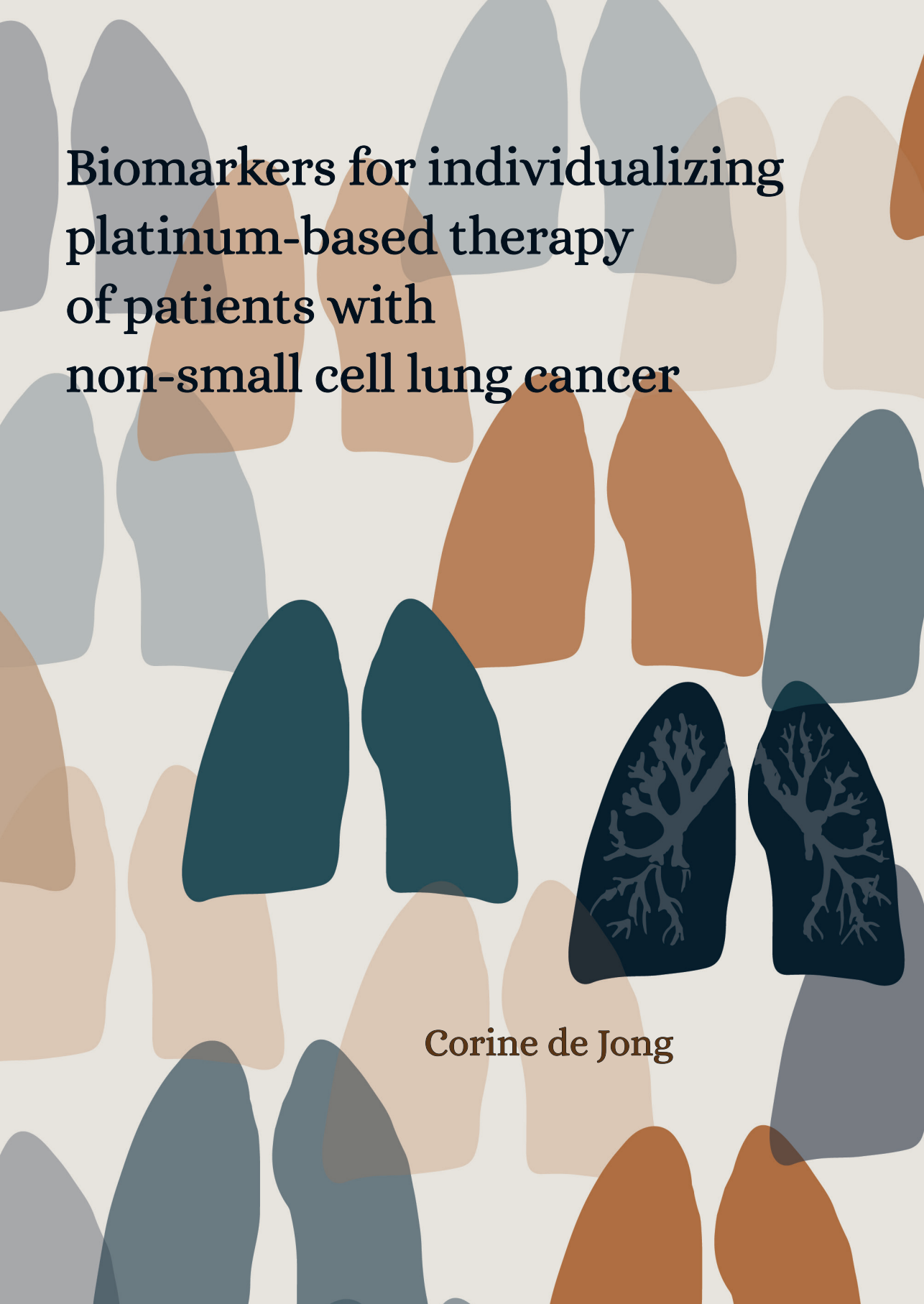
Biomarkers for individualizing platinum-based therapy of patients with non-small cell lung cancer



Corine de Jong

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Biomarkers for individualizing platinum-based therapy of patients with non-small cell lung cancer

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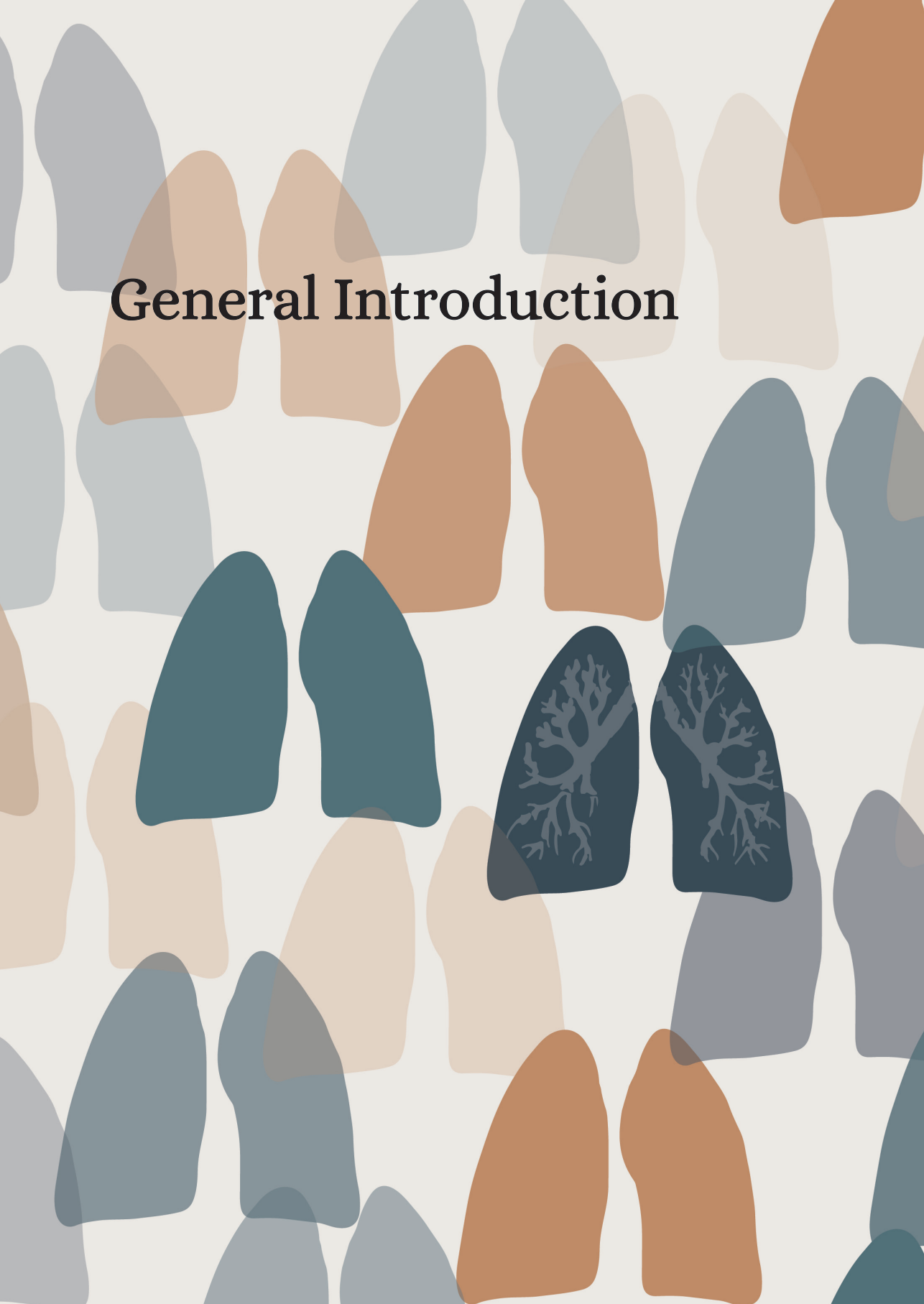
Contents

Chapter 1

General Introduction



General Introduction

Non-small cell lung cancer

Lung cancer remains the leading cause of cancer-related death worldwide, of which non-small cell lung cancer (NSCLC) accounts for nearly 85%.[1] In the Netherlands, more than 14,000 patients are diagnosed with lung cancer yearly.[2,3] Early presentation of lung cancer is characterized by an aspecific symptomatic pattern with a prominent new persisting or worsening cough, chest, back, or shoulder pain, unexplained weight loss, and sudden shortness of breath. Radiological screening and a biopsy are necessary for diagnosis. The Tumor Nodes Metastases (TNM) classification for malignant tumors defines a tumor's anatomical extent and disease stage.[4] The disease stage is the most important prognostic factor and is crucial for determining the optimal treatment regimen.[5–7] However, since many patients have no clinical symptoms in the early stages and there is no routine nationwide screening program, most lung cancers are diagnosed at an advanced stage.[1] At the time of diagnosis, 48% of patients are suffering from stage IV NSCLC, indicating a poor prognosis with a median 1-year survival rate of only 21% for patients diagnosed in 2018.[2] In the near future, the prognosis will improve with the widespread adoption of new therapeutic options.

Non-small cell lung cancer treatment

Treatment options for NSCLC consist, depending on the disease stage, of (a combination of) surgery, radiotherapy, and antineoplastic drugs. Surgery, in combination with (neo) adjuvant platinum-based therapy, is a potentially curative treatment for early-stage NSCLC.[8] In advanced stages, stable disease or tumor response, symptom palliation, and maintaining or improving quality of life or life prolongation are pursued as the primary treatment goals.[5] For these patients, systemic antineoplastic therapy or best supportive care is indicated. For decades, chemotherapeutic regimens for NSCLC have consisted of platinum-agents (cisplatin, carboplatin) combined with an additional antineoplastic agent (such as etoposide, gemcitabine, paclitaxel or pemetrexed), as supported by evidence from multiple clinical studies.[9,10] Identifying targetable mutations (e.g., epidermal growth factor receptor [*EGFR*] mutations and anaplastic lymphoma kinase [*ALK*] rearrangements) has led to new treatment options in recent years.[11] In addition, the recent introduction of immunotherapy has resulted in new treatment perspectives and strategies. Based on the KEYNOTE-189 and KEYNOTE-407 study results,[12-14] pembrolizumab, an anti-programmed death 1 (PD-1) monoclonal antibody, combined with a platinum agent and pemetrexed or paclitaxel is currently considered the first-choice option in metastatic NSCLC. Recent

studies also suggest pembrolizumab monotherapy in the case of tumors with a high expression (tumor proportion score [TPS] ≥ 50%) of programmed death-ligand 1 (PD-L1).[15] However, since merely 30% of tumors exhibit high PD-L1 expression,[16] only a minority of patients with NSCLC are eligible for monotherapy with immunotherapy as a first-line option. Moreover, in rapidly progressive disease, chemotherapy combined with immunotherapy is preferred over monotherapy with immunotherapy because of the difference in time-to-response.[17] In addition, platinum-based therapy is also given as a second-line treatment after targeted therapy or monotherapy with immunotherapy. Therefore, despite the rapid introduction of therapeutic innovations, platinum-based therapy remains a cornerstone of NSCLC treatment.[18]

Platinum-based therapy and toxicity

Platinum agents, such as cisplatin and carboplatin, affect malignant cells by interfering with mitosis and cell division.[19] The mechanism of action of platinum agents is based on the ability to crosslink with the urine bases of deoxyribonucleic acid (DNA) to form DNA adducts. Incorporating platinum agents prevents DNA repair, which subsequently leads to DNA damage and apoptosis.[19] Despite its broad applicability, years of treatment experience, and improved supportive care (e.g., anti-emetics, intravenous fluid repletion), treatment using platinum agents is frequently accompanied by severe side effects.[20] Carboplatin-based or cisplatin-based therapy in patients with advanced NSCLC (stage IV) has exhibited equivalent treatment response in terms of radiological response and overall survival.[21] However, regarding the toxicity profile, there are distinctions between the platinum agents; cisplatin carries a higher toxicity profile than carboplatin.[22] While cisplatin-induced toxicities primarily concern nausea or vomiting, nephrotoxicity, neurotoxicity, and ototoxicity, carboplatin's bone marrow suppressant effect is more prominent.[21] Since treatment-related toxicity can lead to permanent treatment discontinuation, treatment delay, and dose de-escalation, it could also affect the therapy's success rate. Moreover, severe toxicity could significantly affect daily life, leading to treatment-related hospital admissions and negatively impacting quality of life.[23] Although some patients' characteristics (e.g., age, performance status, comorbidities, impaired renal function) are known to be predictive factors for the incidence and severity of toxicity,[24] much remains unknown. Since information from daily clinical practice is scarce, it is currently hardly possible to identify patients at high risk of developing treatment-related toxicity.

Biomarkers for treatment outcomes in platinum-based therapy

In addition to tumor histology and patient characteristics, biomarkers can contribute to selecting patients with the greatest probability of tumor response and/or treatment-related toxicity. A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of a normal biological process, a pathological process, or a response to a therapeutic intervention.[25,26] Likewise, biomarkers can also help identify those at high risk for therapy failure or treatment-related toxicity, supporting clinical decision-making. For example, sometimes a treatment can be optimized by using an individual patient's genetic background, with as leading examples *CYP2C19* genotyping in patients treated with clopidogrel or *DPYD* genotyping in patients treated with fluoropyrimidine therapy, which are both already been implemented in daily clinical practice.[27,28] To lower the risk of developing excessive toxicity, genetic variants of genes involved in the development of toxicity could be of particular interest as such biomarkers. Previous studies involving patients with different kinds of malignancies report genetic variants in organic transporter molecule genes (e.g., *MATE1, OCT2*); DNA repair enzyme genes (e.g., *ERCC1*, *ERCC2*); genes encoding for tumor suppressor proteins (e.g., *TP53*) or metabolic enzymes involved in platinum detoxification (e.g., *GST1*); and other pharmacodynamic genes (e.g., *COMT*), among others, that could be involved in toxicity development.[29] However, relatively few studies have investigated the impact of genetic variants on the development of platinum-related toxicity. In addition, available studies have demonstrated inconsistent findings, potentially due to patient and treatment heterogeneity and variable study designs. Extensive research in a large cohort in a daily clinical practice setting could help close the knowledge gap.

Other parameters of interest for possible association with treatment response and toxicity are based upon body composition. Changes in the body composition of patients with cancer due to cachexia-associated muscle mass loss are prevalent[30], which may be particularly relevant for further individualized drug dosing. A low lean body weight and skeletal muscle mass (SMM) depletion (sarcopenia), combined with low skeletal muscle tissue radiodensity, have been associated with a higher incidence of chemotherapy-induced toxicity.[31] Such measurements can be performed on pretreatment diagnostic imaging, such as computed tomography (CT) scans, and could be valuable during diagnosis and treatment initiation. Nevertheless, monitoring biomarkers during treatment and follow-up is also desirable to perceive and anticipate changes in tumor response and the patient's clinical condition. An example of potential dynamic biomarkers is the serum levels of specific enzymes or proteins, which can be assessed during treatment.

A notable advantage is that biomarkers derived from the standard diagnostic work-up (e.g., pretreatment diagnostic CT scans, regular blood sampling) can easily be added to routine follow-up and quickly adapted into clinical practice when proven reliable.

Individualized platinum-based treatment is warranted

Currently, little is known about the impact of genetic variants, body composition, and serum biomarkers on treatment outcomes in patients with NSCLC receiving platinum-based therapy. Providing this missing information by studying the association between these parameters and platinum-based therapy-related response and toxicity in a daily clinical practice setting, will likely improve personalized anticancer therapy. Novel insights could promote optimal treatment selection for each patient and identify individuals at higher risk of developing toxicity. Consequently, dose reduction or treatment discontinuation could be avoided, influencing the success rate. As an ultimate goal, clinicians can better inform patients about the expected treatment outcomes, supporting clinical decision-making.

Thesis objective

This thesis aims to provide novel insights into the association between genetic, anthropometric, and serum biomarkers for platinum-based therapy-related response and toxicity in patients with NSCLC in daily clinical practice.

Thesis outline

Chapter 2 of this thesis focuses on the association between genetic variants and platinum-based therapy-related toxicity, described in four studies. **Chapter 2.1** presents the design of the PGxLUNG study, a multicenter prospective follow-up study. The study's primary objective is to investigate the association between genetic variants and chemotherapy-induced toxicity in patients with NSCLC receiving first-line platinum-based therapy. Secondary objectives include exploring the association between anthropometric and serum biomarkers for platinum-based therapy-related response and toxicity. **Chapter 2.2** describes a young woman with severe nephropathy following cisplatin-based therapy who was tested for several genetic variants. **Chapter 2.3** investigates the association between genetic variants and cisplatin-induced nephrotoxicity in a large cohort using genome-wide approaches, complemented by a validation study in an independent cohort. **Chapter 2.4** uses a candidate gene approach to examine the association between genetic variants and chemotherapy-induced peripheral neuropathy.

Chapter 3 describes the association between anthropometric and serum biomarkers for platinum-based therapy-related response and toxicity, outlined in two studies. **Chapter 3.1** explores the influence of skeletal muscle mass and density on chemotherapy-induced toxicity based on pretreatment diagnostic CT scans. **Chapter 3.2** describes a retrospective follow-up study investigating the association between pretreatment serum levels of carcinoembryonic antigen (CEA) and lactate dehydrogenase (LDH), and changes from pretreatment levels, with radiological response and overall survival.

Finally, **Chapter 4**, the general discussion of this thesis, reflects on the main findings and provides future perspectives on how to individualize platinum-based therapy in patients with NSCLC.

Declarations

Authors' contributions

C. de Jong wrote the General Introduction of this thesis. Dr. V.H.M. Deneer, dr. G.J.M. Herder and prof. dr. A.C.G. Egberts reviewed the manuscript critically for important intellectual content and approved the final version.

Competing interests

The authors declare that they have no competing interests.

