BREAST CANCER MICROENVIRONMENT CHANGES IN HORMONE RECEPTOR-POSITIVE BREAST CANCER BEFORE AND AFTER ENDOCRINE THERAPY

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Background: Oestrogen receptor-positive (ER+)/human epidermal growth factor receptor 2-negative (HER2-) breast cancer (BC) is considered to be an immunologically cold tumour compared to triple-negative breast cancer. Therefore, the tumour microenvironment (TME) of ER+/HER2- BC is understudied. The aim of this project is to investigate the TME and the immune response during neoadjuvant endocrine therapy (NET) and to correlate this with the treatment response in a real-life setting.

Methods: Expressions of immune checkpoint receptors and immune cells were examined immunohistochemically in pre- and post-NET on a cohort of 44 ER+/HER2-BC patients. They were treated with tamoxifen (N=8), an aromatase inhibitor (N=36), for a median duration of 6 months (range 1-32) months. Monoclonal antibodies for PDL-1, PD-1, TIM-3, LAG-3, CTLA-4, CD4, CD68, FOXP3, RANK and RANKL were used. All stainings were done according to validated protocols. Positivity was defined as staining > 1% on TILs. Response to NET was evaluated according to tumour size change on imaging and Ki-67 change.

Results: The tumour size diameter decreased with a mean of 7.818 mm ($p < 0.005$) during NET, and the value of Ki-67 value decreased significantly after NET ($p < 0.005$). An increase in PD-L1 expression after NET showed a trend towards significance ($p=0.088$), and RANK expression on TILs significantly decreased with a median of 30% ($p=0.0007$) after NET. A good response to NET, defined as a decrease in tumour size and/or decrease of Ki-67, was found to be associated with a longer duration of NET, a change of CD4+T-cells, a change of RANK expression on TILs and a higher number of CD68+ tumour-associated macrophages before the start of NET and RANK expression on TILs before the start of NET.

Conclusion: The immune micro-environment plays an important role in ER+/ HER2- BC. NET influences the composition and/or functional state of the infiltrating immune cells. Furthermore, changes in the immune microenvironment are also associated with treatment response.

A TCF4/BRD4-DEPENDENT REGULATORY NETWORK AS A DRIVER OF PRIMARY RESISTANCE TO IMMUNE CHECKPOINT INHIBITION IN MELANOMA

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Immune checkpoint inhibition (ICI) has become a standard of care for advanced melanoma. However, approximately half of the patients will not respond. Deciphering the mechanisms of treatment resistance remains a key challenge in immune-oncology. We, therefore, conducted a biomarker study to profile the transcriptomic landscape of metastatic melanoma lesions at single-cell resolution. By comparing before and early-on-treatment samples, we unravel the composition of the tumour and its microenvironment in patients treated with anti-PD1 based ICI. Tissue from drug-naive patients with advanced melanoma was processed for single-cell RNA sequencing (scRNAseq). In addition, spatial analyses were performed on embedded material. Patients were stratified according to RECIST1.1 best overall response. In selected cases, pathological response assessment of fully resected lesions was taken into account. We functionally annotated 11 malignant cell clusters corresponding to distinct transcriptional metaprograms. The mesenchymal-like melanoma (MES) phenotype was associated with primary resistance to ICI, whereas the antigen-presentation-like state was associated with response. TCF4, a transcription factor regulated by BET family proteins, was found to govern the MES state. Knockdown of *TCF4* reversed gene expression associated with this state, and induced transcription associated with (tumour-associated) antigen presentation. Consequently, this effect could be phenocopied by treating MES cells with a BET inhibitor. Using scRNAseq, we delineated the ecosystem of melanoma lesions during ICI treatment. We found extensive non-genetically driven heterogeneity, and identified previously undescribed cell states, two of which were associated with divergent treatment outcomes. TCF4 appeared as a key regulator of dedifferentiation, a mechanism of early immune evasion. Our results provide the rationale to further explore the combination of BET inhibition with ICI to overcome primary resistance.

