x103/µL (i.e. grade ≥3 RIL), ALC in week 3 was significantly associated with PFS (adjusted hazard ratio [aHR] 1.64; 95% confidence interval [CI] 1.27-2.13) and OS (aHR 1.56; 95%CI 1.15-2.08), with 5-year PFS of 29% vs. 40% and OS of 38% vs. 51%, respectively.
Conclusions: Reaching grade ≥3 RIL in week 3 of CRT for esophageal cancer is the strongest RIL metric to distinguish survival outcomes. We suggest that this metric should be the target for lymphopenia-mitigating strategies, and propose this metric to be included in future trials.

Therapy

A Carboplatin-induced myelosuppression model to support therapeutic drug monitoring and dose individualization, using Electronic Health Record Data of predominantly Esophagus/stomach and lung cancer patients

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Carboplatin is a cytotoxic anticancer drug used in monotherapy or in combination with other chemotherapeutics and/or immunotherapies to treat different cancer types. Severe myelotoxicity is the most frequent dose limiting carboplatin-induced adverse event. Both therapeutic drug monitoring (TDM) and dose individualization have been proposed to circumvent its onset and preserve treatment efficacy. Pharmacokinetics-Pharmacodynamics (PK-PD) modelling and simulations are a valuable tool to inform personalized dosing strategies in the context of TDM.

Here, a semi-mechanistic population PK-PD model (popPK-PD) of carboplatin effect on platelets (PLT) and neutrophils (NT) was developed on 580 cancer patients of Amsterdam University Medical Center (UMC) (January 2019-June 2022), including 300 lung, 180 oesophagus/stomach and 100 gynaceologic cancer patients. The model was then validated to inform TDM and dose individualization in a validation set of 211 new cancer patients of Amsterdam UMC (June 2022-January 2024).

The final model included the effect of other concomitant chemotherapies as well as patient serum albumin and eGFR as significant covariates to describe interindividual variability of carboplatin-induced myelosuppression. Relative Standard Error values of all model parameters were below 30%. Model suitability to inform carboplatin TDM was tested on the validation population through a full Bayesian approach. Results showed that next cycle outcomes were adequately

predicted as >85% observed NT and PLT fell within the 95% C.I. of posterior prediction.

The popPK-PD model adequately predicted the next cycle carboplatin-induced myelosuppression. Consequently, it can be used to inform carboplatin TDM. Moving forward, this model will be integrated with an AI tool to personalize dose suggestions.

Therapy

HIPEC mechanism and optimise clinic treatment in ovarian cancer

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Introduction: Epithelial ovarian cancer presents at an advanced stage in the majority of women. These women require a combination of surgery and chemotherapy for optimal treatment. Hyperthermic intraperitoneal chemotherapy (HIPEC) along with optimal cytoreductive surgery (CRS) has been shown to be a viable option for the treatment of advanced epithelial ovarian cancer with peritoneal carcinomatosis. Preliminary results of CRS followed by cisplatin-based HIPEC have shown promising results, with improved survival outcomes and tumor regression. However the molecular mechanism has been poorly investigated. The aim of this research was to assess in vitro the effect of temperature and other factors on efficacy of cisplatin-based HIPEC on growth of ovarian cancer cells.

Methods: Ovarian cancer cells were treated with different IC50 doses of Carboplatin and Paclitaxel. Cell cultures are exposed to normothermia and to clinically relevant hyperthermic conditions (42 °C/90 min) with Cisplatin. Cell growth was determined by MTT and colony-formation assays. Cell cycle and live/dead cell analyses were performed by flow cytometry. Western immunoblotting showed DNA damage and the effect on repair pathways. Visualization of DNA double-strand breaks(DSBs) was performed by Y-H2AX experiments.

Results: We found both additive and synergistic effects of hyperthermia added to cisplatin. Cell lines are sensitive to hyperthermia alone. Cisplatin displays a temperature-dependent synergy with heat, resulting in increased DNA damage, and decreased cell growth.

Conclusions: Our in vitro results demonstrate that a hyperthermic 90min exposure of cells to Cisplatin is effective in treating ovarian cancer cell cultures, suggesting that cisplatin-based HIPEC could improve patient outcomes.

Therapy

Upper urinary tract urothelial carcinoma in patients after renal transplantation A retrospective study in a tertiary center and Dutch nationwide database analysis

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To calculate the incidence of UTUC in renal transplant (RTx) recipients in a tertiary referral center and nationwide in the Netherlands. Analyze patient and tumor characteristics within these patients.
Patients diagnosed with primary UTUC in the Amsterdam UMC between 2010 and 2023 were retrospectively analyzed for having RTx preceeding the diagnosis of UTUC. Additionally the nationwide Dutch pathology Registry (PALGA) was searched between 1991 and 2023 for all UTUC cases with RTx preceeding the UTUC diagnosis. Patient, tumor characteristics and treatment information were retrieved. Cumulative incidences, incidence rates and descriptive statistics were calculated.
Between 2010 and 2023, 2351 patients were transplanted at the Amsterdam UMC. Of these patients 4 developed UTUC in the transplant graft post-RTx resulting in a cumulative incidence of 0.17% and incidence rate of 13.1 per 100 000 person-years. The median patient age at diagnosis was 46 years (39-63) and 4/4 cases with high-grade UTUC. Between 1991 and 2023, 24 270 patients were transplanted in the Netherlands. Of these patients 29 developed UTUC in the transplant graft post-RTx resulting in a cumulative incidence of 0.12% and incidence rate of 3.6 per 100 000 person-years. The median patient age at diagnosis was 59 years (51-70) and 25/29 (86%) patients with high-grade UTUC.
We found a higher incidence of UTUC post-RTx, both at a tertiary referral hospital and nationally within the Netherlands, with patients showing a lower median age at diagnosis and a higher percentage of high-grade tumors compared to the general population. These differences are believed to be multifactorial.