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Chapter 2

Genetic biomarkers for platinum-based therapy-related toxicity

Chapter 2.1

Genetic variants as predictors of toxicity and response in patients with non-small cell lung cancer undergoing first-line platinum-based chemotherapy: design of the multicenter PGxLUNG study

Corine de Jong
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Abstract

Introduction: Platinum-based chemotherapy is currently the most frequently applied first-line treatment for patients with advanced non-small cell lung cancer (NSCLC) without targetable mutations or high PD-L1 expression. Unfortunately, chemotherapy-induced toxicity is prevalent and may affect patients' quality of life to a considerable extent. Presumably, genetic variants of genes, coding for proteins involved in the processes of the development of toxicity, may be of interest as predictors of benefits and harms of platinum-based chemotherapy. The primary objective of the study is to investigate the influence of genetic variants on the incidence of chemotherapy-induced toxicity in patients with NSCLC undergoing first-line platinum-based chemotherapy. The main secondary objectives are to study the association between genetic variants and treatment response and to study the association between skeletal muscle mass (SMM) as well as patient-reported health-related quality of life (HRQOL) and treatment response and toxicity.

Methods: In this multicenter prospective follow-up study, a total of 350 patients with NSCLC (stage II-IV) undergoing first-line platinum-based chemotherapy will be included. Blood samples for DNA isolation and genotyping, questionnaires and data on patients risk factors and disease stage will be recorded. The primary endpoint is chemotherapy-induced (non-)haematological toxicity, comprising; nephrotoxicity, neuropathy, esophagitis, ototoxicity, pneumonitis, gastrointestinal toxicity, anemia, leukocytopenia, neutropenia and thrombocytopenia. Secondary endpoints include dose-limiting toxicity, HRQOL, and treatment response (radiological response [RECIST 1.1] and overall survival [OS]).

Discussion: Results of the PGxLUNG study will be primarily used to determine the influence of genetic variants on the incidence of chemotherapy-induced toxicity in patients with NSCLC undergoing first-line platinum-based chemotherapy.

Introduction

Lung cancer remains the leading cause of cancer-related death worldwide, in which non-small cell lung cancer (NSCLC) accounts for nearly 85% of all cases.[1] For decades, therapeutic treatment of NSCLC consisted of platinum-based chemotherapy, which has been shown to be moderately effective on progression-free and overall survival.[2,3] However, identification of targetable mutations (e.g., an epidermal growth factor receptor [*EGFR*] mutation or anaplastic lymphoma kinase [*ALK*] rearrangement) have led to changes in treatment options over the past few years.[4,5] In addition, the introduction of immunotherapy has recently led to new treatment perspectives and strategies. Even though there are promising changes in treatment options for NSCLC, only a minority of patients will benefit from these new first-line therapies. In addition, platinum-based chemotherapy is also given as first-line treatment in combination with immunotherapy, or as second-line treatment after targeted therapy.[6-9] Therefore, although there are rapid transformations in the therapeutic landscape, nowadays of chemotherapy remains the mainstay for treatment of NSCLC patients worldwide. Unfortunately, chemotherapy-induced toxicity is prevalent (20%–30%) and may affect patients' quality of life to a considerable extent.[10-11] Chemotherapy is frequently part of palliative care, and it is therefore of the utmost importance to prevent treatment complications. However, identifying patients who are at high risk of developing serious adverse events is difficult, since predictive tools are lacking. Genetic variants of genes, coding for proteins involved in the processes of development of toxicity, may be of interest as predictors of benefits and harms. Previous studies in patients with different kinds of malignancies report genetic variants in organic transporter molecules genes (*OCT2*), DNA repair enzyme genes (*ERCC1*, *ERCC2*), genes encoding tumor suppressor proteins (*TP53*), or metabolic enzymes involved in platinum detoxification (*GST1*) and other pharmacodynamic genes (*COMT*) among others, may be involved in the development of toxicity.[12-14] Other possible prognostic and predictive parameters for treatment response and toxicity are based upon body composition. This could be of relevance since changes in body composition in patients with cancer are prevalent due to cachexia-associated muscle mass loss.[15] Moreover, low lean body weight, and skeletal muscle mass (SMM) depletion (sarcopenia), together with the radiodensity of skeletal muscle tissue, have been suggested to be associated with a higher incidence of chemotherapy-induced toxicity in cancer patients.[15-19] Hence, currently, little is known about the possible associations between genetic variants as well as skeletal muscle depletion and platinum-based chemotherapy-induced toxicity in patients with NSCLC.

Objectives

The primary objective of the Pharmacogenetics Lung Cancer (PGxLUNG) study is to investigate the influence of genetic variants on the incidence of chemotherapy-induced toxicity in patients with NSCLC undergoing first-line platinum-based chemotherapy in a multicenter prospective follow-up study. The main secondary objectives are to study the association between genetic variants and treatment response, to study the association between skeletal muscle mass (SMM) as well as patient-reported health-related quality of life (HRQOL) and treatment response and toxicity.

Methods/design

Setting

This study is a prospective follow-up study with a multicenter design, conducted in one academic hospital (University Medical Center Utrecht), two teaching hospitals (St. Antonius Hospital Nieuwegein/Utrecht, Meander Medical Center Amersfoort) and three general hospitals (Diakonessenhuis Utrecht, Groene Hart Ziekenhuis Gouda, Ziekenhuis Rivierenland Tiel), all in the Netherlands.

Eligibility

The study population consists of NSCLC patients (stage II–IV) undergoing first-line platinum-based chemotherapy as part of routine patient care. Inclusion criteria: (i) Older than 18 years of age; (ii) radiologically-confirmed NSCLC (stage II–IV); and (iii) first-line treatment with platinum-based (cisplatin or carboplatin) chemotherapy or chemoradiotherapy (according to the contemporary ESMO Clinical Practice Guidelines).[4,5] Patients are platinum-based chemotherapy-naïve and treatment is planned or has been initiated. Exclusion criteria: (i) Cognitive impairment; and (ii) unable to read and write Dutch. All patients receive at least one cycle of a platinum-agent combined with a chemotherapeutic agent (e.g., etoposide, gemcitabine, pemetrexed, paclitaxel), targeted therapy (bevacizumab) and/or immunotherapy (e.g., atezolizumab, nivolumab, pembrolizumab), depending on tumor histology and patient characteristics. Radiotherapy can be either sequential or concurrent, according to the physician's choice. Patients can enroll in the study prior to initiation of chemotherapy or after chemotherapy has been initiated. All treatment procedures (i.e., diagnostic work-up, laboratory tests) will be according to local clinical practice for routine patient care. The end of study is the date of the end of follow-up of the last included patient.

Ethical considerations

The protocol complied with the Good Clinical Practice guidelines and the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013), and was approved by the accredited Medical Research Ethics Committee in Nieuwegein (MEC-U, number R15.056). The study was registered in The Netherlands National Trial Register (NTR) on 26 April 2016 (NTR number NL5373610015). The treating medical doctor will obtain written informed consent from each participant.

Measurements

Blood sampling

An EDTA-blood sample for genotyping will be collected in all patients. For patients who enroll in the study prior to initiation of chemotherapy extra EDTA-blood and serum samples will be collected for measurement of biomarkers possibly associated with treatment response and/or toxicity at four points in time (Table 1). Serum and plasma samples will be processed and stored at –80°C until further analysis. The samples will be coded and stored for a period of 30 years, which provides the opportunity to perform additional research in the future.

Sample processing and genotyping

DNA samples will be obtained from EDTA-blood samples using the EZ1 DNA Blood 200 µl kit (Qiagen, Hilden, Germany). DNA isolation will be performed according to validated in-house protocols of the Pharmacogenetics, Pharmaceutical and Toxicological Laboratory (FarmaToxLab) of the Department of Clinical Pharmacy (ISO15189 certified), St. Antonius Hospital Nieuwegein/Utrecht. Single nucleotide polymorphisms (SNPs) will be genotyped by using Kompetitive allele specific PCR (KASP) at LGC Genomics (Hoddesdon, UK) and by using the Infinium Global Screening Array-24 Kit (Illumina, San Diego, CA) at Life and Brain (Bonn, Germany).

Health-related quality of life

Patients who enroll in the study prior to initiation of chemotherapy will be asked to complete questionnaires regarding HRQOL at treatment initiation and, three, six and 12 months after starting chemotherapy (Table 1). The first hardcopy questionnaire will be handed over by a research nurse. Follow-up questionnaires will be sent as a hardcopy to the patient's home address by the research nurse. To assess HRQOL four instruments

will be used; EQ-5D, EORTC QLQ-C30, EORTC QLQ-LC13 and EORTC QLQ-CIPN20 (Table S1). All questionnaires are widely-used and internationally validated.[20-23]

Endpoints

The primary endpoint is chemotherapy-induced toxicity. Chemotherapy-induced toxicity is defined as haematological and non-haematological toxicity. Non-haematological toxicity comprised nephrotoxicity, neuropathy, esophagitis and pneumonitis. Haematological toxicity includes anemia, leukocytopenia, neutropenia and thrombocytopenia. Chemotherapy-induced toxicity will be assessed using the contemporary Common Terminology Criteria for Adverse Events (CTC-AE) (version 4.03 or higher) or predefined definitions (Table S2).[24] Secondary endpoints comprise SMM, patient-reported HRQOL (Table S1), dose-limiting toxicity defined as "switching treatment" (cisplatin to carboplatin), "treatment delay" (≥ seven days from initially planned), "treatment de-escalation" (dose reduction ≥ 25% of chemotherapeutic agent), early treatment termination and treatment-related hospital admissions (days of hospitalization) (Table S3), changes in biochemical characteristics, biomarker levels and haematological parameters, treatment response in terms of radiological response (according to the World Health Organization (WHO) Response Evaluation Criteria in Solid Tumors (RECIST 1.1))[25], and overall survival (OS).

Data collection

A data management plan, comprising detailed information about data collection, managing and storing of research data has been developed. Clinical data will be extracted from the hospital's electronic information systems and managed using web-based REDCap electronic data capture tools.[26] Beforehand, ranges will be defined in the file for all data values to ensure data validity and integrity. To reduce interobserver variability in gathering and entering data, only four trained individuals will be involved in the data collection process. Data collection will stop one year after start of first-line platinum-based chemotherapy. Patient data from this study will be coded. Only coded data will be analyzed and the results will be published anonymously. The following parameters and endpoints, of which some are considered to be potentially confounding variables, at baseline and at six follow-up time points as shown in Table 1, will be collected:

- Patient demographics: Age at diagnosis, gender, ethnicity, smoking status, alcohol (ab)use;
- Clinical observations: Charlson comorbidity index[27], Eastern Cooperative Oncology Group Performance status (ECOG PS)[28], anthropometric measurements (weight, length, body mass index [BMI]), skeletal muscle measurements by pretreatment and follow-up imaging (using fluorine-18 deoxyglucose positron emission tomography [FDG-PET-] computed tomography [CT] scans as part of standard clinical care);
- Disease characteristics: Disease stage (according to the contemporary TNM Classification of Malignant Tumors, seventh edition or higher)[29-30], histological tumor subtype, manifestation of metastases in the central nervous system;
- Treatment characteristics: Platinum-based agent, dosage, number of cycles, radiotherapy;
- Biochemical characteristics and biomarker levels: Serum creatinine, urea, albumin, magnesium, calcium, lactate dehydrogenase (LDH), carcinoembryonic antigen (CEA), cancer antigen 125 (CA 125);
- Chemotherapy-induced toxicity: Non-haematological toxicity (nephrotoxicity (estimated glomerular filtration rate (eGFR, according to CKD-EPI), serum creatinine)[31,32], neuropathy, esophagitis, ototoxicity, pneumonitis, gastrointestinal toxicity) and haematological toxicity (anemia (Hb level), leukocytopenia (leukocyte count), neutropenia (neutrophils count), thrombocytopenia (platelet count));
- Treatment response: Radiological response and survival status. Radiological response will be measured after two and four chemotherapy cycles (at six and 12 weeks after treatment initiation, respectively) by CT, FDG-PET and/or magnetic resonance imaging (MRI), as part of standard clinical care. Radiological response will be categorized as progressive disease (PD), stable disease (SD), partial response (PR) or complete response (CR), according to RECIST 1.1[25].

Table 1. Schedule of measurements and data collection

Measurements/variables	Prior to cycle 1 Week 0	Prior to cycle 2 Week 3	Prior to cycle 3 Week 6	Prior to cycle 4 Week 9	Follow-up 3 months	Follow-up 6 months	Follow-up 12 months
Blood sampling	X				X[a]	X[a]	X[a]
HRQOL assessment	X[a]				X[a]	X[a]	X[a]
Patient demographics	X						
Disease characteristics	X						
Clinical observations	X	X	X	X	X	X	X
Treatment characteristics	X	X	X	X			
Biochemical characteristics	X	X	X	X	X	X	X
Chemo-induced toxicity		X	X	X	X	X	X
Radiological response			X		X	X	X
Survival status							X

Abbreviations: HRQOL, health-related quality of life.

[a] For patients who enroll in the study prior to initiation of chemotherapy.

Sample size considerations

The sample size calculation is based on a candidate gene approach and on the assumption that approximately 30% of the patients undergoing platinum-based chemotherapy will develop chemotherapy-induced toxicity.[10,11] Common genetic variants will be selected. For example, a genotype or allele frequency of 0.05, 30% of patients with toxicity and a total of 333 patients, implies a detection of true odds ratios (OR) for toxicity of 0.43 or 2.03 in subjects with the genotype or allele of interest relative to subjects without this genotype or allele with a power of 0.8 and a type I error probability of 0.05. Since genetic testing can fail in 3%–5% of the cases, the total number of patients needed in this study is 350.

Data analysis

Standard statistical analysis will be performed by using SPSS version 25.0 or higher (IBM SPSS Statistics) and GraphPad Prism version 8.3 or higher. Standard summary statistics will be used to describe the sample data set. Categorical data will be expressed as frequencies and percentages. Continuous variables will be expressed as mean ± SD or median (ranges). Categorical data will be compared between groups by using the chi-square test and continuous data by Student's t-test or ANOVA when appropriate. In the primary analysis, toxicity will be defined as CTC-AE ≥ grade 1. Depending on the incidence of toxicity grade 2 or higher for the individual endpoints, further stratification will be carried out.

To examine the association between genetic variants and the risk for development of chemotherapy-induced toxicity, different approaches will be used. A candidate gene approach will be used and genome-wide association studies (GWAS) will be performed. Within the candidate gene approach, logistic regression models will be used to test for associations between genetic variants and toxicity expressed as categorical variables and odds ratios (OR) with 95% confidence intervals (CI) will be calculated. If a genetic association is found, correcting for multiple testing will be performed by using the false discovery rate test (q value threshold 0.20).[33] GWAS and quality control will be performed using PLINK version 1.9 or higher. Standard quality control (i.e., by filtering on SNP call rate, Hardy-Weinberg equilibrium, minor allele frequency (MAF) and population stratification (with commonly accepted thresholds based on current literature)) pre- and post-genotype imputation will be applied.[34] Imputation will be conducted on the University of Michigan Imputation Server.[35] To correct for multiple comparisons, conventional methods such as Bonferroni correction (i.e., $p \leq 5 \cdot 10^{-8}$ and $p \leq 5 \cdot 10^{-5}$ for genome-wide significance and near-significance (suggestive) association respectively) will be used to conduct these analyses.

Genetic variants will also be studied for association with radiological response (according to RECIST 1.1)[25] and OS. Individual patient overall survival time will be defined as the time difference between the date of treatment initiation until death. For patients who are alive by the end of follow-up (12 months after chemotherapy initiation) data will be censored. Median overall survival will be plotted in Kaplan-Meier curves and groups will be compared by using the log rank test. Hazard ratios (HR) with 95% CI will be calculated with Cox proportional hazard modeling. The multivariate setting of both logistic regression and Cox proportional hazard regression will be used to take potential confounding variables, specifically for the endpoint in question, into account and to calculate adjusted OR (ORadj) and adjusted HR (HRadj). In addition, when appropriate, stratification analysis (eg, based on platinum-based agent, histological tumor subtype or use of additional radiotherapy) will be performed.

For the analysis of the secondary endpoints, the statistical methods as described above will be used, when appropriate. In addition, univariate and multivariate linear regression analysis will be performed, when indicated.

Discussion

The results of this prospective follow-up study with a multicenter design will be used to determine the influence of genetic variants on the incidence of chemotherapy-induced

toxicity in patients with NSCLC undergoing first-line platinum-based chemotherapy. In addition, the association between genetic variants and treatment response, the association between SMM as well as patient-reported HRQOL with treatment response and toxicity will be assessed. Using a personalized medicine approach, the results may be used in the individualization of therapy based on the patient's clinical risk factors and genotype. Results of the PGxLUNG study may translate into minimisation of harm and contribute to improvement of quality of life of patients with NSCLC undergoing platinum-based chemotherapy, which is still the treatment of first choice for the majority of NSCLC patients worldwide.

Declarations

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Authors' contributions

CJ, GH and VD designed the study protocol. CJ, GH and VD wrote the clinical protocol and obtained authorization from the Medical Research Ethics Committee. CJ wrote the manuscript. GH and VD were responsible for critical revision of the manuscript. The final version of the manuscript was seen and approved by all authors.

Competing interests

The authors declare that they have no competing interests.

Ethics approval

The protocol of the PGxLUNG study complied with the Good Clinical Practice guidelines and the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013), and was approved by the accredited Medical Research Ethics Committee in Nieuwegein (MEC-U, number R15.056).

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Informed consent

All patients provided written informed consent.

Publication policy

The authors aim for open source publications.

Trial registration and status

The Netherlands National Trial Register (NTR), NTR number NL5373610015. Patient recruitment and inclusion took place between February 2016 and August 2019.

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Supplementary materials

Table S1. Patient-reported outcomes;

Table S2. Chemotherapy-induced toxicity;

Table S3. Dose-limiting toxicity.

Table S1. Patient-reported outcomes

Questionnaire	Aim of the questionnaire
EQ-5D	The European Quality of Life five dimension questionnaire (EQ-5D) is used to assesses health-related quality of life (HRQOL) in five socially relevant domains; mobility, self-care, usual activities, pain or discomfort and anxiety or depression.
EQ VAS	The EQ-5D questionnaire is accompanied by a Visual Analogue Scale (EQ VAS) on which the subject is asked to provide a self-assessment of their own health in a range from 0 (worst imaginable health state) to 100 (best imaginable health state).
EORTC QLQ-C30 Version 3.0	The European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) is a questionnaire developed to assess the quality of life of cancer patients.
EORTC QLQ-LC13	The European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire-Lung cancer (EORTC QLQ-LC13) is a questionnaire developed to assess the quality of life of lung cancer patients. It is a disease-specific supplement of the EORTC QLQ-C30 questionnaire.
EORTC QLQ-CIPN20	The European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire-Chemotherapy-induced peripheral neuropathy (EORTC QLQ-CIPN20) is a questionnaire developed to assess the quality of life of cancer patients specified on peripheral neuropathy as a frequently occurring side-effect of chemotherapy.