CTDNA AS A BIOMARKER OF RESPONSE TO IMMUNE CHECKPOINT INHIBITORS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Introduction: Nivolumab and pembrolizumab, two PD1 immune checkpoint inhibitors (ICI), are approved for the treatment of recurrent and/or metastatic (R/M) squamous cell carcinomas of the head and neck (SCCHN). However, the objective response rate is only 15-20%. Circulating tumor DNA (ctDNA) dynamics have the potential to early identify the patients who will benefit from immunotherapy.

Aims: Our primary endpoint is to study if the changes in the proportion of ctDNA (ΔctDNA) during the first 6-8 weeks of ICI treatment can predict the response according to RECISTv1.1. Our secondary objectives are to investigate if ΔctDNA can (i) predict overall survival, (ii) detect disease progression early before imaging, (iii) and identify emerging clones, responsible for relapse.

Methods: Patients with R/M SCCHN treated with ICI are included in a prospective collection of plasma samples, collected before the start of the treatment (baseline (B)), 6-8 weeks after treatment initiation (FU1), and every 2-3 months until progression. We developed a non-personalized method to detect ctDNA: we built a specific gene-panel of 36 frequently mutated genes in R/M SCCHN. All the samples were sequenced with the addition of unique molecular identifiers (UMIs) prior to amplification in order to detect variants present at a very low proportion (Variant Allele Frequency (VAF) ~0.01%). The ΔctDNA is the difference between the mean VAF at baseline and the mean VAF at FU1.

Results: As a proof-of-concept, we have analysed 40 paired samples from 20 patients. ctDNA was detected in 95% of the analysed plasma samples. Our preliminary analysis shows that the ΔctDNA could be representative of the clinical response in at least 75% of our population.

Conclusion: We will confirm our results with samples from 60 patients and analyse the sequential samples during ICI treatment from the entire cohort to evaluate the potential use of ctDNA to detect early disease progression and identify emerging resistant clones.

EVALUATING THE EFFICACY OF PHOTOBIOMODULATION THERAPY FOR MANAGING CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY: A RANDOMIZED, CONTROLLED TRIAL (NEUROLIGHT TRIAL)

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Introduction: Chemotherapy-induced peripheral neuropathy (CIPN) is a common, debilitating side-effect of chemotherapy (CTx). Unfortunately, management of CIPN usually requires a reduction of the CTx dosage or withdrawal of CTx. Photobiomodulation (PBM) therapy is based on applying (near)-infrared light on the target tissue to stimulate cell repair processes. Previous research demonstrated the efficacy of PBM in preventing CIPN. The aim of this trial was to evaluate the efficacy of PBM in managing CIPN, while simultaneously focusing on the patients' quality of life (QoL) and the optimal PBM dosage.

Methods: A randomized controlled trial (RCT) with 60 cancer patients diagnosed with CIPN was performed at the Jessa Hospital (Hasselt, Belgium). Patients received PBM 2x/week for three weeks and were randomized to receive PBM with a fluence of 6 J/cm2 (PBM-1 group, n=28) or 8 J/cm2 (PBM-2 group, n=32). The Numeric Rating Scale (NRS) and Functional Assessment of Cancer Therapy/Gynecologic Oncology Group Neurotoxicity (FACT/GOG- NTX) questionnaires were used to evaluate the patients' pain and QoL, respectively. Outcome measures were collected at baseline, at the final PBM session, and three weeks post-PBM.

Results: The NRS score decreased significantly over time in the PBM-1 group (P=0.015), but not in PBM-2. The post-hoc analysis revealed a significant improvement in the PBM-1 group's pain score between baseline and the follow-up visit (P=0.006). The two-way ANOVA showed no significant main group effect or group-by-time interaction for the FACT/GOG-NTX total score and its neurotoxicity subscale (Ps≥0.35). However, the main effect of time was significant for the FACT/GOG-NTX total score and neurotoxicity subscale (P=0.022, P=0.004, respectively).

Conclusion: This trial shows that PBM can reduce the pain associated with CIPN and improve the patients' QoL. A larger RCT with a more extended follow-up is necessary to support these findings and elucidate the optimal PBM parameters.

LONG-TERM HEALTHCARE UTILIZATION IN OLDER PATIENTS WITH CANCER AND THE PREDICTIVE VALUE OF THE G8 SCREENING TOOL: A LARGE DATA LINKAGE COHORT STUDY IN BELGIUM

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Introduction/aim: The evidence on geriatric screening (GS) for older patients with cancer is expanding. However, long-term outcomes are often missing because of the limited follow-up.