Therapy

Malignant features in addition to size for lateral lymph nodes; its value for risk stratification for lateral local recurrence – a national cohort study

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Introduction: Rectal cancer patients with enlarged lateral lymph nodes (LLNs) have an increased (lateral) local recurrence ((L)LR) risk. However, knowledge regarding prognostic implications of malignant features (loss of fatty hilum, irregular margins, internal heterogeneity and round shape) and the number of LLNs is limited.
Methods: From a national, retrospective cohort study including 3057 patients, 284 patients with visible obturator or internal iliac LLNs on MRI were selected. None of these patients underwent lateral lymph node dissection. Imaging was re-assessed by trained radiologists, who categorized LLNs based on size. Influence of malignant features and the number of LLNs on 4-year LLR was investigated.
Results: Forty-three percent of the patients (122/284) had an enlarged LLN (≥7.0mm) and 157 (55%) had malignant feature(s). In patients with one enlarged LLN (97/122), a single or multiple malignant features were associated with a LLR-rate of 0% and 17%, respectively (p=0.060). Disappearance of multiple malignant features on restaging MRI was associated with 13% LLR-rate compared to 20% for persistent malignant features (p=0.532). The presence of intermediate LLN(s) (5.0-6.9mm) with ≥1 malignant feature had a LLR-rate of 8%, and 13% when the malignant features persisted on restaging MRI (p=0.409). Patients with multiple enlarged LLNs (25/122) had a LLR-rate of 28%, compared to 11% for a single enlarged LLN (p=0.059).
Conclusion: The presence of multiple enlarged LLNs (≥7.0mm), as well as multiple malignant features in an enlarged LLN increase LLR risk. These radiological features can be used for clinical decision making regarding potential benefit of lateral node dissection.

Therapy

Minimally invasive versus open pancreatoduodenectomy for pancreatic and periampullary neoplasm (DIPLOMA-2): an international patient-blinded randomized trial

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Introduction: Minimally invasive pancreatoduodenectomy (MIPD) is increasingly used as an alternative to open pancreatoduodenectomy (OPD). However, the safety of MIPD remains a topic of debate, especially after the recent LEOPARD-2 trial, and the potential benefit in improved time to functional recovery (TTFR) needs to be confirmed. This randomized trial compared MIPD and OPD for 90-day complications and TTFR.

METHODS: International randomized trial in 14 high-volume centers in 6 European countries. Patients with an indication for pancreatoduodenectomy for a resectable (pre-)malignant neoplasm were assigned to either MIPD or OPD (2:1 ratio). Centers participated for either robot-assisted or laparoscopic MIPD. Patients were blinded to the assigned approach until day 5 postoperatively. The primary endpoint was the Comprehensive Complication Index (CCI®), measured to day 90 postoperatively, tested for noninferiority with a predefined margin of 7.5 points. The primary secondary endpoint was TTFR, tested for

superiority.(ISRCTN27483786)
Results: Between 1-1-2022 and 18-7-2023, 288 patients were randomized to MIPD (N=190; 170 robot-assisted) and OPD (N=98). The conversion rate in MIPD was 7.8%.
The primary end point CCI® was 33.4±27.5 in the MIPD group vs. 35.3±25.5 in the OPD group, difference in means [95% CI]: -1.9 [-8.5;4.7]; pnon-inferiority=0.002. The median TTFR was shorter after MIPD (7 days [IQR 5-11] vs. 8 days [IQR 6-14]; p=0.016). No difference in 90-day overall mortality was found (4.7% vs. 2.0%; p=0.26).
CONCLUSION: This first international multicenter randomized trial confirms non-inferiority of MIPD over OPD for 90-day complications in patients with primarily resectable pancreatic and periampullary tumors, and shows faster recovery after MIPD.

Therapy

Snapshot study - DECIDE – Immediate Breast Reconstruction after Mastectomy in the Netherlands

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Introduction: Annually, approximately 6.000 patients with invasive ductal carcinoma in situ (DCIS) or invasive breast cancer undergo a mastectomy in the Netherlands. Preserving the breast contour after a mastectomy can be achieved through immediate or delayed reconstruction and has a positive impact on the quality of life. Data from the National Breast Cancer Audit from 2020 demonstrates a large variation between Dutch hospitals in the application of immediate breast reconstruction (IBR).
Aim: The aim of this study is to provide an updated overview of current incidence and decision-making process regarding IBR after mastectomy.
Methods: The DECIDE study is a national, multicenter SNAPSHOT study. All hospitals providing breast cancer care in the Netherlands will be approached. Female patients undergoing a mastectomy with or without IBR for either DCIS or invasive breast cancer will be included for one year. Data concerning the indication for mastectomy, the reasons for (not) performing IBR, and the final advice for IBR will be collected. We evaluate the final breast contour preservation rate after 5 years.
Results: The primary outcomes are the incidence of IBR after mastectomy and the factors influencing the decision-making process regarding IBR. Secondary outcomes are short-term complications after 60 days and the final breast contour preservation rate after 5 years.
Conclusion: This snapshot study will provide an overview of the current incidence and factors influencing the decision-making process regarding IBR in the Netherlands. We hope to gain knowledge about the possible causes of the variation of IBR and to propose potential solutions.