Table S2. Chemotherapy-induced toxicity

Category	Toxicity	Definition
Non-haematological	**Esophagitis**	A disorder characterized by inflammation of the esophageal wall. Grade 1: Asymptomatic; clinical or diagnostic observations only; intervention not indicated. Grade 2: Symptomatic; altered eating/swallowing; oral supplements indicated. Grade 3: Severely altered eating/swallowing; tube feeding, TPN, or hospitalization indicated. Grade 4: Life-threatening consequences; urgent operative intervention indicated. Grade 5: Death.
	Nephrotoxicity	Acute kidney injury: A disorder characterized by the acute loss of renal function and is traditionally classified as pre-renal (low blood flow into kidney), renal (kidney damage) and post renal causes (ureteral or bladder outflow obstruction). Grade 1: Creatinine level increase of > 0.3 mg/dL (≈ 26 µmol/L); creatinine 1.5-2.0 times above baseline. Grade 2: Creatinine 2 to 3 times above baseline. Grade 3: Creatinine > 3 times above baseline or > 4.0 mg/dL (≈ 353 µmol/L); hospitalization indicated. Grade 4: Life-threatening consequences; dialysis indicated. Grade 5: Death.

Category	Toxicity	Definition
Non-haematological	**Ototoxicity**	Grade 1: No symptoms of hearing loss. Grade 2: Hearing loss, no intervention. Grade 3: Hearing loss, requiring intervention. Grade 4: Profound bilateral hearing loss. Grade 5: -
	Peripheral sensory neuropathy	A disorder characterized by damage or dysfunction of the peripheral sensory nerves. Grade 1: Asymptomatic; loss of deep tendon reflexes or paresthesia. Grade 2: Moderate symptoms; limiting instrumental ADL. Grade 3: Severe symptoms; limiting self-care ADL. Grade 4: Life-threatening consequences; urgent intervention indicated. Grade 5: Death.
	Pneumonitis	A disorder characterized by inflammation focally or diffusely affecting the lung parenchyma. Grade 1: Asymptomatic; clinical or diagnostic observations only; intervention not indicated. Grade 2: Symptomatic; medical intervention indicated; limiting instrumental ADL. Grade 3: Severe symptoms; limiting self-care ADL; oxygen indicated. Grade 4: Life-threatening respiratory compromise; urgent intervention indicated (e.g., tracheotomy or intubation). Grade 5: Death.
	Diarrhea	A disorder characterized by an increase in frequency and/or loose watery bowel movements. Grade 1: Increase of < 4 stools per day over baseline. Grade 2: Increase of 4-6 stools per day over baseline. Grade 3: Increase of ≥ 7 stools per day over baseline; incontinence, hospitalization indicated, limiting self-care ADL. Grade 4: Life-threatening consequences; urgent intervention indicated. Grade 5: Death.
	Nausea	A disorder characterized by a queasy sensation and/or the urge to vomit. Grade 1: Loss of appetite without alteration in eating habits. Grade 2: Oral intake decreased without significant weight loss, dehydration or malnutrition. Grade 3: Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospitalization indicated. Grade 4: - Grade 5: -
	Vomiting	A disorder characterized by the reflexive act of ejecting the contents of the stomach through the mouth. Grade 1: 1-2 episodes (separated by 5 minutes) in 24 hrs. Grade 2: 3-5 episodes (separated by 5 minutes) in 24 hrs. Grade 3: ≥ 6 episodes (separated by 5 minutes) in 24 hrs; tube feeding, TPN or hospitalization indicated. Grade 4: Life-threatening consequences; urgent intervention indicated. Grade 5: Death.

Category	Toxicity	Definition
Haematological	**Anemia**	A disorder characterized by a reduction in the amount of hemoglobin (Hb) in blood. Signs and symptoms of anemia may include pallor of the skin and mucous membranes, shortness of breath, palpitations of the heart, soft systolic murmurs, lethargy, and fatigability. Grade 1: Hb < LLN-6.2 mmol/L. Grade 2: Hb < 6.2-4.9 mmol/L. Grade 3: Hb < 4.9 mmol/L, transfusion indicated. Grade 4: Life-threatening consequences; urgent intervention indicated. Grade 5: Death.
	Neutropenia	A finding based on laboratory test results that indicate a decrease in number of neutrophils in a blood specimen. Grade 1: neutrophils < LLN-1.5 · 10^9/L. Grade 2: neutrophils < 1.5-1.0 · 10^9/L. Grade 3: neutrophils < 1.0-0.5 · 10^9/L. Grade 4: neutrophils < 0.5 · 10^9/L. Grade 5: -
	Platelet count decreased	A finding based on laboratory test results that indicate a decrease in number of platelets in a blood specimen. Grade 1: Platelet count < LLN-75 · 10^9/L. Grade 2: Platelet count < 75 · 10^9/L-50 · 10^9/L. Grade 3: Platelet count < 50 · 10^9/L-25 · 10^9/L. Grade 4: Platelet count < 25 · 10^9/L. Grade 5: -

Abbreviations: ADL, Activities of daily living. Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc. Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden; Hb, Hemoglobin; LLN, Lower limit of normal; TPN, total parenteral nutrition.

Table S3. Dose-limiting toxicity

Toxicity	Definition
Treatment delay	The intended treatment as stated in the medical record is later started or resumed. The reason for rescheduling will be categorized in the database (e.g., due to chemotherapy-induced toxicity, disease progression, at patients request, other reasons). Treatment delay will be defined as treatment ≥ 7 days later than initially planned.
Treatment de-escalation	The intended treatment as stated in the medical record is not given according to the standard treatment protocol (e.g., dose reduction ≥ 25% or early treatment termination). The reason for treatment de-escalation will be categorized in the database (e.g., due to chemotherapy-induced toxicity, disease progression, at patients request, other reasons).
Switching treatment	The intended treatment as stated in the medical record is not completed, however the treatment is not discontinued but changed (e.g., cisplatin switched to carboplatin). The reason for switching treatment will be categorized in the database (e.g., due to chemotherapy-induced toxicity, disease progression, at patients request, other reasons).
Hospital admission	Unplanned treatment-related hospital admissions (days of hospitalization).

Chapter 2.2

Pharmacogenetic analysis of irreversible severe cisplatin-induced nephropathy: a case report of a 27-year-old woman

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Abstract

In this report we describe a young patient diagnosed with bulky FIGO stage IIIb squamous cell cervix carcinoma with severe and irreversible nephropathy after three weekly low-doses of cisplatin. Besides several known risk factors such as hypomagnesemia and hypoalbuminemia, the patient also proved to be homozygously polymorphic for two polymorphisms within the *COMT* gene (c.615+310C>T and c.616–367C>T). As *COMT* polymorphism has been associated with cisplatin-induced ototoxicity, its effect on nephrotoxicity of cisplatin should be the subject of further investigation.

Case report

Cisplatin is a widely used anticancer drug for the treatment of various solid tumors, including gastric, ovarian, testicular and lung cancer. Treatment with cisplatin is frequently associated with severe side effects such as nephrotoxicity, neurotoxicity and ototoxicity.[1] Despite intensive prophylactic measures, kidney damage occurs in one-third of patients and remains the most important complication that may limit further treatment.[2] Susceptibility to cisplatin nephrotoxicity is known to vary between individuals. Identified risk factors include co-administration with nephrotoxic agents, smoking, age, hypomagnesemia and hypoalbuminemia.[3] In addition, genetic variations in genes involved in the pharmacological pathway of cisplatin may affect response and toxicity. In particular, polymorphism in genes involved in cisplatin cellular uptake such as the organic cation transporter 2 (*OCT2*); metabolism, i.e., glutathione S-transferases 1 (*GST1*); DNA repair, like the excision repair cross-complementation groups (*ERCC1*, *ERCC2*); and other pharmacodynamic candidate genes such as catechol-O-methyltransferase (*COMT*), have shown to be associated with nephrotoxicity.[4-7] Although cisplatin toxicity is in most cases largely reversible, this report describes a young patient with persistent severe nephropathy after three doses of low-dose cisplatin therapy.

A 27-year-old Caucasian woman was referred to our hospital with vaginal bleeding and abdominal pain. The patient had no further medical history besides an asymptomatic pelvic kidney and no history of smoking or intake of any nephrotoxic agent. She was diagnosed with bulky FIGO stage IIIb squamous cell cervix carcinoma with pelvic and presacral lymph nodes with right-sided hydro-nephrosis. Renal function improved after double J ureteral stent placement (serum creatinine level 87 µmol/L). Treatment was started with induction chemotherapy consisting of three cycles carboplatin (with a target area under the curve (AUC) of five) plus paclitaxel (175 mg/m^2) once every 3 weeks. The second and third cycle of carboplatin/paclitaxel were both postponed for 1 week due to haematological toxicity with stable creatinine clearance. Radiologic evaluation after three cycles showed partial response of the primary tumor and lymph nodes remained stable. One month after the last cycle of carboplatin/paclitaxel, chemoradiation was initiated. Definitive chemoradiotherapy comprised weekly intravenous administration of cisplatin 40 mg/m^2 and 25 fractions of 1.8 Gy radiotherapy besides 3 · 8 Gy brachytherapy in weeks five, six and seven. After three cycles of cisplatin, serum creatinine level increased to 147 µmol/L and platelets decreased to 40 · 10^9/L. Cisplatin therapy was discontinued but both radiation and brachytherapy were continued. At day 31, the patient was hospitalized for 16 days because of further deterioration of kidney function (AKI grade 3, creatinine

432 µmol/L) and progressive pancytopenia (leukocytes 1.7 · 10^9/L, neutrophils 0.84 · 10^9/L, haemoglobin 4.9 · 10^9/L, platelets 21 · 10^9/L) (Figure 1). In addition, hypoalbuminaemia (30 g/L) and hypomagnesaemia (0.66 mmol/L) were noted. At time of hospital discharge, the patient's serum creatinine level was still 228 µmol/L. Six months later, no improvement of renal function had occurred – the serum creatinine levels remained above 200 µmol/L (AKI grade 2) (Figure 1).

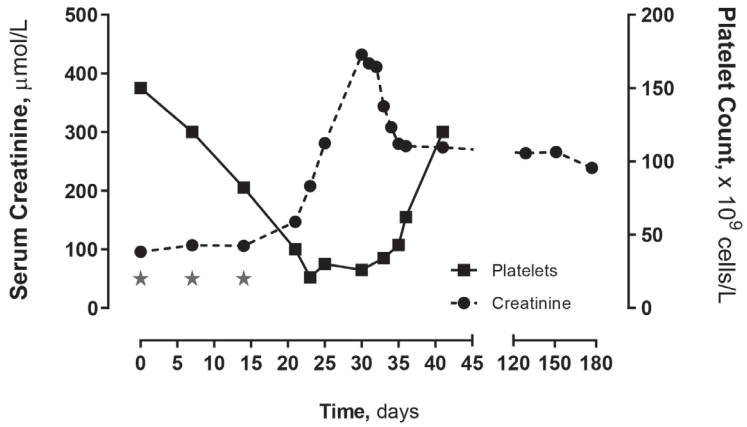

Figure 1. Serum creatinine levels and platelet counts in the peripheral blood of the patient after cisplatin therapy. Time is measured in days after the start of the chemotherapy, which is day 0. Stars indicate administration of cisplatin 40 mg/m^2 on day 0, 7 and 14.

In order to elucidate potential causes of the observed irreversible nephropathy, a pharmacogenomic analysis was performed, for which informed consent for genotyping and publication as case report was obtained from the patient. Polymorphisms in five candidate genes (*COMT*, *ERCC1*, *ERCC2*, *GSTP1*, *OCT2*) were determined by PCR (Taqman assay).

The tested polymorphisms in *ERCC1* (c.197G>T (rs3212986)), *ERCC2* (c.934C>T (rs1799793)), *GSPT1* (313A>G (rs1695)) and *OCT2* (c.808G>T (rs316019)) proved to be wild-type. Interestingly, however, both tested polymorphisms in *COMT* proved to be homozygously polymorphic (*COMT* c.615+310C>T (rs4646316) and c.616-367C>T (rs9332377)). Of note, both polymorphisms have previously been associated with cisplatin-induced ototoxicity.[8,9] The COMT enzyme is dependent on the S-adenosylmethionine (SAM) methyl donor substrate in the methionine pathway and involved in the inactivation of catecholamine neurotransmitters. Despite the fact that its precise function with regard to hearing loss of cisplatin has not yet fully been unraveled, a putative mechanism for cisplatin toxicity could be mediated through increased levels of SAM as result of reduced

COMT activity. In a recent mice model study, administration of both SAM and cisplatin increased cisplatin toxicity by 3–6.2-fold compared to cisplatin alone, as monitored by renal dysfunction.[10] Furthermore, whether *COMT* polymorphisms are also associated with nephrotoxicity of cisplatin in humans has thus far not yet been studied. We prudentially hypothesize that based on the known association of *COMT* polymorphism with ototoxicity, plus the observed homozygosity of both polymorphisms in this young patient that led to reduced COMT activity, this may have contributed to the irreversible and severe kidney damage. With minor allele frequencies of the *COMT* polymorphisms of 16% and 24%, respectively[9], it would be interesting to explore the effect of these polymorphisms on cisplatin-induced nephrotoxicity in a *COMT* knock-out mice model and in an appropriate patient population. Besides a potential genetic susceptibility, several other risk factors may have additionally contributed to kidney damage in this young woman. Cisplatin has a high plasma protein binding of more than 90%; malnutrition and hypoalbuminaemia may consequently result in a higher fraction of unbound cisplatin, with a potentially increased risk of toxicity. Hypomagnesaemia was noted, which is also associated with nephrotoxicity.[11] It is not likely that the existing hydronephrosis, for which a double J stent was placed successfully, contributed to kidney failure. Since pelvic kidney-sparing radiotherapy was performed, radiation damage is not likely. Besides, no other concomitant nephrotoxic drugs were used.

In summary, homozygosity of two *COMT* polymorphisms (c.615+310C>T and c.616–367C>T) was demonstrated in a patient with persisting nephrotoxicity after three low doses of cisplatin. Besides additional risk factors, including hypomagnesaemia and hypoalbuminaemia, *COMT* polymorphisms may have contributed to the severe kidney damage. Based on the known association of *COMT* polymorphism with cisplatin-induced ototoxicity, association analysis with nephrotoxicity should be the subject of further investigation.

Declarations

Authors' contributions

CJ wrote the manuscript. SS, GC, AB, MT, JS and MD were responsible for critical revision of the manuscript. The final version of the manuscript was seen and approved by all authors.

Competing interests

The authors declare that they have no competing interests.

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Informed consent

Written informed consent was obtained from the patient.

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Chapter 2.3

Association between genetic variants and cisplatin-induced nephrotoxicity: a genome-wide approach and validation study

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Abstract

Background: This study aims to evaluate genetic risk factors for cisplatin-induced nephrotoxicity by investigating not previously studied genetic risk variants and further examining previously reported genetic associations.

Methods: A genome-wide study (GWAS) was conducted in genetically estimated Europeans in a discovery cohort of cisplatin-treated adults from Toronto, Canada, followed by a candidate gene approach in a validation cohort from the Netherlands. In addition, previously reported genetic associations were further examined in both the discovery and validation cohorts. The outcome, nephrotoxicity, was assessed in two ways: (i) decreased estimated glomerular filtration rate (eGFR), calculated using the Chronic Kidney Disease Epidemiology Collaboration formula (CKD-EPI) and (ii) increased serum creatinine according to the Common Terminology Criteria for Adverse Events v4.03 for acute kidney injury (AKI-CTCAE). Four different Illumina arrays were used for genotyping. Standard quality control was applied for pre- and post-genotype imputation data.

Results: In the discovery cohort (n = 608), five single-nucleotide polymorphisms (SNPs) reached genome-wide significance. The A allele in rs4388268 (minor allele frequency = 0.23), an intronic variant of the *BACH2* gene, was consistently associated with increased risk of cisplatin-induced nephrotoxicity in both definitions, meeting genome-wide significance (β = −8.4, 95% CI −11.4–−5.4, $p = 3.9 \cdot 10^{-8}$) for decreased eGFR and reaching suggestive association (OR = 3.9, 95% CI 2.3–6.7, $p = 7.4 \cdot 10^{-7}$) by AKI-CTCAE. In the validation cohort of 149 patients, this variant was identified with the same direction of effect (eGFR: β = −1.5, 95% CI −5.3–2.4, AKI-CTCAE: OR = 1.7, 95% CI 0.8–3.5). Findings of our previously published candidate gene study could not be confirmed after correction for multiple testing.

Conclusions: Genetic predisposition of *BACH2* (rs4388268) might be important in the development of cisplatin-induced nephrotoxicity, indicating opportunities for mechanistic understanding, tailored therapy and preventive strategies.