This study aims to evaluate the long-term healthcare utilization in older patients after cancer diagnosis in Belgium, and the association with baseline GS results.

Methods:
Older patients with a new cancer diagnosis who underwent G8 geriatric screening in previous studies (2009-2015) and who survived ≥3 months post-G8 were included. The clinical data were linked to cancer registry data and healthcare reimbursement data for long-term follow-up. The occurrence of the outcomes was assessed up to 3 years post-G8. Their association with baseline G8 was estimated using adjusted rate ratio (aRR) from Poisson regression and using reverse Kaplan-Meier analysis.

Results:
In total, 6,391 patients with a median age of 77 years old were included. Breast, colon and lung cancer were the most common diagnoses, and 64.3% (n=4,110) had an abnormal baseline G8 score (≤14/17). In the first 3 months after cancer diagnosis, healthcare utilization peaked and then decreased over time except for general practitioner contacts and home care days, for which it remained high throughout the whole 3 years. Patients with an abnormal baseline G8 score had more hospital admissions (aRR, 1.21;p<0.001), hospital days (aRR, 1.66;p<0.001), emergency department visits (aRR, 1.40;p<0.001), intensive care days (aRR, 1.46;p<0.001), general practitioner contacts (aRR, 1.19;p<0.001), home care days (aRR, 1.58;p<0.001), and nursing home admissions (16.7% vs 3.1%;p<0.001) over the 3 years. Of the 2,281 patients with a normal baseline G8 score, 62.3% continued to live at home independently at 3 years and 22.0% were not alive anymore. Of the 4,110 patients with an abnormal baseline G8 score, only 25.7% continued to live at home independently and 53.3% were not alive anymore.

Conclusion: G8 geriatric screening at cancer diagnosis predicts healthcare utilization in the following 3 years.

IDENTIFICATION OF NEW BIOMARKERS TO BETTER PREDICT AND IMPROVE RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN TRIPLE NEGATIVE BREAST CANCERS

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Introduction: The implementation of neoadjuvant chemotherapy (NAC) as the standard of care for patients with high-risk early-stage or locally advanced breast cancer (BC) is one of the major changes in the BC clinical landscape. Pathological complete response (pCR) after NAC is a prognostic factor with an improvement in event-free and overall survival. Nevertheless, achieving pCR upon NAC only occurs in 30% of the cases. Among the different BC subtypes, triple negative BC (TNBC) display the poorest prognosis and the highest risk of relapse, highlighting the urgent need for new predictive biomarkers to better select patients and adapt NAC according to the chances of response. Here, we aim to reveal specific transcriptomic and metabolic signatures correlated with distinct responses to NAC in treatment-naive TNBC, and to compare gene expression profiles of resistant TNBC (i.e. escaping from NAC treatment) with initial (treatment-naive) tumors.

Methods: 2D and 3D cultures of “early TNBC” cell lines, patient-derived tumor organoids and human clinical specimens were used in combination with drug testing, RNA-seq and metabolomics studies.

Results: We have first shown that our TNBC cell models exhibit distinct responses to NAC. Treatment with NAC induces major metabolic alterations in glycolysis and mitochondrial respiration, as revealed by changes in glucose consumption, lactate secretion and oxygen consumption. Sequential treatment with NAC and specific metabolic inhibitors exhibits potent growth-inhibitory effects on TNBC cell lines and organoids. Clinical BC specimens have also been collected from diagnostic biopsies and from surgical pieces upon incomplete response to NAC. Through bulk transcriptomic analyses, we have reported changes in expression o genes related to tumor cell metabolism.

Conclusion: Altogether, our data may lead to the identification of predictive biomarkers and/or therapeutic avenues that enable the delay of TNBC progression or overcome therapy resistance.

KI-67 INDEX AFTER NEOADJUVANT ENDOCRINE THERAPY AS A PROGNOSTIC BIOMARKER IN PATIENTS WITH ER+/HER2- EARLY BREAST CANCER: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Introduction/aim: Neoadjuvant treatment distinguishes responders from non-responders, but pathologic complete response is uncommon in estrogen receptor-positive/HER2 negative (ER+/HER2-) early breast cancer (eBC). Therefore, we aim to assess the prognostic value of Ki-67 after neoadjuvant endocrine therapy (NET).