Therapy

Preliminary results of the PERFU study: PERsonalized predication and regulation of 5-FluoroUracil exposure.

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5-fluorouracil (5-FU) is the cornerstone of modern treatment regimens for pancreatic and colorectal cancer. The starting dose of 5-FU is currently based on body surface area (BSA) and dihydropyrimidinedehydrogenase (DPYD) genotyping. Despite this approach, inter- and intra-individual variability of exposure remains high. The aim of this study is to validate whether therapeutic drug monitoring (TDM) is an effective tool to regulate individual 5-FU exposure.

The primary objective of this study is to determine the percentage of patients that achieve optimal 5-FU exposure within two dose cycles, defined by an AUC between 20-30 mg*h/l or dose limiting toxicity.

Mono-center intervention study in patients undergoing 5-FU chemotherapy in combination with leucovorin, irinotecan and/or oxaliplatin. Blood samples were taken at 2h and/or 45h during a 46-hour continuous infusion, and toxicity was assessed according to the CTCAE vs 5.0. A Kaldate et al. based dose algorithm was applied to advise dose adjustments.

27 patients were enrolled from 2020 until 2024. For this interim analysis, 15 patients with 5-FU measurements in the first 2 cycles, were included. In 67% of these patients, optimal 5-FU exposure was achieved after two dose cycles. In 27% of the patients dose limiting toxicity prevented dose increases while AUCs

remained <20 mg*h/l. The risk of grade ≥3 toxicity was 21%, 25% and 50% at AUCs of <15, 15-20, and 20-30 mg*h/l, respectively.

In our setting, the additional value of 5-FU TDM was limited. Further research is needed to validate these findings and reconsider the current AUC target between 20-30 mg*h/l.

Therapy

Use of novel protein degraders (PROTAC) as functional enhancers of chimeric antigen receptor (CAR) T cells.

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The success of adoptive cellular immunotherapy with genetically engineered T cells to bear a chimeric antigen receptor (CAR-T cells) has shaken the treatment landscape of hematological malignancies. CARs are synthetic receptors that mediate antigen recognition, T cell activation and cytotoxicity. Second generation CARs, that provide combined activation and co-stimulatory signals, induced impressive clinical responses in chemotherapy resistant B cell leukemias and lymphomas. Despite the high remission rates a significant portion of patients still eventually experience tumor relapse. A major factor limiting the efficacy of CAR-T cells is overactivation leading to functional dysfunction, reduced persistence in the bloodstream of patients and subsequent escape of the disease.
This study is based on strong preliminary data suggesting that CDK6 can supress T cell receptor (TCR) signalling and that low-dose pharmacologic inhibition or degradation of CDK6 can enhance T cell functions. We hypothesize the CDK6 may have similar effect on CAR signalling and that inhibition of CDK6 may release and enhance CAR T cell effector functions without compromising their persistence. The aim of this study is to pharmacologically target CDK4/6 at various stages of CAR T cell therapy and investigate, whether they can improve CAR T cell manufacturing and anti-tumor function. We will pharmacologically degrade CDK4/6 with PROteolysis TArgeted Chimera (PROTAC) molecules.

Therapy

Effect of R-CHOP on anti-CD19 CAR T-cell Metabolic Fitness in high-risk DLBCL

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Despite the successes of Chimeric Antigen Receptor (CAR) T-cell therapy in several hematological malignancies, in relapsed/refractory diffuse large B-cell lymphoma (DLBCL) only ~40% of patients have durable response to anti-CD19 directed CAR T-cell therapy. This study aims to investigate whether treatment with R-CHOP negatively affect metabolic fitness of CD19 CAR T cells derived from patients with confirmed high-risk DLBCL, potentially reducing CAR T-cell efficacy.
Cryopreserved high-risk DLBCL peripheral blood mononuclear cells (PBMCs) (N=13) were included and compared to age-matched healthy donors (N=10). Anti-CD19 CAR T-cells were manufactured according to protocol described previously. Flow cytometry was used to assess CAR T-cell phenotype and metabolic characteristics. Cytotoxic potential against Raji cells was determined using bioluminescence. CAR T-cells were stimulated with irradiated Raji to assess proliferative capacity.
Results show that upon activation, CD25 expression was significantly lower on patient CD3+ T-cells compared to age-matched healthy donors (33.5% vs 67.5%, p=0.0001). Transduction efficiency was significantly better in healthy donors compared to DLBCL patient samples (74% vs 40%, p=0.0005). No significant differences in CAR T-cell phenotype, mitochondrial characteristics or cytotoxic capacity were found. However, fold proliferation was significantly higher in age-matched healthy donor CAR T-cells compared to DLBCL CAR T-cells (2.324 vs 1.096, p=0.0001).
Comparing DLBCL-derived CAR T-cells with age-matched healthy donor CAR T-cells shows that DLBCL patient CD3+ T-cells show inferior activation upon stimulation, which may result in lower transduction efficiency. Additional DLBCL patient samples need to be included to elucidate the mechanisms that cause lower T-cell activation and inferior CAR T-cell proliferative capacity.