Introduction

Since its approval by the FDA in 1978[1], cisplatin has remained a backbone antineoplastic agent used to treat various cancers, such as head and neck, ovarian, testicular, cervical, bladder, gastroesophageal, breast and lung cancer[2,3]. Cisplatin binds to the N7 reactive center on purine residues after entering the cell and exerts its cytotoxic effects via DNA damage in cancer cells, blocking cell division and resulting in apoptotic cell death.[2] Cisplatin also causes endoplasmic reticulum and mitochondria dysfunction.[4] However, its effectiveness also coincides with numerous acute and long-term adverse effects[4,5] such as bone marrow suppression, nausea and vomiting, nephrotoxicity, ototoxicity, and neurotoxicity, which may hamper the antineoplastic potential for the individual patient.[2,6] Approximately one-third of patients develop any kind of nephrotoxicity after a single dose of 50–100 mg/m^2 cisplatin[7], while up to 90% of patients experience hypomagnesemia, which may exacerbate cisplatin nephrotoxicity, if no corrective measures are initiated.[8] Clinically, nephrotoxicity can lead to various renal manifestations such as acute kidney injury, hypomagnesemia, distal renal tubular acidosis, hypocalcemia, renal salt wasting, renal concentrating defect, hyperuricemia, transient proteinuria, erythropoietin deficiency, thrombotic microangiopathy, and chronic kidney disease (CKD).[9] Ultimately, CKD may result in significantly elevated cardiovascular mortality risk and further preclude patients from subsequent cisplatin or other cancer therapies.[10] Four potential mechanisms of cisplatin-induced nephrotoxicity have been suggested[11]: (i) proximal tubular injury; (ii) oxidative stress; (iii) inflammation; and (iv) vascular injury in the kidney. Strategies to prevent cisplatin-induced nephrotoxicity are commonly applied in clinical settings, including intravenous fluid repletion with or without magnesium supplementation and mannitol forced diuresis in select patients.[12] However, the risk of kidney damage remains to a significant extent. Non-genetic risk factors for cisplatin-induced nephrotoxicity have been identified, including older age, low functional status, malnourishment, hypovolemia, presence of chronic comorbid illnesses (e.g., vascular disease, diabetes mellitus, and liver dysfunction), pre-existing (chronic) kidney disease, concurrent nephrotoxic drug exposure (e.g., iodinated contrast, chronic use of non-steroid anti-inflammation drugs (NSAIDs), and gemcitabine), electrolyte disturbances (low serum magnesium levels), alcohol ingestion, and high cisplatin doses per administration (≥ 50 mg/m^2), greater frequency of administration, greater cumulative dose, and insufficient intravenous fluid during cisplatin administration.[4] However, studies that have investigated genetic contributions to the development of cisplatin-induced nephrotoxicity have shown inconsistent findings, potentially due to significant patient and treatment heterogeneity along with variability in candidate gene study designs.[13] Nevertheless, a variation in *SLC22A2* rs316019, a gene involved in platinum uptake by the kidney, was associated with different nephrotoxicity definitions in four independent candidate gene studies.[13] Furthermore,

variants of *ERCC1* (rs11615 and rs3212986) and *ERCC2* (rs13181 and rs1799793), two genes involved in DNA repair, were found to be associated with increased risks of nephrotoxicity in two independent candidate gene studies.[13-16] At this stage, a genome-wide approach is preferred to identify unreported genetic associations as well as to confirm previous reported findings. Compared to the candidate gene approach, genome-wide association studies (GWASs) offer an unbiased method to identify genetic variants through scanning of the genome. This includes the identification of novel causal genetic variants providing an opportunity to improve mechanistic understanding of cisplatin-induced nephrotoxicity.[17-19] To our knowledge, to date, only candidate gene studies and not GWASs have been performed to evaluate genetic risk factors for cisplatin-induced nephrotoxicity. In addition, understanding the potential contribution of genetic variants in the occurrence of cisplatin-induced nephrotoxicity could help physicians identify individuals at risk of nephrotoxicity and may assist in guiding optimal drug and dose selection and preventive strategies. Utilizing patients' genetic information could thus enable safer, more effective, and more cost-effective treatment.[20] This study evaluated the relationship between genetic risk factors and cisplatin-induced nephrotoxicity by investigating genetic risk variants not previously studied through the use of GWAS. An independent validation cohort using a candidate gene approach was used to confirm genetic variations (single-nucleotide polymorphisms (SNPs)) associated with the risk of cisplatin-induced nephrotoxicity from the GWAS. In addition, previously reported genetic associations were further examined in both the discovery and validation cohorts.

Materials and methods

Study design and patients

Discovery cohort

A retrospective analysis was performed in a discovery cohort, which consisted of two groups of patients newly diagnosed with head and neck cancer and one group of patients diagnosed with esophageal cancer, all of whom were treated at Princess Margaret Cancer Centre in Toronto, Canada between July 2002 and December 2017. The inclusion criteria for patients in the discovery cohort were as follows: (i) ≥ 18 years of age; (ii) had received high-dose (≥ 75 mg/m^2) cisplatin administered in three-week intervals for at least one cycle, either as a single agent or in combination with either other antineoplastic agents and/or radiation for curative intent; (iii) estimated glomerular filtration rate (eGFR) ≥ 60 mL/min/1.73 m^2 prior to cisplatin therapy; and (iv) were previously cisplatin naïve. Patients without cisplatin administration data, non-genotyped patients, and patients of non-European ancestry were excluded from further analyses. Study procedures were approved by the Review Ethics Board of the University Health Network, Toronto, Canada (CAPCR06-639, CAPCR07-0521) and

implemented in accordance with the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013). All patients provided the informed written consent.

Validation cohort

Patients diagnosed with non-small-cell lung cancer (NSCLC) included in the PGxLUNG study were identified as an independent cohort for the purpose of validating the association between any identified variant and nephrotoxicity.[21] Patients of the PGxLUNG study were recruited from one academic hospital (University Medical Center Utrecht), two teaching hospitals (St. Antonius Hospital Nieuwegein/Utrecht, Meander Medical Center Amersfoort) and three general hospitals (Diakonessenhuis Utrecht, Groene Hart Ziekenhuis Gouda, Ziekenhuis Rivierenland Tiel), all in the Netherlands, between February 2016 and August 2019. The inclusion criteria for this multicenter prospective follow-up study were as follows: (i) ≥18 years of age; (ii) had radiologically confirmed NSCLC (stage II-IV); (iii) planned or initiated first-line treatment with platinum-based (cisplatin or carboplatin) chemotherapy or chemoradiotherapy (according to the contemporary ESMO Clinical Practice Guidelines); and (iv) were previously platinum-based chemotherapy-naïve. For the analyses as part of this study, patients who did not receive cisplatin and patients of non-European ancestry were excluded. Study procedures were approved by the accredited Medical Research Ethics Committee in Nieuwegein (MEC-U, number R15.056) and implemented in accordance with the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013). All patients provided the informed written consent. Because the inclusion/exclusion and treatments were not identical to the discovery cohort, we have termed this a validation (and not replication) cohort.

Clinical data collection

Information on age, gender, weight, height, body surface area (BSA), type of cancer, baseline albumin, concomitant therapy, comorbidities, cisplatin administration (timing and dose) and serum creatinine (SCr) was extracted from the hospitals' electronic medical record systems. Cisplatin dosage (mg/m^2) was acquired by dividing the actual cisplatin dose administered (mg) by the BSA.

Cisplatin-induced nephrotoxicity phenotype

Cisplatin-induced nephrotoxicity was defined using two phenotype definitions: (i) the SCr-based CTCAE 4.03[22] definition of "acute kidney injury" (AKI-CTCAE) as a categorical variable (grade 1 [creatinine level increase of > 0.3 mg/dL (≈ 26 µmol/L); creatinine 1.5-2.0 above

baseline] or higher was defined as nephrotoxicity) and (ii) difference between baseline and lowest eGFR (delta) during the follow-up period as a continuous variable. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula (CKD-EPI) as per the Kidney Disease: Improving Global Outcomes (KDIGO) recommendation.[23] Baseline values were defined as the SCr and eGFR measurements taken closest to the first cisplatin administration (within 30 days before the first cisplatin administration). The follow-up period for the assessment of nephrotoxicity in the discovery and validation cohort was 90 and 21 days after the last cisplatin dose, respectively. Given such a range in kidney function follow up period, AKI-CTCAE can also be defined as acute kidney disease/disorder as per KDIGO Clinical Practice Guideline for Acute Kidney Injury.[24] The follow-up period for the validation cohort was shorter to avoid treatment bias, since some patients in the validation cohort, but not in the discovery cohort, were allowed to switch to carboplatin during therapy, typically 21 days after the last cisplatin dose. In contrast, this switch was not allowed in the patients of the discovery dataset, where we could capture a longer follow-up period of 90 days.

Genotyping and imputation

DNA was extracted from peripheral blood. Four chips were used for genotyping: the Consortium-OncoArray 500K and OncoArray 500K (Illumina, San Diego, CA, USA) at the Center for Inherited Disease Research (CIDR; Johns Hopkins, Baltimore, MD, USA) for head and neck cancer patients, the Human Omni 1M Quad Beadchip at the US National Cancer Institute (Bethesda, MD, USA) for esophageal cancer patients and the Infinium Global Screening Array-24 Kit (Illumina, San Diego, CA, USA) at Life and Brain (Bonn, Germany) for NSCLC patients. Different genotyping chips were used because this study consists of several independent cohorts that were merged into a discovery and a validation cohort. Sample quality control (QC) was performed for each chip with the following criteria: sample call rate > 98%, heterozygosity ± 3 SD from the sample's heterozygosity rate mean, and pi-hat 98%, minor allele frequency (MAF) > 0.05, Hardy–Weinberg equilibrium $p > 10^{-6}$ (in patients without nephrotoxicity for the AKI-CTCAE phenotype and for the eGFR phenotype) and $p > 10^{-10}$ (in patients with nephrotoxicity for the AKI-CTCAE phenotype). Imputation using these QC-passed SNPs was conducted on the University of Michigan Imputation Server[25] using the Minimac4 1.2.1, 1000G Phase 3 v5 reference panel, GRCh37/hg19 array build and Eagle v2.4 phasing. Those SNPs with imputation quality (Rsq) > 0.8 and MAF > 0.05 were retained for association analysis. QC was performed using PLINK v.1.9 and 2.[26,27]

Statistical analysis

Genome-wide approach: discovery cohort

The sample size needed for the discovery cohort was calculated using GAS Power Calculator[28], assuming an additive model, type I error rate of $5 \cdot 10^{-8}$, MAF of 20%, cisplatin-induced nephrotoxicity prevalence of 30% and genotype relative risk of 2.0. A minimum of 680 subjects was required to achieve 80% power. The GWAS assumed additive SNP effects for the AKI-CTCAE phenotype and linear additive effects for the eGFR phenotype. The GWAS was conducted on imputed SNPs and adjusted for 10 genetic MDS components as well as baseline eGFR, gender, age at cisplatin initiation, cumulative dose of cisplatin, cardiovascular disease status, diabetes mellitus status, and chronic NSAID usage. Logistic regression and multiple linear regression analysis were conducted to evaluate the association between genetic variants and the AKI-CTCAE (dichotomous categorical outcome) and eGFR phenotypes (continuous outcome), respectively. Association analysis was performed using PLINK 1.9.[26,27] Multiple cohort analyses were conducted by combining GWAS results from each genotyping chip in a meta-analysis using the inverse variance method with fixed effect model performed by METAL[29] to overcome issues that might arise from including different genotyping platforms and to increase the power of this study. The Manhattan plot and the Q–Q plot of the GWAS meta-analysis results were visualized using R version 3.4 (http://www.R-project.org/, accessed on 20 February 2021). The genome-wide significance association and suggestive association were set at $p \leq 5 \cdot 10^{-8}$ and $p \leq 10^{-5}$, respectively.

Candidate gene approach: validation cohort

SNPs meeting at least the suggestive association threshold ($p \leq 10^{-5}$) for each phenotype in the discovery cohort were assessed in the validation cohort. The strength of the association between genotypes and nephrotoxicity phenotypes were evaluated with regression analysis and expressed as odds ratios and β with a 95% confidence interval (CI) for the AKI-CTCAE phenotype and eGFR phenotype, respectively. Association analysis was conducted on imputed SNPs and was adjusted for 10 genetic MDS components as well as gender, age at cisplatin initiation, cumulative dose of cisplatin and Charlson comorbidity index[30] (including diabetes mellitus and cardiovascular disease status). The false discovery rate (FDR) was used for correction in multiple testing based on the Benjamini–Hochberg procedure available in PLINK.[31] Association analysis was performed using PLINK 1.9[27], and significant association was set at adjusted $p < 0.05$. The sample size needed for the validation cohort was calculated using GPower[32] based on 80% power, 5% alpha and the results of our discovery dataset (i.e., effect sizes and allele frequency). The minimum sample sizes for AKI-CTCAE and eGFR outcomes were 141 and 153 patients, respectively.

Sensitivity analysis in the discovery cohort

A sensitivity analysis was carried out in the discovery cohort subjects in which the Charlson comorbidity index data were available. The GWAS was conducted in the same manner as the primary association analysis except the Charlson comorbidity index was incorporated into the model, instead of the specific variables of cardiovascular disease and diabetes mellitus status.

Association of previously investigated SPNs based on the systematic review

The relationships between known genetic variants identified in our previously published systematic review[13] and cisplatin-induced nephrotoxicity were also evaluated in the same manner with both discovery and validation cohort analysis.

Population impact measures

The potential impact of pharmacogenetic testing, in terms of preventing one patient from having an adverse event, can be expressed as the number needed to genotype (NNG). Furthermore, the number needed to treat (NNT) can be calculated as the number of patients who need an intervention to prevent one patient from having an adverse event, with patients being those who carry the genetic variant indicating the need for alternative treatment. The NNG and NNT on the SNP with strongest evidence were determined using the combined dataset (discovery and validation cohort) to estimate the efficiency of genotyping and treatment modification based on the formula described by Tonk *et al*.[33]

Results

Population characteristics of discovery and validation cohorts

The study flow chart is shown in Figure 1A (discovery cohort) and Figure 1B (validation cohort). After performing pre- and post-imputation QC and through the MDS approach, data from 608 and 149 patients of European genetic ancestry were available for the discovery cohort and validation cohort, respectively (Figure S1). The demographic and clinical characteristics of the cohorts are shown in Table 1, while the clinical characteristics categorized by type of cancer (discovery cohort only) are available in the supplement (Table S1). The majority of patients in the discovery cohort were diagnosed with head and neck cancer (470 patients, 77.3%).

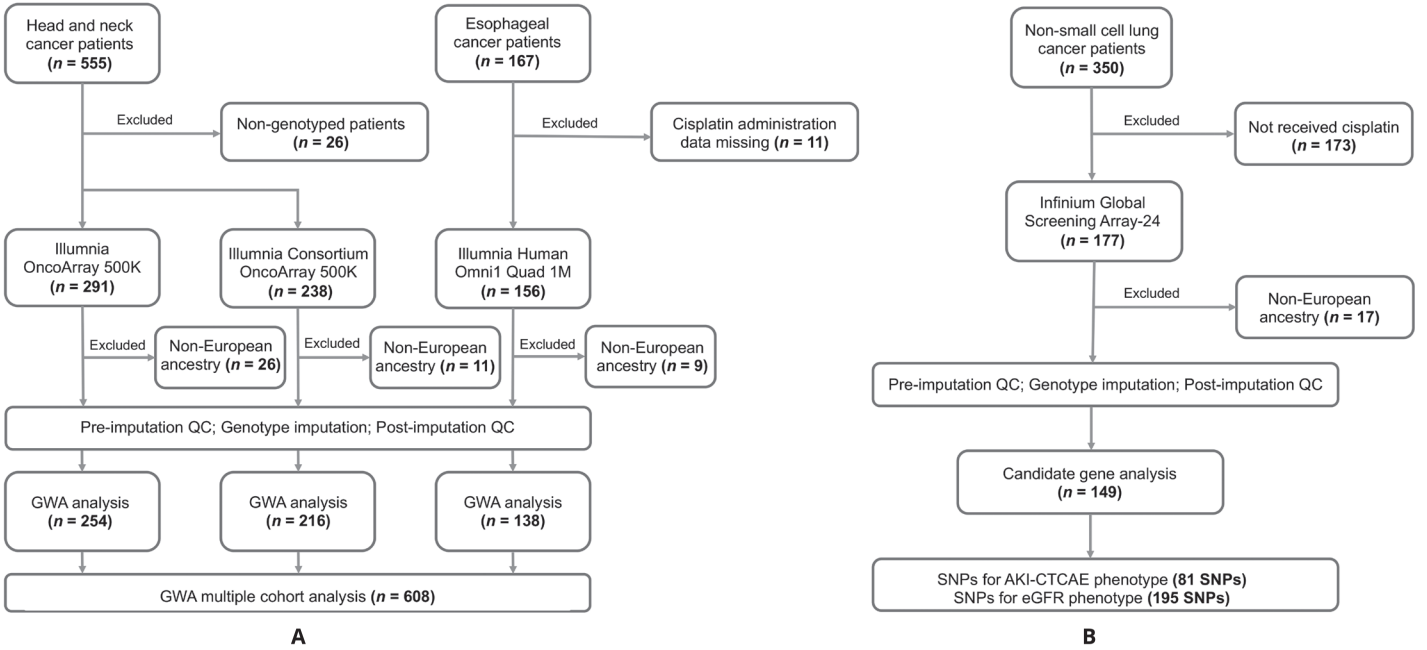

Figure 1. Flowchart of this study. **A.** Discovery cohort (*n* = 608). The discovery cohort consisted of head and neck cancer patients (*n* = 555) and esophageal cancer patients (*n* = 167). Three arrays were used for genotyping. After pre-imputation QC, imputation and post-imputation QC, data of 608 patients in total were included for analysis. **B.** Validation cohort (*n* = 149). The validation cohort consisted of non-small-cell lung cancer patients (*n* = 350). After pre-imputation QC, imputation, post-imputation QC and exclusion for chemotherapeutic therapy, 149 patients in total were included for analysis. Abbreviations: AKI-CTCAE, Common Terminology Criteria for Adverse Events v4.03 for acute kidney injury; GWA, genome-wide association; SNPs, single-nucleotide polymorphisms; and QC, quality control.

Table 1. Demographic and clinical characteristics

Characteristics	Discovery cohort (*n* = 608)	Validation cohort (*n* = 149)	*p*-value
Age at cisplatin initiation in years, mean ± SD	57.9 ± 7.9	62.8 ± 9.4	< 0.01*
Male, *n* (%)	500 (82.2)	71 (47.7)	< 0.01*
Cardiovascular disease, *n* (%)	156 (25.7)	NA	NA
Diabetes mellitus, *n* (%)	44 (7.2)	NA	NA
Charlson comorbidity index[a], *n* (%)			
2–3	206 (40.5)	71 (47.7)	< 0.01*
4–5	247 (48.5)	43 (28.9)	
≥ 6	56 (11.0)	35 (23.4)	
Missing data	99	0	
Chronic NSAID users, *n* (%)	42 (6.9)	NA	NA
Concurrent administration of other antineoplastics, *n* (%)	138 (22.7)	149 (100)	< 0.01*
Received radiotherapy, *n* (%)	534 (87.8)	87 (58.4)	< 0.01*
Albumin baseline, median mmol/L (IQR)	42 (40–44)	39.0 (33.0–42.0)	< 0.01*
Baseline eGFR, median mL/min/1.73 m^2 (IQR)	94.0 (83.4–101.4)	90.0 (80.0–90.0)	< 0.01*

Abbreviations: NA, information not available; NSAID, non-steroidal anti-inflammatory drug; SD, standard deviation; eGFR, estimated glomerular filtration rate; IQR, interquartile range.