Methods: We did a systematic literature search of PubMed, Embase, CENTRAL, and conference proceedings up to 28/06/22 to identify clinical trials or observational studies reporting an association of low versus high Ki-67 index after NET with recurrence-free survival (RFS) and/or overall survival (OS) in women with ER+/HER2- eBC (CRD42022333735). We performed a sensitivity analysis of the studies measuring Ki-67 index after NET in a pre-planned core biopsy. As heterogeneity between studies did not reach statistical significance, we used a fixed effects model to combine RFS and OS hazard ratios (HR) with 95% confidence intervals (CI).

Results: We included 11 studies reporting data from 4,231 patients. The majority of studies were clinical trials (n=4,143), including only post-menopausal women (n=3,953) treated with aromatase inhibitors (n=3,359). Three studies evaluated Ki-67 after NET in a pre-planned core biopsy after a window of treatment of two to four weeks (n=3,348), while the other eight evaluated Ki-67 in the surgery specimen (n=883) after 2 to 24 weeks of NET. The median follow-up for RFS ranged between 37 and 95 months, and between 62 and 84 months for OS. Higher Ki-67 index after NET was significantly associated with worse RFS (HR 2.43, 95%CI 1.99-2.98) and OS (2.66, 95%CI 1.65-4.28). The sensitivity analysis of the three studies evaluating Ki-67 after NET in a pre-planned core biopsy showed the same association with RFS (HR 2.41, 95%CI 1.77-3.30).

Conclusion: Ki-67 index after NET is associated with survival outcomes, even after a short course of two to four weeks of NET, reinforcing the prognostic value of this biomarker in women with ER+/HER2- eBC.

EVEROLIMUS EFFECTIVENESS AFTER PROGRESSION ON CDK4/6 INHIBITORS FOR PATIENTS WITH ENDOCRINE RECEPTOR-POSITIVE, HER2-NEGATIVE, ADVANCED BREAST CANCER (EVERGREEN)

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Background: Endocrine therapy (ET) in combination with CDK4/6 inhibitors (CDK4/6i) is a standard treatment option for endocrine receptor-positive, HER2-negative, advanced breast cancer (ABC). There is no direct evidence supporting an optimal treatment sequence after progression to CDK4/6i. We aim to assess the efficacy and safety of everolimus (EVE)-based therapy immediately after CDK4/6i.

Methods: All patients (pts) with ABC treated with EVE+ET as immediate next line after exposure and disease progression on CDK4/6i were included in this retrospective monocentric study. Study endpoints were progression-free survival (PFS), time-to-chemotherapy (TTC), and overall survival (OS), all from the start date of EVE. Survival curves were estimated using the Kaplan-Meier method with confidence interval (CI) of 95%, and the log-rank test was used to compare survival distributions.

Results: In total, 27 pts were included in this analysis. Median age was 60 years-old (interquartile range [IQR] 50-68), 78% had ECOG performance status 0-1, 96% were postmenopausal, and 44% had de novo ABC. The median time between the completion of adjuvant ET and the diagnosis of ABC was 3.3 years (IQR 0-4.1 years). Seventy percent received ≤2 prior treatment lines in the metastatic setting, including 15% with prior exposure to chemotherapy. Median EVE treatment duration was 4.5 months (mos) (95% CI, 2.8-7.1). Median PFS (mPFS) was 5.3 mos (95%CI, 3.3-7.9). mPFS was significantly longer in patients with recurrent compared to *de novo* ABC (7.1 vs. 3.1 mos; p=0.03). There were no PFS differences according to the presence of visceral metastases. mTTC was 7.1 mos and mOS was 28 mos. EVE dose reductions were required in 37% of patients. The rate of AE grade 3 was 7%, there were no grade 4-5 AE, and 26% of patients had adverse events (AE) leading to EVE discontinuation.

Conclusion: EVE is an effective treatment that deserves to be explored in larger trials as an option immediately after progression to CDK4/6i in selected pts.