Therapy

Surgery versus thermal ablation for small-size colorectal liver metastases (COLLISION): A randomized, international, multicenter, phase III trial

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Background: The standard of care for patients with colorectal liver metastases (CRLM) is surgical resection (SR). However, growing evidence suggests thermal ablation (TA) to have a superior safety profile, lower costs, and shorter hospital stay, while rivaling SR in terms of local control and overall survival (OS). This study explored the potential non-inferiority of TA compared to SR for patients with small-size (≤3cm) CRLM.
Methods: In this international, phase 3 RCT, patients with CRLM were recruited from 14 centers. Patients with ≤10 CRLM were randomly assigned (1:1) to undergo SR or TA. The primary outcome was OS. Secondary outcomes include distant and local progression-free survival (PFS), local-control, safety, length-of-hospital-stay, quality-of-life and cost-effectiveness.
Results: A total of 341 patients were enrolled; 299 were randomized: 147 assigned to TA, 148 to SR. After a median follow-up time of 28.8 months there was no difference regarding OS (HR 1.042; 95% CI, 0.689-1.576; p = 0.846). Procedure related mortality was 2.1% (n=3) for resection vs. 0% (n=0) for TA. The total number of adverse events (p = <0.001), length-of-hospital-stay (p = <0.001) and local control also favored TA (HR 0.184; 95% CI, 0.040-0.838; p = 0.029). No differences were found regarding local (HR 0.833; 95% CI, 0.473-1.469; p = 0.528) and distant PFS (HR 0.982; 95% CI, 0.739-1.303; p = 0898).
Conclusion: In conclusion, transitioning from SR to TA as standard of care for patients with small-size CRLM would reduce complications, shorten hospital stay and improve local control, without compromising disease-free and OS.

Therapy

Nationwide use and outcome of pancreatectomy for IPMN: should guidelines take the type of pancreatic resection required into account?

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Current guidelines on intraductal papillary mucinous neoplasm (IPMN) do not take the location of IPMN and the associated required pancreatic resection into account when advising pancreatic resection. This could be relevant as outcomes may differ, although large multicenter studies on the outcome of pancreatoduodenectomy, left-sided pancreatectomy, and total pancreatectomy for IPMN are lacking. Therefore, this nationwide study aimed to evaluate the outcomes associated with pancreatectomy for IPMN.
Nationwide, retrospective analysis of all consecutive patients after pancreatectomy for pathology proven IPMN from the prospective Dutch Pancreatic Cancer Audit (2014-2020). Primary outcomes were in-hospital/30-day mortality and major morbidity (Clavien-Dindo ≥3).
Overall, 396 patients underwent pancreatectomy for IPMN, including pancreato-duodenectomy (PD; n = 246, 62%), left-sided pancreatectomy (LP; n = 130, 33%), and total pancreatectomy (TP; n = 20, 5%). The rate of in-hospital/30-day mortality was 2.0% and did not differ significantly between groups (2.8%, 0%, 5.0%, p = 0.257). The rate of major morbidity was 32% and did not differ significantly (36%, 25%, 25% p = 0.545). The rates of postoperative pancreatic fistula (POPF; 17%, 15%, NR, p = 0.140) and bile leak (12%, NR, 5%, p = 0.694) did not differ significantly between groups, whereas delayed gastric emptying was reported more often after PD (DGE; 23%, 2%, 20%, p = 0.0001).
This study found no significant differences in terms of mortality and major morbidity among three types of pancreatectomy for IPMN. These findings do not justify an altered threshold for surgical resection based the type of pancreatic resection required for IPMN.

Therapy

Robotic versus open pancreatoduodenectomy in patients with primary resectable cancer in the pancreatic head (DIPLOMA-2x2): A "roll-over" international multicenter patient- and pathologist-blinded randomized controlled trial

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Background: Robot-assisted pancreatoduodenectomy (RAPD) is rapidly becoming part of clinical practice in patients with resectable cancer in the pancreatic head, aiming for enhanced postoperative recovery compared to open pancreatoduodenectomy (OPD). The recently completed DIPLOMA-2 trial assessed the safety of MIPD (including robot-assisted) versus OPD for all indications. The oncological safety of RAPD in patients with cancer in the pancreatic head has yet to be determined.

Methods/design: An international randomized controlled, patient- and pathologist-blinded, non-inferiority trial in 16 high-volume centers in Europe, will be performed as an extension of the DIPLOMA-2 trial (ISRCTN27483786). In total, 396 adult patients with an indication for elective pancreatoduodenectomy for suspected or proven primary resectable cancer in the pancreatic head (i.e. pancreatic ductal adenocarcinoma and distal cholangiocarcinoma), eligible for both approaches are randomly allocated for RAPD or OPD (2:1). Recruitment is expected to be completed in July 2025, follow-up October 2025. Primary outcome is radical (R0) resection rate with a sample size based on 5% one-sided α, 80% power, expected R0-resection rate 55% with OPD and 62% RAPD, and 7% non-inferiority margin. Second primary outcome is time to functional recovery. Secondary outcomes include lymph node retrieval, start and tolerance of adjuvant therapy, disease recurrence, overall survival, intra- and postoperative outcomes, healthcare resource utilization, and quality of life. Long-term follow-up is up to 5 years.
Discussion: The DIPLOMA-2x2 trial is the first RCT to assess the oncological safety of RAPD versus OPD for patients with cancer in the pancreatic head, in high-volume centers in an enhanced recovery setting.
Trial registration:ISRCTN27483786