[a] Charlson comorbidity index score provides a simple means to quantify the effect of comorbid illnesses, including cardiovascular diseases, chronic obstructive pulmonary disease, liver disease and diabetes mellitus among others and accounts for the aggregate effect of multiple concurrent diseases. A higher score indicates more comorbidities.

* *p*-value < 0.05 based on independent t-test or Mann–Whitney *U* Test (for continuous independent variable) and Fisher's Exact Test or chi-square test (for categorical independent variable).

Within the discovery cohort, no statistically significant differences were found in gender and percentage of patients with diabetes mellitus between head and neck and esophageal cancer patients. However, mean ± SD age at cisplatin initiation was higher in esophageal cancer patients compared to head and neck cancer patients (59.8 ± 9.6 vs. 57.4 ± 7.3 years). In contrast, the percentage of patients with cardiovascular disease, chronic NSAID users, and treated with radiotherapy were higher in head and neck cancer patients (28.1% vs. 17.4%; 8.3% vs. 2.2%; 98.3% vs. 52.2%, respectively). Among the 509 subjects where data were available to calculate the Charlson comorbidity index score, there were no statistically significant differences in Charlson comorbidity index score between the head and neck and esophageal cancer patient subgroups (see Table S1). Albumin and eGFR baseline were statistically (but not clinically relevant) significantly higher in head and neck cancer patients (median: 42 vs. 41 mmol/L; 94.3 vs. 92.2 mL/min/1.73 m^2, respectively). Compared to the discovery cohort, patients in the validation cohort were statistically significantly older at cisplatin initiation (mean ± SD: 62.8 ± 9.4 vs. 57.9 ± 7.9 years), more frequently female (82.2% vs. 47.7%), had more comorbidities (Charlson comorbidity index

score ≥ 6: 23.4% vs. 11.0%) and were less often treated with concurrent radiotherapy (58.4% vs. 87.8%). The baseline albumin and eGFR in the discovery cohort was statistically (but not clinically) significantly higher than in the validation cohort (median: 42 vs. 39 mmol/L; 94 vs. 90 mL/min/1.73 m^2, respectively; Table 1).

Cisplatin-induced nephrotoxicity in the discovery and validation cohorts

In the discovery cohort, 93 patients (15.3%) developed grade 1 or higher AKI-CTCAE during cisplatin therapy (Table 2). Data on treatment characteristics and distribution of outcomes within the discovery cohort are shown in Table S2. In both head and neck cancer and esophageal cancer subgroups, subjects with cardiovascular disease, diabetes mellitus, and those who in chronic use of NSAIDs more frequently developed nephrotoxicity (Table S1). The head and neck cancer subgroup received cisplatin as a single agent with a higher cumulative dose of cisplatin (median: 198.2 vs. 173.8 mg/m^2) and a higher percentage of radiotherapy-treated subjects (98.3% vs. 52.2%). However, the incidence of nephrotoxicity between the two types of cancer was similar (14.9% vs. 16.7%). The percentage of patients with more comorbidities, chronic NSAID use or who had received concurrent administration of other antineoplastics, was higher in patients who developed nephrotoxicity. No statistically significant differences in age at cisplatin initiation or albumin baseline were found between the group of patients with and without nephrotoxicity, both in head and neck and esophageal cancer patients (Table S3). As shown in Table 2, patients in the validation cohort more frequently developed grade 1 or higher AKI-CTCAE compared to discovery cohort patients (26.8% vs. 15.3%). In both the discovery and validation cohort patients, most of the AKI-CTCAE occurred as grade 1 (11.7% and 22.1%, respectively). Validation cohort patients received a non-significantly higher cumulative dose of cisplatin (median: 224.5 vs. 196.7 mg/m^2). Validation cohort patients tended to receive a greater number of chemotherapy cycles than patients in the discovery cohort (median: 3 vs. 2 cycles). The reduction in the eGFR was statistically (but not clinically relevant) greater in the validation cohort (median: 11 vs. 7 mL/min/1.73 m^2) while the median reduction in eGFR between discovery and validation cohort in patients with and without nephrotoxicity was not statistically different.

Association analysis in the discovery cohort

After QC processing and initial association analysis, more than 6.5 million SNPs were included in the GWAS meta-analysis of the discovery cohort. The Manhattan plot and Q–Q plot of the analysis can be found in Figure 2A-B. No genomic inflation was observed in the GWAS for the

AKI-CTCAE phenotypes as none of the tested SNPs surpassed the genome-wide significance threshold ($p \leq 5 \cdot 10^{-8}$). However, 81 SNPs exceeded the suggestive association *p*-value ($p \leq 10^{-5}$) with most of the signals in SNPs at chromosomes 4, 6, and 11. Details of the top 20 SNPs associated with grade 1 or higher AKI-CTCAE can be found in Table S4.

Table 2. Treatment characteristics and distribution of outcomes

Characteristics	**Discovery cohort (*n* = 608)**	**Validation cohort (*n* = 149)**	***p*-value**
Cumulative cisplatin dose, median mg/m^2 (IQR)	196.7 (173.0–248.0)	224.5 (150.1–274.8)	0.297
Cycles of cisplatin-based chemotherapy, *n* (%)			< 0.01*
1	50 (8.2)	28 (18.8)	
2	313 (51.5)	23 (15.4)	
3	201 (33.1)	55 (36.9)	
≥ 4	44 (7.2)	43 (28.9)	
AKI-CTCAE, *n* (%)[a]			< 0.01*
Grade 0 (no nephrotoxicity)	515 (84.7)	109 (73.2)	
Grade 1	71 (11.7)	33 (22.1)	
Grade 2	17 (2.8)	4 (2.7)	
Grade 3	5 (0.8)	3 (2.0)	
Grade 4	0 (0)	0 (0)	
Any grade	93 (15.3)	40 (26.8)	
eGFR reduction, median, mL/min/1.73 m^2 (IQR)[b]	7.0 (0.6–18.9)	11.0 (1.0–25.5)	< 0.01*
Patients without nephrotoxicity	5.5 (0.0–14.3)	7.0 (0.0–16.0)	0.502
Patients with grade ≥ 1 AKI-CTCAE	30.6 (15.3–42.9)	34.5 (25.3–41.5)	0.173

Abbreviations: IQR, interquartile range; eGFR, estimated glomerular filtration rate.

[a] Highest AKI-CTCAE grade between cisplatin initiation and the last day of follow-up.

[b] Differences between baseline eGFR and eGFR nadir recorded from cisplatin initiation until the last day of follow-up.

* *p*-value < 0.05 based on Mann–Whitney *U* Test (for continuous independent variable) and chi-square test (for categorical independent variable).

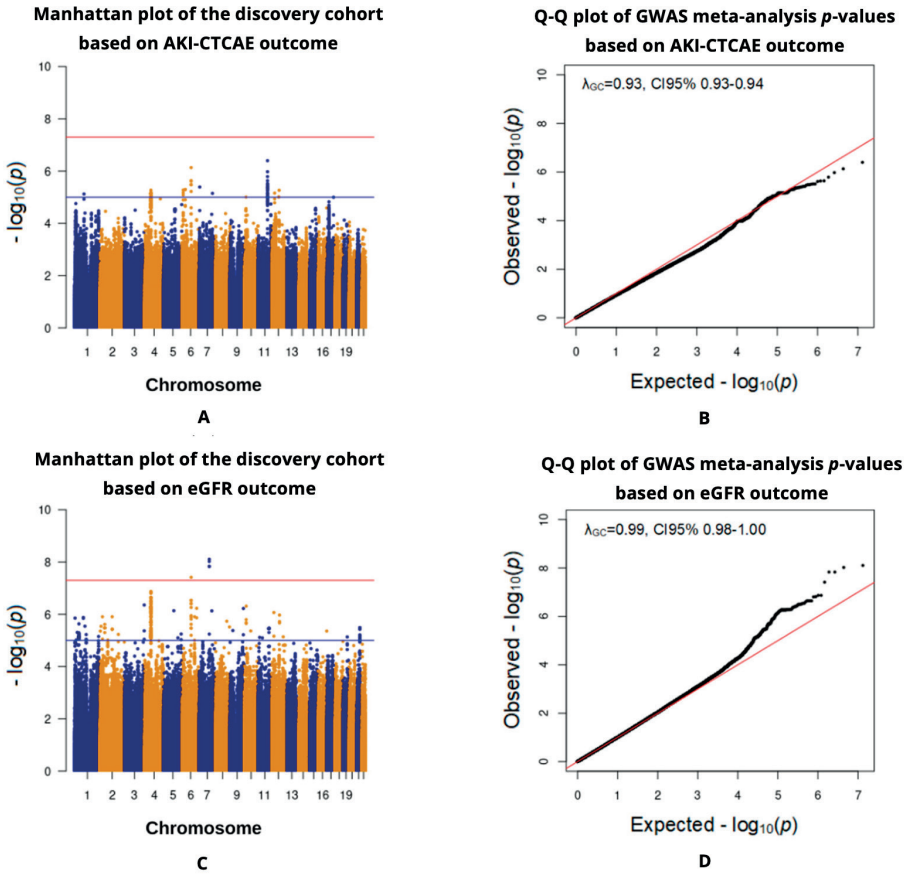

Figure 2. Genome-wide meta-analysis results of cisplatin-induced nephrotoxicity using AKI-CTCAE and eGFR phenotypes. **A.** Manhattan plot showing logistic regression results using the AKI-CTCAE phenotype; −log10 *p*-values are plotted against the respective chromosomal position of each SNP. **B.** A quantile–quantile (Q–Q) plot showing the distribution of *p*-values in the GWAS using the AKI-CTCAE phenotype. **C.** Manhattan plot showing logistic regression results using the eGFR phenotype. **D.** Q–Q plot showing the distribution of *p*-values in the GWAS using the eGFR phenotype.

The Manhattan plot and Q–Q plot of the analysis based on eGFR outcome can be found in Figure 2C-D. Four intronic SNP variants and one variant sitting outside of a known gene that exceeded the genome-wide significance threshold were identified (see Table S5): two SNPs were associated with lower risk for eGFR reduction, *ARPC1A* rs199659233 and rs556958738 (β = 28.7, 95% CI 18.7–38.6, $p = 1.5 \cdot 10^{-8}$) and three SNPs were associated with higher risk for eGFR reduction, *TMEM225B* rs17161766 (β = −28.9, 95% CI −38.8–−19.1, $p = 7.8 \cdot 10^{-9}$), chr7:98951080 (β = −27.2, 95% CI −36.5–−17.9, $p = 9.5 \cdot 10^{-9}$), and *BACH2* rs4388268 (β = −8.4, 95% CI −11.4–−5.4, $p = 3.9 \cdot 10^{-8}$). 190 SNPs met suggestive association

p-value threshold. Of these 195 SNPs, 11 intron variants SNPs are located on chromosome 7, except for rs4388268, which is located on chromosome 6. Of the five SNPs with genome-wide significance, only *BACH2* rs4388268 was consistently surpassed post-imputation QC in three genotyping arrays of the discovery cohort. The remaining four SNPs surpassed the QC in only one of the three datasets. In addition, *BACH2* rs4388268 was consistently associated with a decreased eGFR in the discovery cohort with genome-wide significant association (β = −8.4, 95% CI −11.4–−5.4, $p = 3.9 \cdot 10^{-8}$) and with higher risk of the AKI-CTCAE with suggestive association (OR = 3.9, 95% CI 2.3–6.7, $p = 7.4 \cdot 10^{-7}$) (Table 3). The sensitivity analysis in 509 subjects with Charlson comorbidity index data confirmed consistent direction of association and similar effect sizes of *BACH2* rs4388268 with previous analysis with regard to cisplatin-induced nephrotoxicity. The variant was consistently associated with a decreased eGFR (β = −8.1, 95% CI −11.4–−4.8, $p = 1.4 \cdot 10^{-6}$) and with higher risk of the AKI-CTCAE (OR = 3.6, 95% CI 1.7–5.4, $p = 3.8 \cdot 10^{-5}$) (Table S6, Figure S2).

Association analysis in the validation cohort: GWAS results

Following analysis of the discovery cohort, SNPs surpassing the suggestive association threshold (81 SNPs for AKI-CTCAE and 195 SNPs for eGFR outcome in which 32 SNPs were overlapped) were further tested in the validation cohort. Although no statistically significant association was validated, the association of *BACH2* rs4388268 was associated in the same direction as in the discovery cohort for both the AKI-CTCAE (OR = 1.7, 95% CI 0.8–3.5) and eGFR outcomes (β = −1.5, 95% CI −5.3–2.4; Table 4).

Table 3. Association between *BACH2* rs4388268 and cisplatin-induced nephrotoxicity in the discovery cohort

Chromosome: Location: Allele[a]	Functional consequences	Outcome	Effect size (95% CI)[b]	*p*-value	Direction[c]
6:90734908:G:A	Intron variant	AKI–CTCAE	3.9 (2.3–6.7)	$7.4 \cdot 10^{-7}$	+ + +
		eGFR reduction	−8.4 (−11.4–−5.4)	$3.9 \cdot 10^{-8}$	− − −

[a] Chromosome: base pair:Allele1:Allele2.

[b] OR for AKI-CTCAE phenotype and β for eGFR phenotype.

[c] Three symbols depict the direction of association in the three datasets included in the discovery cohort. The first symbol is for head and neck cancer genotyped with Illumina OncoArray (*n* = 254), the second symbol is for head and neck cancer genotyped with Illumina Consortium OncoArray (*n* = 216), and the third symbol is for esophageal cancer (*n* = 138). For AKI–CTCAE outcome: (−) protective effect; (+) risk effect. For eGFR reduction outcome: (−) reduced eGFR; (+) increased eGFR.

Table 4. Association analysis in the validation cohort

RsID	Genes	Chromosome: Location: Allele[a]	Effect size (95% CI)[b]	Unadjusted *p*-value	Adjusted *p*-value	Functional consequences
Analysis of SNPs that meet at least the suggestive association threshold in the discovery cohort						
AKI-CTCAE phenotype[c]						
rs4388268	*BACH2*	6:90734908:G:A	1.7 (0.8–3.5)	0.19	0.70	Intron variant
eGFR phenotype[c]						
rs17161766	*TMEM225B*	7:99177716:G:A	NA	NA	NA	Intron variant
NA	NA	7:98951080:C:CTTAT	NA	NA	NA	NA
rs199659233	*ARPC1A*	7:98959960:T:C	NA	NA	NA	Intron variant
rs556958738	*ARPC1A*	7:98959961:T:C	NA	NA	NA	Intron variant
rs4388268	*BACH2*	6:90734908:G:A	−1.5 (−5.3–2.4)	0.45	0.99	Intron variant
Analysis of known SNPs from systematic review						
AKI-CTCAE phenotype						
rs316019	*SLC22A2*	6:160670282:A:C	1.2 (0.4–3.6)	0.73	0.82	Missense variant
rs13181	*ERCC2*	19:45854919:T:G	0.6 (0.38–1.1)	0.095	0.24	Stop gained
rs1799793	*ERCC2*	19:45867259:C:T	0.5 (0.3–1.1)	0.075	0.24	Missense variant
rs3212986	*ERCC1*	19:45912736:C:A	0.9 (0.4–1.9)	0.82	0.82	3 prime UTR variant
rs11615	*ERCC1*	19:45923653:A:G	1.4 (0.7–2.5)	0.35	0.59	Synonymous variant
eGFR phenotype						
rs316019	*SLC22A2*	6:160670282:A:C	1.9 (−3.4–7.2)	0.49	0.82	Missense variant
rs13181	*ERCC2*	19:45854919:T:G	0.09 (−3.2–3.4)	0.96	0.96	Stop gained
rs1799793	*ERCC2*	19:45867259:C:T	−0.3 (−3.8–3.3)	0.89	0.96	Missense variant
rs3212986	*ERCC1*	19:45912736:C:A	−4.4 (−8.1–−0.7)	0.02	0.10	3 prime UTR variant
rs11615	*ERCC1*	19:45923653:A:G	−1.7 (−4.8–1.5)	0.31	0.77	Synonymous variant

[a] Chromosome: base pair:Allele1:Allele2.

[b] OR for AKI-CTCAE phenotype and β for eGFR phenotype.

[c] No significant association was found based on both AKI-CTCAE and eGFR phenotypes; NA, information not available. SNPs did not pass the quality control.

Association of previously investigated SNPs with cisplatin-induced nephrotoxicity based on the systematic review

A candidate gene approach was also used to study five SNPs identified from our previous systematic review[13]: *ERCC1* rs11615, *ERCC1* rs3212986, *ERCC2* rs13181, *ERCC2* rs1799793 and *SLC22A2* rs316019. However, in the discovery cohort, no significant or suggestive associations were found between these SNPs and either renal toxicity outcome. In the validation cohort, allele C *ERCC1* rs3212986 was associated with eGFR reduction (β = −4.4, 95% CI −8.1–−0.7). However, the association was no longer statistically significant after multiple-testing adjustment (Table 4).

BACH2 rs4388268 and risk of nephrotoxicity

In the discovery cohort, *BACH2* rs4388268 was the SNP most consistently associated, with increased risk of cisplatin-induced nephrotoxicity in both outcomes and across the genotyping platforms, and it met genome-wide significance for the eGFR outcome and suggestive association for AKI-CTCAE. In our validation cohort, this variant was also consistently associated in the same direction for both AKI-CTCAE and eGFR phenotypes although the results were not statistically significant. Closer examination of this variants in both discovery and validation cohorts, revealed that patients with an additional copy of the A allele at rs4388268 were at higher risk for cisplatin-associated nephrotoxicity defined as grade 1 or higher AKI-CTCAE (Figure S3). In the discovery cohort, the incidence of grade 1 or higher AKI-CTCAE was 10.6% for patient with a GG genotype, while the incidence was 24.7% for patients with an AG genotype 36.4% for AA genotype. In the validation cohort, the incidence rates in GG, AG and AA genotype were 24%, 30.4% and 50%, respectively. In the discovery cohort, an additional copy of the A allele also increased the median eGFR reduction from 6.2 to 9.6 to 13.3 mL/min/1.73 m^2 for GG homozygotes, AG heterozygotes and AA homozygotes, respectively (Figure S4). A similar trend in eGFR reduction was not observed in the validation cohort. An additional copy of the A allele reduced the median eGFR reduction from 10 to 9 mL/min/1.73 m^2 for GG and AG heterozygotes, respectively. The eGFR reduction then increased to 13.5 mL/min/1.73 m^2 for AA homozygotes in the validation cohort. However, the overall trend in the combined dataset still showed continuous reduction (Figure S4) with median eGFR reduction 6.6 for GG, 9.6 for AG and 13.3 mL/min/1.73 m^2 for AA genotype. A carrier of allele A may experience a reduction in eGFR up to 66 mL/min/1.73 m^2. The median eGFR reductions for each rs4388268 genotype in overall, discovery, and validation cohorts are available in Table S7. The NNG and NNT for rs4388268 in the discovery cohort were

44 and 8, respectively while in the validation cohort they were 36 and 7, respectively (Supplementary S1). When both datasets were combined, the NNG and NNT were 42 and 8, respectively (Supplementary S1).

Discussion

Main findings

To our knowledge, this is the first GWAS with a validation study in an independent cohort exploring the association between genetic variants and cisplatin-induced nephrotoxicity in cancer patients. rs4388268, an intron variant SNP in the *BACH2* gene, warrants further investigation due to its consistent association with increased risk of cisplatininduced nephrotoxicity in both AKI-CTCAE and eGFR outcomes and in both discovery and validation cohorts of European ancestry patients. In addition, from five SNPs identified from systematic review, only *ERCC1* rs3212986 was associated with a higher risk of cisplatin-induced nephrotoxicity in the validation cohort of NSCLC patients.

BACH2 rs4388268 is a common intron variant located in chromosome 6, not only in the European population (MAF = 0.23) but also in the global population (MAF = 0.29).[34] The frequency of homozygous AA carriers is relatively high, although the European population tends to have a lower frequency than the global population (0.058 vs. 0.103).[35] Expression quantitative trait loci (eQTLs) data were checked to examine if direct association between genetic variation markers and gene expression levels existed. However, no significant eQTLs were found for this SNP in all tissue types available at Genotype-Tissue Expression project portal (GTEx), meaning that the alternative allele of rs4388268 has no statistically significant effect on any tissue-specific gene expression levels compared to the reference allele.[36] In addition, its low RegulomeDB score of 5 suggests that limited data are available (only transcription factor (TF) binding or Dnase peak available).[37] The scoring scheme of RegulomeDB ranging from 1 to 7 and refers to the available datatypes for a single coordinate. The highest level of evidence (score 1a) reached when the SNP has the following data: eQTL, TF binding, matched TF motif, matched DNase Footprint and DNase peak.[37] The *BACH2* gene regulates B cell differentiation and function and is therefore biologically relevant for autoimmune disease pathogenesis. Variants in this gene have been previously associated with an increased risk of autoimmune diseases such as Addison's disease[38], rheumatoid arthritis[39], inflammatory bowel disease[40] and hyperthyroidism[41,42]. One study found BACH2, a transcription regulator protein, to be highly expressed in bone marrow and lymphoid tissue but moderately expressed in

kidney tubule.[43] Another study using mouse fibroblast cell line NIH3T3 reported *Bach2* as a rapid and highly sensitive reporter of DNA damage and demonstrated that *Bach2* overexpression is harmful to cell survival while silencing stimulates cell growth and shows protection from acute oxidative stress.[44] A recently published study[45] also showed that aged *Bach2*$^{\Delta CD4}$ mice displayed prominent IgG deposits in kidney glomeruli suggesting an autoimmunity process. Since cisplatin is mainly excreted through the kidneys, variants in *BACH2* might play a role in the pathogenesis of cisplatin-induced nephrotoxicity, though through which mechanism (cell proliferation, DNA damage, or autoimmunity) is unclear and warrants further investigation. AKI-CTCAE is commonly used in clinical settings and previous candidate gene studies to measure kidney function. In addition to assessing AKI-CTCAE, this study also evaluated the change in eGFR as a continuous outcome, since age, gender, race, and body weight affect SCr concentration independently from GFR.[23] Genome-wide significance signals were identified for the eGFR outcome, while the AKI-CTCAE outcome only showed SNPs with suggestive association. This is understandable since categorizing a continuous outcome results in loss of information; thus, the statistical power to detect a relation between the SNPs and kidney function was reduced.[46] Moreover, we corrected the association with known risk factors of cisplatin-induced nephrotoxicity. Approximately 15% of the patients in the discovery cohort and 25% of the patients in the validation cohort developed AKI-CTCAE, mainly grade 1, which is lower than the average percentage reported previously.[7] This might be due to effective mitigation strategies such as intravenous fluid repletion, magnesium supplementation and/or the mannitol administration protocol implemented in patient cohorts receiving high-dose cisplatin. In addition, we could not validate the findings of the previously published candidate gene study on *ERCC1* (rs11615 and rs3212986), *ERCC2* (rs13181 and rs1799793), and *SLC22A2* (rs316019) in our head and neck and esophageal cancer discovery cohort. However, our NSCLC validation cohort showed that rs3212986, a 3 prime UTR variant of *ERCC1*, was associated with a higher risk of cisplatin-induced nephrotoxicity, a result that was in line with previous studies.[47,48] Polymorphisms in *ERCC1* might exhibit the renal tubular damage caused by cisplatin through altered DNA repair mechanisms in the kidney. eQTL data in renal tubular tissue were available to confirm the impact of this SNP on *ERCC1* gene expression.[49] As for other SNPs, inconsistencies in the direction of association were discovered when comparing the association in the validation cohort with previous studies.[13] One possible explanation for the lack of association for these SNPs is population stratification. However, the SNPs of interest, especially five SNPs identified from our systematic review, were also studied in European ancestry subjects and still showed association, except for rs316019 which also studied in East Asian populations.[13]

In fact, the allele frequency of rs316019 is comparable between European and East Asian population (0.10 vs. 0.11%).[34] Other possible explanations for this lack of association are lack of study power, heterogeneity in outcome (i.e., differences in outcome definition and/or differences in cut-off value to be considered as a case) and differences in cancer type which eventually lead to differences in cisplatin-based regimen.

A recently published GWAS reported that rs1377817, a SNP intronic to *MYH14*, was associated with a high residual serum platinum level and possibly correlated to the development of several cisplatin-related toxicities such as tinnitus and Raynaud's phenomenon.[50] Our previously published candidate gene study also found that addition of allele A at *SLC22A2* rs316019 was associated with an increased risk of grade 1 or higher AKI-CTCAE.[51] However, in the present study significant associations were not found between those SNPs and either of our renal toxicity outcomes, although non-significant associations were in the same direction.

Compared to the discovery cohort, the follow-up period of the validation cohort was shorter. The reason for this is the fact that one-third of the NSCLC patients in the validation cohort were switched to carboplatin-based chemotherapy during treatment and effectively started 21 days after the last administration of cisplatin. These patients were switched to carboplatin for different reasons, but mostly due to cisplatin-induced toxicity. Meanwhile, only 2% of the subjects switched to carboplatin in the discovery cohort. To avoid treatment bias, the follow-up period of 21 days after the last administration of cisplatin was selected instead of 90 days as in the discovery cohort. Since the time-to-AKI is expected to be less than 21 days after cisplatin administration[52], this is arguably an acceptable follow-up duration, although different from the follow-up duration of the discovery cohort.

Differences in clinical characteristics between the discovery and the validation cohort, such as age at cisplatin initiation and number of comorbidities, potentially caused a higher incidence of cisplatin nephrotoxicity in the validation cohort. Such differences may also explain the non-significant contribution of genetic factors on cisplatin nephrotoxicity in the validation cohort. The clinical characteristics could be seen as effect modifiers since such factors were unlikely to confound the association between SNPs and cisplatin nephrotoxicity. Despite the differences in type of cancer (which led to different clinical characteristics), such approach could open possibility to gain more knowledge on the clinical relevance of genetic predisposition on cisplatin nephrotoxicity in different patient populations.

Potential clinical relevance

In clinical practice, occurrence of AKI-CTCAE grade 1 or higher will frequently result into clinical interventions such as delaying chemotherapy, cisplatin dose reduction up to 75% or treatment switch (e.g., to carboplatin). Our results indicate the possible involvement of genetic variants in platinum renal disposition. Genetic polymorphisms in *BACH2* were associated with higher risk of cisplatin-induced nephrotoxicity among European ancestry patients. This finding, together with proven clinical risk factors, may facilitate the identification of individuals at high risk of nephrotoxicity despite adequate volume status, magnesium supplementation and mannitol in high-dose cisplatin.

Based on the NNG and NNT in our combined cohort of patients of European ancestry, for every 42 cisplatin-candidate patients who are genotyped, 8 patients will carry a minor allele A of rs4388268. What we demonstrated was that carrying the minor allele A may contribute to susceptibility to nephrotoxicity and interindividual differences in clinical management. Thus, an intervention such as the need to delay, reduce or switch treatment may be considered for almost 20% of patients who are cisplatin candidates, which could have a significant impact on clinical care.

Strengths and limitations

The present study has several strengths. Firstly, to our knowledge, this is the first GWAS study to investigate the association between genetic variants and cisplatin-induced nephrotoxicity. Secondly, we were able to perform a validation (but not replication) study in an independent cohort. We recognize that both validation and replication will eventually become essential to confirm associations discovered via GWASs, to rule out associations due to bias, to improve effect estimation and to improve understanding of the biological underpinnings.[53] This is a first step towards these goals. Thirdly, the variables collected in our discovery cohort and validation cohort were based on real-world data. Therefore, the results of this study reflect the actual clinical setting, which strengthens the possibility of extrapolating our findings. Finally, although not statistically significant, the effect sizes of the validation study were in same direction as in the discovery cohort, despite the differences in clinical characteristics, type of malignancies, chemotherapy regimen and period of follow-up, suggesting a consistent association between particular genetic variants and cisplatin-induced nephrotoxicity.

The present analysis has some limitations, which illustrate the difficulties of performing such pharmacogenomic studies. First, this study had a relatively small occurrence of

grade 2 or higher AKI-CTCAE. Thus, although SNPs were identified that reached genome-wide significance across mild nephrotoxicity, suggesting a strong genetic signal in the development of cisplatin-induced nephrotoxicity, further analysis in this more severe nephrotoxicity group was not feasible. In addition, we had anticipated a higher rate of nephrotoxicity (based on data from older studies) that never materialized. Consequently, the study power was lower than expected. Second, our outcomes relied on the widely used SCr-based nephrotoxicity grading. Serum creatinine is not an ideal biomarker for drug-induced kidney injury because it is influenced by renal and non-renal factors independent of kidney function.[54] In addition, creatinine (to a small extent) competes with cisplatin for excretion as both are substrates of the organic cation transporter 2 (OCT2).[55] Third, dehydration and chemotherapy-induced nausea and vomiting cases were difficult to detect due to the retrospective nature of the discovery cohort. As for the validation cohort, such data were only partially recorded. Information regarding hydration protocols or other prophylaxis against nephrotoxicity was not available for both cohorts as well. Finally, our study focused on populations of European descent. Thus, further independent investigation should be conducted to assess if the results are transferrable to a more diverse population.

This study highlights both the benefits and limitations of using real-world observational data in pharmacogenomic studies: (i) we utilized pragmatic if imperfect surrogate markers of outcome (e.g., SCr-based changes) that may lead to variability in results; (ii) heterogeneity of populations could lead to heterogeneous results, including variability in eligibility criteria (study population), underlying clinical risks of the drug toxicity (e.g., differences across study cohorts in terms of age, and gender), and treatment regimens (doses and frequency of administration, concurrent drugs and/or radiation); and (iii) the need to validate and replicate results. In our study, we have restricted the focus on validation of the genetic associations but not true replication of results. Despite all of these issues, we were still able to identify a previously unknown variant in *BACH2* as a putative marker of nephrotoxicity.

Future research

Future studies should focus on functional validation of the *BACH2* role in cisplatin-induced nephrotoxicity, for example through experimental studies in knock-out mice and/or in vitro studies allowing unraveling the molecular pathway. The current issues with using SCr as the basis of nephrotoxicity is a pragmatic approach, but confirmatory studies may require the further development of more sensitive markers of kidney injury.

Regardless, if further validated or even replicated in other large datasets of prospective studies with more clinical similarities (e.g., same type of cancers), a clinical study to investigate the potential use of *BACH2* variants in guiding selection of platinum agents (i.e., between cisplatin and carboplatin) to avoid both acute and chronic nephrotoxicity without compromising the platinum's effectiveness (i.e., radiological response and overall survival) would be a future step. In addition, prospective observational studies that defines nephrotoxicity through highly sensitive and specific urinary biomarkers such as kidney injury molecule-1 (KIM-1), β2-microglobulin (B2M), cystatin C, clusterin, calbindin, neutrophil gelatinase-associated lipocalin (NGAL) and trefoil factor-3 (TFF-3)[54] would enhance understanding of cisplatin-induced nephrotoxicity as showed in a recent pharmacokinetic study[56] and a candidate gene study[57] alongside pragmatic studies such as ours that uses what is currently available in clinical practice.

Conclusions

The present GWAS and validation study suggest that genetic predisposition could be important in the development of nephrotoxicity among cisplatin users. *BACH2* rs4388268, a common intronic variant, increased the risk of cisplatin-induced nephrotoxicity nearly 4- and 1.7-fold in the discovery and validation cohorts, respectively. These results need further functional and pharmacokinetic/dynamic validation to reveal the mechanistic basis on how the variant may be involved in cisplatin-induced nephrotoxicity. Further replication in an independent cohort is also necessary before this finding can be utilized to personalize cisplatin therapy. In the validation cohort, one of the previously studied candidate SNPs, *ERCC1* rs3212986, was associated with eGFR reduction although the association was no longer statistically significant after multiple-testing adjustment. Nevertheless, genetic predisposition of *BACH2* could be important in the development of cisplatin-induced nephrotoxicity and providing opportunities for mechanistic understanding, potential individualized platinum selection and preventive strategies.

Declarations

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Authors' contributions

Conceptualization, ZZ, CJ, SV, RM, TE, GH, VD, AZ and GL; methodology, ZZ, CJ, WX, SV, RM, TE, GH, VD, AZ and GL; validation, ZZ and CJ; formal analysis, ZZ and CJ; investigation, ZZ and CJ; resources, GH, VD, AZ and GL; data curation, ZZ, CJ and WX; writing original draft preparation, ZZ and CJ; writing, review and editing, ZZ, CJ, WX, SV, RM, DP, MM, KK, DC, BO, SH, AS, AH, DG, JA, SB, AH, JK, RW, GD, AK, CR, SH, FM, AL, AH, JB, BC, TE, GH, VD, AZ and GL; visualization, ZZ and CJ.; supervision, SV, RM, TE, GH, VD, AZ and GL; project administration, CJ, GH, VD, AZ and GL; funding acquisition, ZZ, GH, VD and GL.
CJ and NC share first authorship since these authors contributed equally to this work.
AZ and GL share last authorship since these authors contributed equally to this work.
All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

The protocol of both the discovery and the validation cohort (PGxLUNG study) of this study complied with the Good Clinical Practice guidelines of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013), and was approved by the Review Ethics Board of the University Health Network, Toronto, Canada (CAPCR06-639, CAPCR07-0521) and the accredited Medical Research Ethics Committee in Nieuwegein (MEC-U, number R15.056), respectively.

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Informed consent

All patients provided written informed consent.

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Supplementary materials

Figure S1. Multidimensional scaling (MDS) plot of 1KG against the subjects of the discovery cohort (A, B and C) and the validation cohort (D) for each genotyping chip;

Table S1. Demographic and clinical characteristics in the discovery cohort: head and neck cancer and esophageal cancer patients;

Table S2. Treatment characteristics and distribution of outcomes in the discovery cohort: head and neck cancer vs. esophageal cancer patients;

Table S3. Demographic and clinical characteristics of patients without nephrotoxicity and patients with grade 1 or higher AKI-CTCAE, both in discovery and validation cohort;

Table S4. Top twenty SNPs from genome-wide meta-analysis of cisplatin-induced AKI-CTCAE in the discovery cohort;

Table S5. Top twenty SNPs from genome-wide meta-analysis of cisplatin-induced eGFR reduction in the discovery cohort;

Table S6. Association between *BACH2* rs4388268 and cisplatin-induced nephrotoxicity in subjects of discovery cohort with available Charlson comorbidity index data (n = 509);

Table S7. Median of eGFR reduction for each *BACH2* rs4388268 genotype in the overall, discovery, and validation cohort;

Figure S2. Genome-wide meta-analysis results of cisplatin-induced nephrotoxicity using AKI-CTCAE and eGFR phenotypes in subjects of discovery cohort with available Charlson comorbidity index data (n = 509);

Figure S3. AKI-CTCAE status for each *BACH2* rs4388268 genotype;

Figure S4. eGFR differences (∆eGFR) for each *BACH2* rs4388268 genotype;

Supplementary S1. Calculations number needed to genotype (NNG) and number needed to treat (NNT) on *BACH2* rs4388268 based on formula provided by Tonk, *et al*. (2017).