Therapy

Qualitative research into the wishes and obstacles regarding contact and information provision in patients treated with immunotherapy

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Patiënts with melanoma, renal cell or urothelial cell carcinoma who are treated with immunotherapy (Ipilimumab, Nivolumab or Pembrolizumab) at the Amsterdam UMC do not always contact the hospital if symptoms of side effects occur. As a result, side effects may be detected later than desirable and intervention may be necessary. If the side effect is detected early, the correct treatment can be started earlier, which may prevent further burden on the patiënt, discontinuation or adjustment of treatment with immunotherapy and burden on the healthcare system. This research provides us with insight into the wishes and obstacles that patiënts experience with regard to contact and information provision during treatment with immunotherapy. This allows the quality of care to be improved by optimizing the provision of information and support to patiënts treated with immunotherapy.

Therapy

Investigating T-cell Metabolism in Multiple Myeloma

Chloe O'Neill[1]

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Introduction:
Bispecific antibodies (BsAbs) are efficacious for the treatment of relapsed/refractory multiple myeloma (RRMM) patients. However, continuous exposure of T-cells to BsAbs induces features of T-cell exhaustion. Effective T-cell metabolism is crucial for the function, proliferation and differentiation of T-cells, however little is known about T-cell metabolism in different stages of disease.

Methods:
We used PBMC from newly-diagnosed relapsed/refractory (NDMM), daratumumab-naïve, daratumumab-RRMM and BsAb-treated patients and HD in a 48 hour stimulation assay with CD3/CD28 beads. Metabolic and phenotypic readouts were performed by FACS. ELISA and CBA assays were used for cytokine analysis.

Results:
MM patients have a reduced percentage of naïve CD8+ and CD4+ T-cells and an increased percentage of EM and TEMRA CD8+ and EM CD4+ T-cells over time, compared to HD. MM patients have reduced granzyme B and cytokine production compared to HD, with the lowest production in BsAb patients. MM patients have increased expression of exhaustion markers in comparison to HD, with the highest levels seen in BsAb patients. BsAb patients demonstrate reduced glucose uptake in CD8+ and CD4+ T-cells compared to all other groups. MM patients had a higher percentage of depolarized mitochondria in their CD4+ T-cells compared to HD and had higher ROS production in CD8+ and CD4+ T-cells in their unstimulated samples compared to HD. This may indicate dysfunctional glycolytic and mitochondrial metabolism in BsAb patients.

Conclusion:
MM patients experience changes in the composition of their T-cell subsets overtime which is accompanied by reduced cytokine production, increased

expression of exhaustion markers and changes in T-cell metabolism.

Therapy

Critical illness affects the pharmacokinetics and pharmacodynamics of imatinib: an analysis of invasively ventilated COVID-19 ARDS patients

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Introduction: A severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection can lead to COVID-19 acute respiratory distress syndrome (C-ARDS). The potential effect of imatinib on C-ARDS was studied in two placebo controlled RCTs. Imatinib was originally registered for chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GIST)effectiveness. In these disease there a clear relationship between total imatinib trough concentrations and effectiveness has been established, but the association in C-ARDS patients is unknown. When exploring imatinib pharmacokinetic-pharmacokinetic (PK-PD) relationships, the influence of protein binding and its main metabolite, DM-imatinib in severe disease, should be considered.
Aims: Investigate the associations between various potentially active forms of imatinib and clinical outcomes in invasively ventilated patients with COVID-19.
Methods: Associations between individually predicted daily through concentrations (Cdaily) and WHO-score and ratio between partial oxygen pressure, fraction inspired (P/F), length of ICU stay and number of ventilator free days were explored.
Results: There were statistically significant associations between CdailyT, CdailyU and CdailyPM and WHO-score and CdailyT and CdailyPM and P/F. CdailyT, CdailyU and CdailyPM associated with ICU length of stay and VFD.
Conclusion: Higher exposure of all pharmacologically active forms of imatinib was associated with worsening of clinical outcomes. This could have been an effect of critical disease on PK of imatinib and on clinical outcome. In these analyses, no clinical benefit from increased imatinib exposures in invasively ventilated COVID-19 patients was observed.

Therapy

Upper tract urothelial cancer scoping review and Delphi consensus project on the follow-up protocols after kidney-sparing surgery. Researching rare diseases.

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Context
Upper tract urothelial carcinoma (UTUC) can be efficiently and safely managed through kidney-sparing surgery (KSS) in selected patients groups. However, the most effective and efficient postoperative surveillance strategy remains undetermined.
Objective
To provide a comprehensive synopsis of follow-up strategies and survival outcomes in patients diagnosed with UTUC treated by KSS.
Evidence acquisition
Following the systematic methodology outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews, we conducted searches in four databases (MEDLINE (Ovid), Embase (Ovid), the Cochrane Library, and Web of Science) up until December 11th 2023.
Evidence synthesis
A total of 3,121 articles underwent screening, of which 19 studies were selected for inclusion in this review. The follow-up schedules after KSS exhibited considerable variability among the included studies. Diagnostic modalities employed consisted of CT-urography (present in 84% of protocols), X-urography (21%), ultrasound (21%), thoracic imaging (26%), voided urine cytology (89%), selective upper tract cytology (5.3%), cystoscopy (84%) and ureterorenoscopy (53%) at varying frequencies. At 5 years follow-up reported recurrence free survival rate ranged from 30 to 86%, overall survival was 50-92% and metastasis-free survival was 77-90%.
Conclusions
This review unveils significant heterogeneity in clinical practices and survival outcomes, indicating disparities between real-world approaches and guidelines' recommendations. The lack of consensus on follow-up schemes is evident, emphasizing the necessity for future initiatives aimed at developing a comprehensive protocol. Our goal is to concretize and standardize surveillance practices following KSS. We aim to attain consensus on post-KSS surveillance through a Delphi Consensus Project.