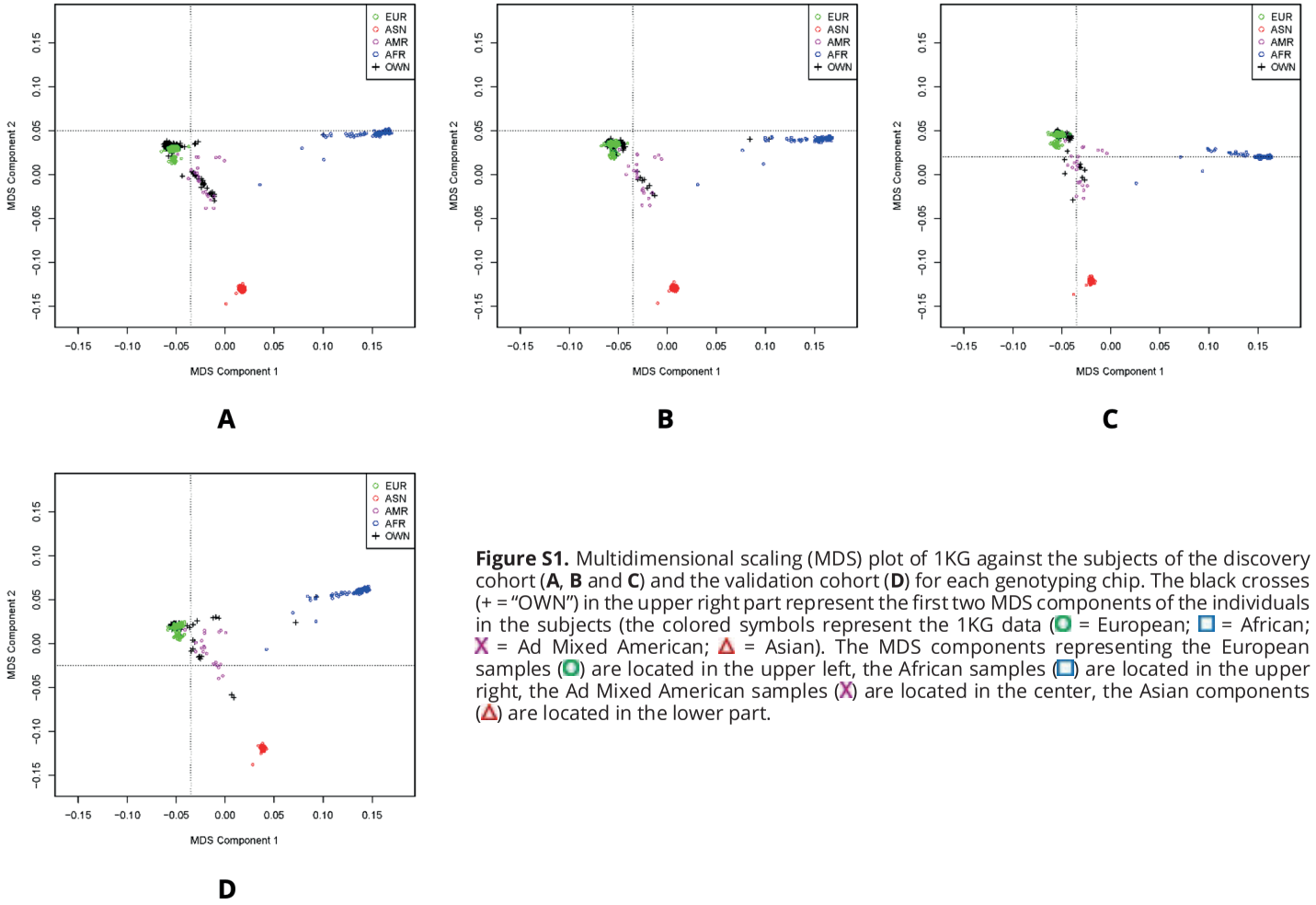

Figure S1. Multidimensional scaling (MDS) plot of 1KG against the subjects of the discovery cohort (**A**, **B** and **C**) and the validation cohort (**D**) for each genotyping chip. The black crosses (+ = "OWN") in the upper right part represent the first two MDS components of the individuals in the subjects (the colored symbols represent the 1KG data (○ = European; □ = African; X = Ad Mixed American; △ = Asian). The MDS components representing the European samples (○) are located in the upper left, the African samples (□) are located in the upper right, the Ad Mixed American samples (X) are located in the center, the Asian components (△) are located in the lower part.

Table S1. Demographic and clinical characteristics in the discovery cohort: head and neck cancer and esophageal cancer patients

Characteristics	Head and neck cancer patients			Esophageal cancer patients			*p*-value[a]
	Total (*n* = 470)	Nephrotoxicity (grade ≥ 1 AKI-CTCAE)		Total (*n* = 138)	Nephrotoxicity (grade ≥ 1 AKI-CTCAE)		
		No (*n* = 400)	Yes (*n* = 70)		No (*n* = 115)	Yes (*n* = 23)	
Age at cisplatin initiation in years, mean ± SD	57.4 ± 7.3	57.3 ± 7.3	57.0 ± 7.3	59.8 ± 9.6	60.2 ± 9.4	58.0 ± 10.6	< 0.01*
Male, *n* (%)	387 (82.3)	328 (82)	59 (84.3)	113 (81.9)	92 (80)	21 (91.3)	0.90
Cardiovascular disease, *n* (%)	132 (28.1)	106 (26.5)	26 (37.1)	24 (17.4)	15 (13)	9 (39.1)	0.01*
Diabetes mellitus, *n* (%)	35 (7.4)	25 (6.3)	10 (14.3)	9 (6.5)	5 (4.3)	4 (17.4)	0.85
Charlson comorbidity index[b], *n* (%)							
2–3	175 (42.2)	156 (44.3)	19 (30.2)	31 (33.0)	28 (36.4)	3 (17.6)	0.23
4–5	197 (47.5)	163 (46.3)	34 (54.0)	50 (53.2)	39 (50.6)	11 (64.7)	
≥ 6	43 (10.4)	33 (9.4)	10 (15.9)	13 (13.8)	10 (13.0)	3 (17.6)	
Missing data	55	48	7	44	38	6	
Chronic NSAID users, *n* (%)	39 (8.3)	32 (8)	7 (10)	3 (2.2)	2 (1.7)	1 (4.3)	0.01*
Received other antineoplastic, *n* (%)	0 (0)	0 (0)	0 (0)	138 (100)	115 (100)	23 (100)	NA
Received radiotherapy, *n* (%)	462 (98.3)	393 (98.3)	69 (98.6)	72 (52.2)	63 (54.8)	9 (39.1)	< 0.01*
Albumin baseline, median mmol/L (IQR)	42 (41-44)	42 (40-44)	43 (41-44)	41 (39-43)	41 (39-43)	41 (39-43)	< 0.01*
Baseline eGFR, median mL/min/1.73 m^2 (IQR)	94.3 (85.2-101.5)	94.6 (84.8-101.5)	93.8 (88.3-101.5)	92.2 (77.4-100.4)	92.2 (79-99.9)	90.6 (68.3-105.7)	0.02*

Abbreviations: NA, information not available; NSAID, non-steroidal anti-inflammatory drug; SD, standard deviation; eGFR, estimated glomerular filtration rate; IQR, interquartile range.

[a] *p*-value of comparison head and neck and esophageal cancer patients.

[b] Charlson comorbidity index score provides a simple means to quantify the effect of comorbid illnesses, including cardiovascular diseases, chronic obstructive pulmonary disease, liver disease and diabetes mellitus among others and accounts for the aggregate effect of multiple concurrent diseases. A higher score indicates more comorbidities.

* *p*-value < 0.05 based on independent t-test or Mann-Whitney *U* Test (for continuous independent variable) and Fisher's Exact Test or chi-square test (for categorical independent variable).

Table S2. Treatment characteristics and distribution of outcomes in the discovery cohort: head and neck cancer vs. esophageal cancer patients

Characteristics	**Head and neck cancer patients (*n* = 470)**	**Esophageal cancer patients (*n* = 138)**	***p*-value**
Cumulative cisplatin dose, median mg/m^2 (IQR)	198.2 (179.6-250)	173.8 (140.6-222.8)	< 0.01*
Cycles of cisplatin-based chemotherapy, *n* (%)			< 0.01*
1	35 (7.4)	15 (10.9)	
2	275 (58.5)	38 (27.5)	
3	155 (33)	46 (33.3)	
≥ 4	5 (1.1)	39 (28.3)	
AKI-CTCAE, *n* (%)[a]			0.61
Grade 0 (no nephrotoxicity)	400 (85.1)	115 (83.3)	
Grade 1	51 (10.9)	20 (14.5)	
Grade 2	14 (3)	3 (2.2)	
Grade 3	5 (1.1)	0 (0)	
Grade 4	0 (0)	(0)	
Any Grade	70 (14.9)	23 (16.7)	
eGFR reduction, median, mL/min/1.73 m^2 (IQR)[b]	6.6 (0.5-18.6)	8.9 (1.1-19.3)	0.28
Patients without nephrotoxicity	5.1 (0.0-13.6)	6.8 (0.0-16.1)	0.22
Patients with grade ≥ 1 AKI-CTCAE	32.1 (19.4-46.6)	25.5 (10.1-38.7)	0.14

Abbreviations: IQR, interquartile range; eGFR, estimated glomerular filtration rate.

[a] Highest AKI-CTCAE grade between cisplatin initiation and the last day of follow-up.

[b] Differences between baseline eGFR and lowest eGFR recorded from cisplatin initiation until the last day of follow-up.

* *p*-value < 0.05 based on Mann-Whitney *U* Test (for continuous independent variable) and chi-square test (for categorical independent variable).

Table S3. Demographic and clinical characteristics of patients without nephrotoxicity and patients with grade 1 or higher AKI-CTCAE, both in discovery and validation cohort

Characteristics	Discovery cohort (*n* = 608)	Nephrotoxicity (grade ≥ 1 AKI-CTCAE)		Validation cohort (*n* = 149)	Nephrotoxicity (grade ≥ 1 AKI-CTCAE)		*p*-value[a]
		No (*n* = 515)	Yes (*n* = 93)		No (*n* = 109)	Yes (*n* = 40)	
Age at cisplatin initiation in years, mean ± SD	57.9 ± 7.9	57.9 ± 7.9	58.0 ± 8.2	62.8 ± 9.4	62.8 ± 9.6	62.8 ± 9.2	< 0.01*
Male, *n* (%)	500 (82.2)	420 (81.6)	80 (86.0)	71 (47.7)	52 (47.7)	19 (47.5)	< 0.01*
Cardiovascular disease, *n* (%)	156 (25.7)	121 (23.5)	35 (37.6)	NA	NA	NA	NA
Diabetes mellitus, *n* (%)	44 (7.2)	30 (5.8)	14 (15.1)	NA	NA	NA	NA
Charlson comorbidity index[b], *n* (%)							< 0.01*
2–3	206 (40.5)	184 (42.9)	22 (27.5)	71 (47.7)	51 (46.8)	20 (50.0)	
4–5	247 (48.5)	202 (47.1)	45 (56.3)	43 (28.9)	30 (27.5)	13 (32.5)	
≥ 6	56 (11.0)	43 (10.0)	13 (16.3)	35 (23.4)	28 (25.7)	7 (17.5)	
Missing data	99	86	13	0	0	0	
Chronic NSAID users, *n* (%)	42 (6.9)	34 (6.6)	8 (8.6)	NA	NA	NA	NA
Received other antineoplastic, *n* (%)	138 (22.7)	115 (22.3)	23 (24.7)	149 (100)	109 (100)	40 (100)	< 0.01*
Received radiotherapy, *n* (%)	534 (87.8)	456 (88.5)	78 (83.9)	87 (58.4)	73 (67.0)	14 (35.0)	< 0.01*
Albumin baseline, median mmol/L (IQR)	42 (40–44)	42 (40–44)	42 (41–44)	39 (33–42)	39 (32–42)	39 (35–42)	< 0.01*
Baseline eGFR, median mL/min/1.73 m^2 (IQR)	94.0 (83.4–101.4)	94.0 (83.1–101.2)	93.7 (86.3–101.7)	90.0 (80.0–90.0)	90.0 (79.5–90.0)	90.0 (81.0–90.0)	< 0.01*

Abbreviations: NA, information not available; NSAID, non-steroidal anti-inflammatory drug; SD, standard deviation; eGFR, estimated glomerular filtration rate; IQR, interquartile range.

[a] *p*-value of comparison head and neck and esophageal cancer patients.

[b] Charlson comorbidity index score provides a simple means to quantify the effect of comorbid illnesses, including cardiovascular diseases, chronic obstructive pulmonary disease, liver disease and diabetes mellitus among others and accounts for the aggregate effect of multiple concurrent diseases. A higher score indicates more comorbidities.

* *p*-value < 0.05 based on independent t-test or Mann-Whitney *U* Test (for continuous independent variable) and Fisher's Exact Test or chi-square test (for categorical independent variable).

Table S4. Top twenty SNPs from genome-wide meta-analysis of cisplatin-induced AKI-CTCAE in the discovery cohort

rsID	Gene	Chromosome: Location:Allele[a]	OR (95% CI)	*p*-value	Direction[b]	Heterogeneity I^2	Heterogeneity *p*-value	Functional consequences	eQTL from GTEx database	RegulomeDB score
NA	NA	11:94417672:AC:A	3.4 (2.1-5.5)	$5.0 \cdot 10^{-7}$	+++	53.9	0.11	NA	NA	NA
rs4388268	*BACH2*	6:90734908:G:A	3.9 (2.3-6.7)	$7.4 \cdot 10^{-7}$	+++	0	0.98	intron variant	NS	5
rs72965891	*LOC105369438*	11:94415630:T:C	0.3 (0.2-0.5)	$1.1 \cdot 10^{-6}$	---	60	0.08	intron variant	NS	5
rs11020896	*LOC105369438*	11:94416887:T:C	0.3 (0.2-0.5)	$1.6 \cdot 10^{-6}$	---	28.7	0.25	intron variant	NS	No data
rs16882364	*BACH2*	6:90735491:A:G	0.2 (0.1-0.4)	$2.3 \cdot 10^{-6}$	---	29.2	0.24	intron variant	NS	2b
rs7110345	*LOC105369438*	11:94395048:G:A	3.0 (1.9-4.6)	$2.3 \cdot 10^{-6}$	+++	77.8	0.01	intron variant	NS	No data
rs12664728	*BACH2*	6:90737315:G:A	5.4 (2.7-10.9)	$2.5 \cdot 10^{-6}$	+++	30.1	0.24	intron variant	NS	6
NA	NA	11:94449088:GC:G	3.0 (1.9-4.8)	$3.1 \cdot 10^{-6}$	+++	38.5	0.20	NA	NA	NA
rs7350489	*AMOTL1; LOC105369438*	11:94452476:C:T	3.0 (1.9-4.8)	$3.1 \cdot 10^{-6}$	+++	38.5	0.20	intron variant	NS	No data
NA	NA	11:94451910:GTTGA:G	3.0 (1.9-4.8)	$3.2 \cdot 10^{-6}$	+++	38.1	0.20	NA	NA	NA
rs16882357	*BACH2*	6:90731445:T:C	0.2 (0.1-0.4)	$3.3 \cdot 10^{-6}$	---	25.7	0.26	intron variant	NS	5
rs12664550	*BACH2*	6:90732877:T:C	0.2 (0.1-0.4)	$3.3 \cdot 10^{-6}$	---	25.7	0.26	intron variant	NS	6
rs60917421	*LOC105369438*	11:94395777:T:C	0.4 (0.2-0.5)	$3.5 \cdot 10^{-6}$	---	78.2	0.01	intron variant	NS	2b
rs11020924	*AMOTL1; LOC105369438*	11:94453131:A:G	0.3 (0.2-0.5)	$3.9 \cdot 10^{-6}$	---	32.2	0.23	intron variant	NS	6
rs2068908	*AMOTL1; LOC105369438*	11:94458154:G:A	3.0 (1.9-4.7)	$3.9 \cdot 10^{-6}$	+++	32.2	0.23	intron variant	NS	6
rs11020920	*AMOTL1; LOC105369438*	11:94447905:T:C	0.3 (0.2-0.5)	$4.0 \cdot 10^{-6}$	---	32.1	0.23	intron variant	NS	6
rs4486099	*TNRC18*	7:5373370:A:C	2.9 (1.9-4.6)	$4.1 \cdot 10^{-6}$	+++	62.8	0.07	intron variant	NS	5
rs10831271	*AMOTL1; LOC105369438*	11:94448263:C:T	3.0 (1.9-4.7)	$4.1 \cdot 10^{-6}$	+++	32.4	0.23	intron variant	NS	5
NA	NA	11:94431032:TAAAG:T	3.0 (1.9-4.8)	$4.3 \cdot 10^{-6}$	+++	37.3	0.20	NA	NA	NA
rs7130432	*LOC105369438*	11:94395496:A:G	0.4 (0.2-0.6)	$4.3 \cdot 10^{-6}$	---	79.3	0.01	intron variant	significant with expression of *KDM4D* gene in vagina tissue	5

Abbreviations: NA, information not available; NS, no significant data.

[a] Chromosome: base pair:Allele1:Allele2.

[b] Three symbols depicted the direction of association in three datasets included in the discovery cohort. The first symbol was for head and neck cancer genotyped with Illumina OncoArray (*n* = 254), the second symbol was for head and neck cancer genotyped with Illumina Consortium OncoArray (*n* = 216), and the third symbol was for esophageal cancer (*n* = 138). (-) protective effect; (+) risk effect; (?) results not known.