Therapy

Local Treatment of Colorectal Liver Metastases in the Presence of Extrahepatic Disease: Survival Outcomes from the Amsterdam Colorectal Liver Met Registry (AmCORE)

Hannah Schulz[1]

[1] None

Purpose: The simultaneous presence of colorectal liver metastases (CRLM) and extrahepatic metastases in patients with colorectal cancer (CRC) can be considered a relative contraindication for local treatment with curative intent. This study aims to assess the survival outcomes of patients with CRLM and extrahepatic metastases after comprehensive local treatment of all metastatic sites.
Material and methods: Patients with CRLM who received local treatment of all metastatic sites were extracted from the prospective AmCORE registry database and subdivided into two groups: CRLM only vs CRLM and extrahepatic metastases. To address for potential confounders multivariate analysis was performed. The primary endpoint was overall survival (OS).
Results: In total, 881 patients with CRLM only and 60 with CRLM and extrahepatic disease were included, median OS was 55.7 months vs 42.7 months respectively. OS was significantly lower in patients with concomitant extrahepatic metastases (HR 1.477; 95% CI 1.029-2.121; p = 0.033), the survival-curve shows a plateau. Extrahepatic manifestations were pulmonary (43.3%), peritoneal (16.7%) and non-regional lymph node metastases (10.0%). In patients with pulmonary and non-regional lymph node metastases, OS did not significantly differ from patients with CRLM only disease; concomitant peritoneal metastases showed an inferior OS (HR 1.976; 95% CI 1.017-3.841, p = 0.041).
Conclusions: In this comparative series OS was inferior for patients with multi-organ metastatic CRC versus patients with CRLM alone. Nonetheless, the long-term survival-curve plateau seems to justify local treatment in a subset of patients with multi-organ metastatic CRC, especially for patients with CRLM and pulmonary or lymph node metastases.

Therapy

Adaptive Frequentist Designs in the Randomized HOVON-87/NMSG18 Clinical Trial for patients with Multiple Myeloma

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Background:
The median costs of phase III trials are $19 million [Moore, 2018]. Adaptive trial designs facilitate anticipation on outcomes and early stopping for efficacy or futility, potentially mitigating costs. The phase III HOVON-87/NMSG18 trial showed no statistically significant difference in progression-free survival between melphalan-prednisone with lenalidomide or thalidomide (MPR-R versus MPT-T) in multiple myeloma (MM) [Zweegman, 2016]. We retrospectively introduced interim analyses studying three adaptive designs, to address how these might have changed trial conduct.

Methods:
Group sequential and super superiority designs allow early trial termination for strong efficacy or futility. We introduced redefined HR boundaries for efficacy and futility at 33% and 67% of events, with a gamma spending function. A sample size re-estimation design allows for early termination and/or inclusion of more patients, determined by the conditional power at 67% of events.

Results:
Group sequential
The HR remained between predefined boundaries during interim analyses, suggesting trial continuation, without early termination.

Sample size re-estimation
The interim conditional power for benefit of MPR-R suggested early trial termination. Although inclusion would be complete, termination would have resulted in 27 months follow-up reduction.

Super superiority
A larger sample size would be needed because of the null-hypothesis (HR=0.95). The futility bound for benefit of MPR-R was exceeded at the second interim analysis, resulting in a follow-up time reduction.

Summary:
Retrospectively applying three frequentist adaptive designs to a 'negative' MM trial, shows that a group sequential design would not have led to trial adaptation. However, sample size re-estimation and super superiority designs suggested earlier trial termination because of futility, leading to reduction of follow-up time.

Therapy

Omission of axillary lymph node dissection in cN2-3 breast cancer patients with an excellent response on primary systemic treatment is safe

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Introduction: Oncological outcomes of omitting axillary lymph node dissection (ALND) in patients with node-positive breast cancer (cN+) after primary systemic therapy (PST) are lacking. This study presents the oncologic outcome of node-positive breast cancer patients with >3 suspicious axillary lymph nodes treated according to the MARI-protocol.

Methods: Patients with cN+ breast cancer and >3 suspicious axillary lymph nodes on FDG-PET/CT pre-PST, who were treated according to the MARI protocol were prospectively included between 2014 and 2021. After PST, the MARI node was excised; patients with a pathological complete response (ypN0) of the MARI node received locoregional radiotherapy (LRRT). Patients with residual disease (ypN+) received ALND plus LRRT. Primary endpoint was axillary recurrence rate (ARR). Secondary endpoints were 5-year invasive disease free survival (5y iDFS) and 5-year overall survival (5y OS).

Results: Of the 218 included patients. Median (IQR) age was 50 (42 – 57) years. 47% of patients (103/218) had ypN0 and were treated with LRRT alone, whereas 53% of patients (115/218) had ypN+ and underwent ALND plus LRRT. Median (IQR) follow up was 44 (26 – 62) months. Axillary recurrence rate was 2.9% (n = 3) in the ypN0 group and 3.5% (n =4) in the ypN+ group. Invasive DFS and OS was worst in ypN+ patients who underwent ALND plus LRRT (82% vs. 89% and 90% vs. 95%).

Conclusion: Omission of axillary lymph node dissection after PST in selected node positive breast cancer patients with >3 suspicious lymph nodes using the MARI protocol is associated with an excellent oncologic outcome.

Therapy

Neoadjuvant therapy versus upfront surgery in patients with left-sided resectable pancreatic adenocarcinoma: an observational international multicenter study

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Purpose: To investigate the association of neoadjuvant therapy with overall survival (OS) for patients with left-sided (i.e., pancreatic body/tail) resectable pancreatic cancer compared to upfront surgery.

Methods: Retrospective international multicenter study (76 centers; 18 countries; 4 continents), including all consecutive patients who underwent distal pancreatectomy for pathology-proven left-sided resectable pancreatic cancer, either after neoadjuvant therapy or with upfront surgery (2013-2019). Primary endpoint was OS from diagnosis. Time-dependent Cox regression analysis was performed to investigate the association of neoadjuvant therapy with OS, adjusting for confounders at time of diagnosis. Adjusted OS probabilities were calculated.

Results: Overall, 2,288 patients were included of whom 289 patients (13%) received neoadjuvant therapy. Most common neoadjuvant regimens used were (m)FOLFIRINOX (38%) and gemcitabine-nab-paclitaxel (22%). Neoadjuvant therapy

was independently associated with prolonged OS (HR=0.69 [95%CI 0.58-0.83]). Adjusted median OS after neoadjuvant therapy was longer compared to upfront surgery (53 vs. 37 months; P=0.0003) with adjusted 5-year OS rates of 47% versus 36% (P=0.0002). Interaction analysis showed a stronger effect of neoadjuvant therapy in patients with a larger tumor size (P=0.002) and higher serum CA19-9 (P=0.001). In contrast, the effect of neoadjuvant therapy was not increased in case of splenic vein/artery, retroperitoneal, or multivisceral involvement.

Conclusions: Neoadjuvant therapy is associated with prolonged OS compared to upfront surgery in patients with left-sided resectable pancreatic cancer, particularly with larger tumors or increased serum CA19-9 at diagnosis. A randomized controlled trial by intention-to-treat is needed to confirm the value of neoadjuvant therapy for these patients.

Therapy

Phase I trial investigating neo-adjuvant DURvalumab administered IntraTumorally (DURVIT) in early-stage cervical cancer: safety, toxicity and immunological effects.

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Activation of T-cells in tumor-draining lymph nodes is vital for the efficacy of immune checkpoint inhibitors targeting PD-(L)1. Intratumoral administration of lower doses of immune checkpoint inhibitors may result in optimal access to tumor and draining lymph nodes, while minimizing systemic treatment-related adverse events (AE). We assessed the safety, toxicity and immunological effects of neo-adjuvant, intratumorally injected durvalumab, a PD-L1 checkpoint inhibitor, in early-stage cervical cancer.

A phase I study was performed in patients with early-stage cervical cancer, scheduled for radical hysterectomy and pelvic lymphadenectomy. Durvalumab was injected intratumorally two weeks before surgery at escalating doses of durvalumab (5/10/20 mg) in a 3+3 trial design. Primary endpoints were safety and toxicity. Secondary endpoints were immunological effects. Immune responses were analyzed by flow cytometry.

Twelve patients were included: three patients received 5 mg, three patients 10 mg and six patients 20 mg durvalumab. Eight patients experienced treatment-related AE, not exceeding grade 1-2. One patient at 20 mg had immune-related thyroiditis, resulting in grade 2 hypothyroidism. PD-L1 expression was identified in 6/12 pre-treatment tumor samples. Comprehensive flow cytometric analyses revealed increased activation of CD8+ T-cells in patients treated with low-dose durvalumab. At a higher dose level, increased rates of regulatory T-cells, and decreased CD8+ T-cell/Treg ratios, were observed.

This is the first study to investigate neo-adjuvant, intratumorally administered, anti-PD-L1 antibodies in early-stage cervical cancer. This strategy is safe, since no toxicity exceeding grade 2 was observed. Increased Treg frequencies suggest PD-L1 blockade should be combined with Treg-reducing treatment strategies in future studies.

Therapy

Remote patient monitoring using mHealth technology in cancer care and research: patients' perspectives, willingness, and current use

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Purpose. Though there is an increasing interest in the potential of mHealth as monitoring tools for cancer patients, sparse knowledge is available about their perspectives regarding its adoption. This study aimed to gain insight on cancer patients' willingness, perspectives and current use of mHealth monitoring tools.

Methods. Cross-sectional data was collected via a nation-wide survey (n=4196) and semi-structured interviews (n=13). Participants were eligible if they were 18 years or older with a current or past diagnosis of cancer. Descriptive statistics were made using Chi-squared tests and logistic regression. The interview guide was based on the Technology Acceptance Model.

Results. Of the survey respondents, 3346 (79.7%) used smartphones, 2367 (56.4%) tablets and 1285 (30.6%) wearables. A total of 2927 (69.8%) respondents were willing to use an app and 2477 (59.1%) a wearable for digital monitoring. Younger age and higher education level were significantly associated with higher willingness to use apps ($p < 0.001$) and wearables ($p < 0.001$), whereas gender, cancer type and travel time were not. In the interviews, the main barriers for mHealth use included persistent reminders of illness, receiving unwanted notifications, and the acknowledgment that mHealth shouldn't substitute human doctors. Conversely, patients recognized the potential for time savings and reducing the communication threshold through mHealth, viewed active monitoring as non-burdensome, and expressed willingness to adopt such a platform if they perceived personal or societal relevance.

Conclusion. Patients' attitudes were positive towards the implementation of remote digital monitoring, showing promising prospects for future research of mHealth in oncology.

Therapy

Zanubrutinib in Monoclonal Immunoglobulin M-Related Light Chain Amyloidosis (IgM amyloidosis): tolerability and efficacy

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IgM amyloidosis is rare and mostly associated with IgM MGUS or WM. While there are few data to guide treatment, intensive chemotherapy is recommended to achieve a deep hematological response. However, this is too toxic for a significant proportion of patients due to age and amyloid-related morbidities. Bruton tyrosine kinase inhibitors are highly effective in WM. However, in a small case series with ibrutinib in IgM amyloidosis, serious (cardiac) side effects were observed, translating into poor tolerability. Zanubrutinib could potentially be a more feasible approach, with a more favorable (cardio)toxicity profile and trend towards deeper responses.

Data of all consecutive IgM amyloidosis patients treated with zanubrutinib were retrospectively collected.

Seven patients were included. Median follow up was 6.0 months (range 3.3 – 16.8) and median duration of therapy was 6.0 months (range 2.2 – 11.5) with treatment ongoing in 4 of 7 patients. Best hematologic response was a VGPR in 3 out of 7 patients. Of the 4 remaining patients, 2 only recently started therapy and have not reached a response, 1 patient switched to next line due to lack of hematological response, and 1 patient died of heart failure. Grade ≥3 AEs were reported in four individual patients and did not lead to permanent discontinuation.

Zanubrutinib led to a VGPR in at least 3 out of 7 patients. Of importance, 6 out of 7 patients experienced no cardiac events. Based on these preliminary data, zanubrutinib might represent a treatment option for IgM amyloidosis patients that are unfit for intensive chemotherapy.

Therapy

Reduction of colorectal anastomotic leakage by optimizing the intraoperative condition: the DoubleCheck study

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In order to predict colorectal anastomotic leakage (CAL) or even prevent its occurrence, the aim of the current study was to introduce a bundle of pre- and perioperative interventions to minimize exposure to modifiable risk factors and determine its effect on CAL. This open-labelled, two stepped, interventional study was performed between January 2016 and December 2023 in fourteen international hospitals. Six intraoperative modifiable risk factors for CAL were identified and, subsequently, a bundle of care optimizing the intraoperative condition was introduced in patients undergoing colorectal surgery and consisted of correction of anemia, glucose measurement, attaining normothermia, administration of antibiotics within 60 to 15 minutes preoperatively, refraining from vasopressive agents and epidural analgesia if possible. Primary outcome was the occurrence of risk factors just prior to the creation of the anastomosis, secondary outcomes were CAL and mortality. Univariate and multivariate logistic regression analysis were performed to establish the relationship between the intervention protocol, the intraoperative condition defined by exposure to the six factors and CAL. The pre-intervention group consisted of 1572 patients versus 902 patients in the optimization group. The pre-intervention group had a mean of 1.84 risk factors versus 1.63 in optimized patients (P <0.001). CAL was significantly lower in the optimized group (8.6% vs 6.2%, P = 0.039). The reduction of CAL was associated with the intervention bundle in multivariate regression analysis (OR 1.521, 95% CI 1.01-2.29, P = 0.045). This study showed that optimizing the intraoperative condition reduced the colorectal anastomotic leak rate.

Therapy

A nationwide real-world evaluation of cytoreductive nephrectomy in patients with synchronous metastatic renal cell carcinoma in the era of new systemic treatment options.

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Introduction

Deferred cytoreductive nephrectomy (dCRN) has become the main standard for metastatic renal cell carcinoma (mRCC). Nevertheless, upfront cytoreductive nephrectomy (uCRN) is still part of the daily practice and questioned again in the recent immunotherapy (IO) era. The aim of this nationwide real-world study was to evaluate the overall survival (OS) of patients with uCRN versus first-line systemic therapy (ST) with or without dCRN in the IO era.

Methods

In this historic cohort study all patients diagnosed with synchronous mRCC between 2018 and 2020 were identified from the NCR. The OS of patients receiving first-line ST was compared to patients with uCRN using propensity score-based inverse probability of treatment weighting (IPTW)-adjusted Kaplan-Meier curves and Cox regression analysis. Included confounders were T-stage, N-stage, age, year of diagnosis, IMDC risk category, performance status, histology, number of metastatic sites, and the presence of lung, liver, brain or bone metastases.

Results

Out of 956 included patients, 742 (78%) received first-line systemic therapy and 214 (22%) underwent uCRN. Of those receiving systemic therapy, 8% (61 of 742) received a dCRN, and 61% (130 of 214) of patients with uCRN were subsequently treated with ST. IPTW adjusted median OS was 20 months for patients with ST vs 28 months with uCRN. The IPTW-adjusted HR was 0.64 (95%CI 0.52-0.79).

Conclusions

The OS of patients with synchronous mRCC treated with uCRN was improved compared to patients treated with first-line ST. However, this study has limitations due to the potential for unmeasured confounding factors. Randomized trials are needed to reassess the role of CRN.