Table S5. Top twenty SNPs from genome-wide meta-analysis of cisplatin-induced eGFR reduction in the discovery cohort

rsID	Genes	Chromosome :Location :Allele[a]	β	SE	*p*-value	Direction[b]	Heterogeneity I^2	Heterogeneity *p*-value	Functional consequences	eQTL from GTEx database	RegulomeDB score
rs17161766*	*TMEM225B*	7:99177716:G:A	-28.9	5.01	$7.8 \cdot 10^{-9}$	NA-NA	0	1	intron variant	significant with e · pression of *ZSCAN25, GS1-259H13.2, BUD31* in various tissues but not in kidney	4
NA*	NA	7:98951080:C:CTTAT	-27.2	4.74	$9.5 \cdot 10^{-9}$	NA-NA	0	1	NA	NA	NA
rs199659233*	*ARPC1A*	7:98959960:T:C	28.7	5.06	$1.5 \cdot 10^{-8}$	NA+NA	0	1	intron variant	NA	6
rs556958738*	*ARPC1A*	7:98959961:T:C	28.7	5.06	$1.5 \cdot 10^{-8}$	NA+NA	0	1	intron variant	NA	NA
rs4388268*	*BACH2*	6:90734908:G:A	-8.4	1.52	$3.8 \cdot 10^{-8}$	---	0	0.53	intron variant	NS	5
rs1826059	NA	4:64014716:A:G	7.3	1.38	$1.4 \cdot 10^{-7}$	+++	0	0.71	NA	NS	No data
NA	NA	4:64016970:CTT:C	7.3	1.38	$1.4 \cdot 10^{-7}$	+++	0	0.71	NA	NA	NA
rs62320477	NA	4:64018447:G:A	-7.3	1.39	$1.5 \cdot 10^{-7}$	---	0	0.89	NA	NS	No data
NA	NA	4:63915271:A:AT	7.1	1.35	$1.6 \cdot 10^{-7}$	+++	0	0.67	NA	NA	NA
rs6834243	NA	4:64012390:T:C	7.2	1.39	$2.3 \cdot 10^{-7}$	+++	0	0.70	NA	NS	6
rs59242959	NA	4:64013730:G:A	-7.2	1.39	$2.3 \cdot 10^{-7}$	---	0	0.70	NA	NS	No data
NA	NA	4:64017361:C:CTGGGTT	-7.2	1.39	$2.3 \cdot 10^{-7}$	---	0	0.70	NA	NA	NA
rs62320476	NA	4:64018284:C:T	-7.2	1.39	$2.3 \cdot 10^{-7}$	---	0	0.70	NA	NS	No data
rs62320470	NA	4:64009273:T:A	-7.2	1.39	$2.5 \cdot 10^{-7}$	---	0	0.69	NA	NS	No data
rs138024145	NA	4:63893286:C:A	-7.0	1.35	$2.9 \cdot 10^{-7}$	---	0	0.76	NA	NA	No data

rsID	Genes	Chromosome :Location :Allele[a]	β	SE	*p*-value	Direction[b]	Heterogeneity I^2	Heterogeneity *p*-value	Functional consequences	eQTL from GTEx database	RegulomeDB score
rs12664728	*BACH2*	6:90737315:G:A	-10.8	2.10	$2.9 \cdot 10^{-7}$	---	61.6	0.07	intron variant	NS	6
rs16882364	*BACH2*	6:90735491:A:G	10.7	2.09	$3.0 \cdot 10^{-7}$	+++	61.8	0.07	intron variant	NS	2b
rs35533931	NA	4:64028203:C:T	-7.2	1.41	$3.1 \cdot 10^{-7}$	---	0	0.68	NA	NS	No data
rs976921	NA	4:64076807:T:C	7.0	1.36	$3.2 \cdot 10^{-7}$	+++	0	0.62	NA	NS	No data
rs2007396	NA	4:64076658:T:C	7.0	1.37	$3.2 \cdot 10^{-7}$	+++	0	0.63	NA	NS	6

Abbreviations: NA, information not available.

[a] Chromosome: base pair:Allele1:Allele2.

[b] Three symbols depicted the direction of association in three datasets included in the discovery cohort. The first symbol was for head and neck cancer genotyped with Illumina OncoArray (*n* = 254), the second symbol was for head and neck cancer genotyped with Illumina Consortium OncoArray (*n* = 216), and the third symbol was for esophageal cancer (*n* = 138). (-) reduced eGFR; (+) increased eGFR; (NA) results not known since the SNP did not surpass the post-imputation QC in that particular dataset.

* Reached genome-wide-significance (*p*-value ≤ $5 \cdot 10^{-8}$).

Table S6. Association between *BACH2* rs4388268 and cisplatin-induced nephrotoxicity in subjects of discovery cohort with available Charlson comorbidity index data (*n* = 509)

Chromosome: Location: Allele[a]	Functional consequences	Outcome	Effect size (95% CI)[b]	*p*-value	Direction[c]
6:90734908:G:A	Intron variant	AKI–CTCAE	3.6 (1.7–5.4)	$3.8 \cdot 10^{-5}$	+ + +
		eGFR reduction	−8.1 (−11.4–−4.8)	$1.4 \cdot 10^{-6}$	– – –

[a] Chromosome: base pair:Allele1:Allele2.

[b] OR for AKI-CTCAE phenotype and β for eGFR phenotype.

[c] Three symbols depict the direction of association in the three datasets included in the discovery cohort. The first symbol is for head and neck cancer genotyped with Illumina OncoArray (*n* = 254), the second symbol is for head and neck cancer genotyped with Illumina Consortium OncoArray (*n* = 216), and the third symbol is for esophageal cancer (*n* = 138). For AKI–CTCAE outcome: (−) protective effect; (+) risk effect. For eGFR reduction outcome: (−) reduced eGFR; (+) increased eGFR.

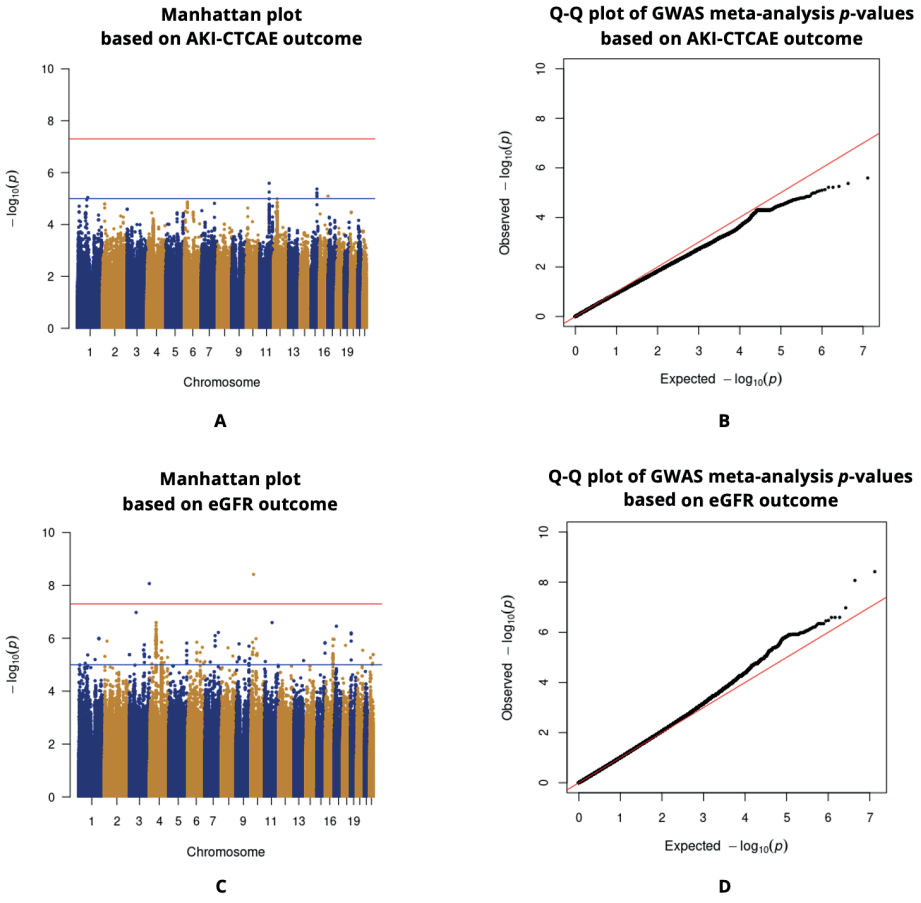

Figure S2. Genome-wide meta-analysis results of cisplatin-induced nephrotoxicity using AKI-CTCAE and eGFR phenotypes in subjects of the discovery cohort with available Charlson comorbidity index data (*n* = 509). **A.** Manhattan plot showing logistic regression results using the AKI-CTCAE phenotype; −log10 *p*-values are plotted against the respective chromosomal position of each SNP. **B.** A quantile-quantile (Q-Q) plot showing the distribution of *p*-values in the GWAS using the AKI-CTCAE phenotype. **C.** Manhattan plot showing logistic regression results using the eGFR phenotype. **D.** Q-Q plot showing the distribution of *p*-values in the GWAS using the eGFR phenotype.

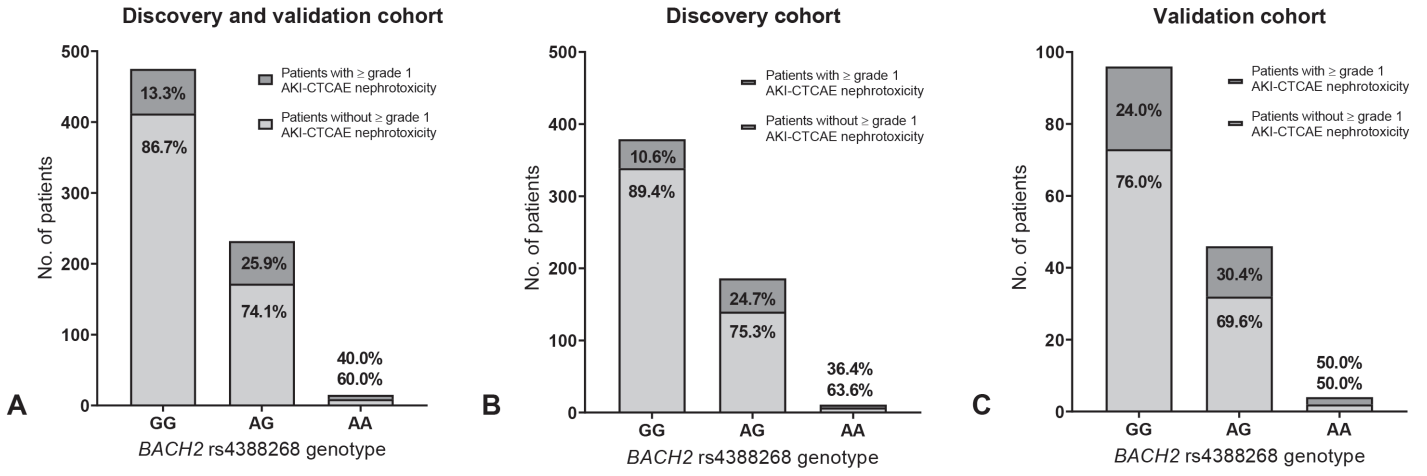

Figure S3. AKI-CTCAE status for each *BACH2* rs4388268 genotype. **A.** AKI-CTCAE status for each *BACH2* rs4388268 genotype in the overall cohort (*n* = 757). **B.** AKI-CTCAE status for each *BACH2* rs4388268 genotype in the discovery cohort (*n* = 608). **C.** AKI-CTCAE status for each *BACH2* rs4388268 genotype in the validation cohort (*n* = 149).

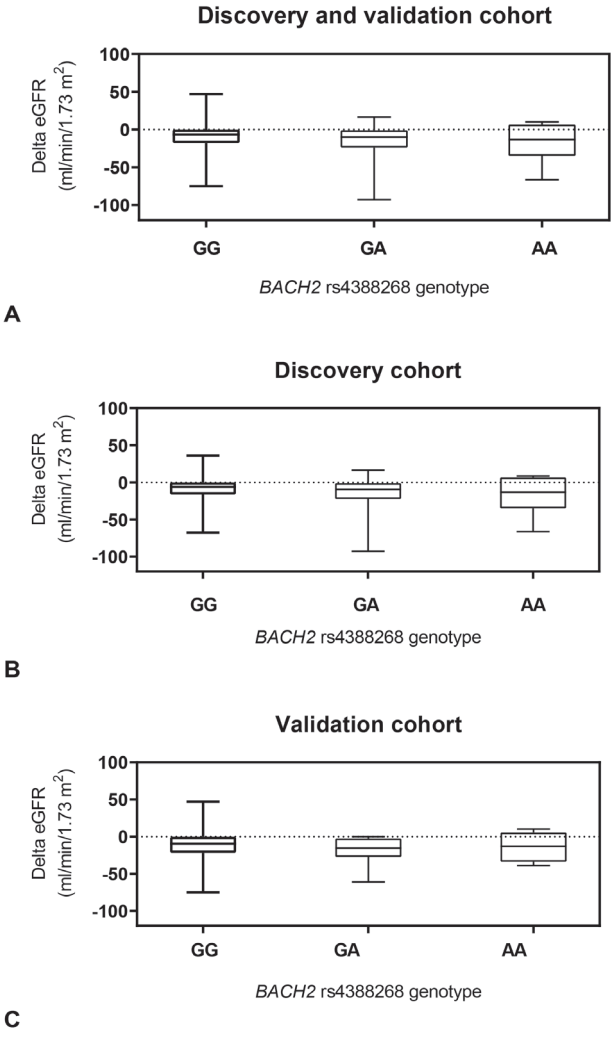

Figure S4. eGFR differences (ΔeGFR) for each *BACH2* rs4388268 genotype. **A.** eGFR differences (ΔeGFR) for each *BACH2* rs4388268 genotype in the overall cohort (*n* = 757). **B.** eGFR differences (ΔeGFR) for each *BACH2* rs4388268 genotype in the discovery cohort (*n* = 608). **C.** eGFR differences (ΔeGFR) for each *BACH2* rs4388268 genotype in the validation cohort (*n* = 149).

Table S7. Median of eGFR reduction for each *BACH2* rs4388268 genotype in the overall, discovery, and validation cohort

Group	GG		AG		AA	
	n (%)	Reduction of eGFR mL/min/1.73 m2 median (IQR)	*n* (%)	Reduction of eGFR mL/min/1.73 m2 median (IQR)	*n* (%)	Reduction of eGFR mL/min/1.73 m2 median (IQR)
Discovery and validation cohort (*n* = 757)[a]	475 (65.8)	6.6 (0.0–17.0)	232 (32.1)	9.6 (1.10–21.2)	15 (2.1)	13.3 (3.0–34.9)
Patients without nephrotoxicity (*n* = 624)	412 (66.2)	5.1 (0–13.7)	172 (27.7)	6.8 (0.6–16.7)	9 (1.4)	8.0 (7.4–12.9)
Patients with grade ≥ 1 AKI-CTCAE (*n* = 133)	63 (47.7)	31.8 (24.2–44.8)	60 (45.5)	30.2 (17.2–38.0)	6 (4.5)	36.9 (34.5–44.4)
Grade 1 (*n* = 104)	49 (47.6)	29.9 (24.2–38.7)	46 (44.7)	27.6 (16.9–35.4)	5 (4.9)	34.9 (34.5–39)
Grade 2 (*n* = 21)	10 (47.6)	53.2 (42.8–56)	11 (52.4)	45.5 (15.3–57.6)	0 (0)	0 (0)
Grade 3 (*n* = 8)	4 (50)	63.5 (25.8–72.5)	3 (37.5)	66.2 (48.6–92.8)	1 (12.5)	66.4
Discovery cohort (*n* = 608)[b]	379 (65.8)	6.2 (-16.2–0.0)	186 (32.3)	9.6 (1.1–22.4)	11 (1.9)	13.3 (6.5–34.8)
Patients without nephrotoxicity (*n* = 515)	339 (65.8)	5.1 (1.1–13.7)	140 (27.2)	6.6 (0.7–16.7)	7 (1.4)	12.8 (7.4–13.3)
Patients with grade ≥ 1 AKI-CTCAE (*n* = 93)	40 (43.0)	30.6 (16.4–42.9)	46 (49.5)	27.5 (14.0–42.0)	4 (4.3)	39.6 (34.7–55.4)
Grade 1 (*n* = 71)	33 (46.5)	30.5 (24.2–35.7)	32 (45.1)	26.2 (8–35.6)	3 (4.2)	34.9 (34.5–44.4)
Grade 2 (*n* = 17)	6 (35.3)	47.4 (4.9–54.4)	11 (64.7)	45.5 (15.3–57.6)	0 (0)	0 (0)
Grade 3 (*n* = 5)	1 (20)	5.5	3 (60)	66.2 (48.6–92.8)	1 (20)	66.4
Validation cohort (*n* = 149)[c]	96 (65.8)	10.0 (0.0–21.0)	46 (31.5)	9.0 (0.0–19.0)	4 (2.7)	13.5 (5.0–46.8)
Patients without nephrotoxicity (*n* = 109)	73 (68.2)	5.0 (0.0–13.0)	32 (29.9)	8.0 (0.0–17.5)	2 (1.9)	-1.0 (-10.0–8.0)
Patients with grade ≥ 1 AKI-CTCAE (*n* = 40)	23 (59.0)	40.0 (26.0–56.0)	14 (35.9)	33.0 (26.0–35.0)	2 (5.1)	28.5 (18.0–39.0)
Grade 1 (*n* = 33)	16 (50)	28 (24.5–40)	14 (43.8)	33 (26–35)	2 (6.3)	28.5 (18–39)
Grade 2 (*n* = 4)	4 (100)	57 (53.5–60)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3 (*n* = 3)	3 (100)	70 (57–75)	0 (0)	0 (0)	0 (0)	0 (0)

[a] Missing genotype in 35 patients.

[b] Missing genotype in 32 patients.

[c] Missing genotype in 3 patients.

Supplementary S1. Calculations number needed to genotype (NNG) and number needed to treat (NNT) on *BACH2* rs4388268 based on formula provided by Tonk, *et al*. (2017)

1. Discovery cohort

Genotype	With nephrotoxicity	Without nephrotoxicity
AA	4	7
AG	46	140
GG	40	339

Allele	With nephrotoxicity	Without nephrotoxicity	Total
A	54	154	208
G	126	818	944
Total	180	972	1152

Type of effect sizes	Value
RR	1.95
RD	0.13
NNT	7.93
NNG	43.91

2. Validation cohort

Genotype	With nephrotoxicity	Without nephrotoxicity
AA	3	1
AG	8	22
GG	14	53

Allele	With nephrotoxicity	Without nephrotoxicity	Total
A	14	24	38
G	36	128	164
Total	50	152	202

Type of effect sizes	Value
RR	1.68
RD	0.15
NNT	6.72
NNG	35.7

3. Combined cohort

Genotype	With nephrotoxicity	Without nephrotoxicity
AA	7	8
AG	54	162
GG	54	392

Allele	With nephrotoxicity	Without nephrotoxicity	Total
A	68	178	246
G	162	946	1108
Total	230	1124	1354

Type of effect sizes	Value
RR	1.89
RD	0.13
NNT	7.68
NNG	42.27

Chapter 2.4

Association between genetic variants and peripheral neuropathy in patients with NSCLC treated with first-line platinum-based therapy

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Abstract

Background: Chemotherapy-induced peripheral neuropathy (CIPN) is a common, disabling, often irreversible side effect in non-small cell lung cancer (NSCLC) patients treated with platinum-based therapy. There is increasing evidence for associations between genetic variants and susceptibility to CIPN. The aim of this study was to further explore genetic risk factors for CIPN by investigating previously reported genetic associations in an independent cohort of NSCLC patients treated with platinum-based therapy.

Methods: A multicenter prospective follow-up study (PGxLUNG, NTR number NL5373610015) in NSCLC patients (stage II-IV) treated with first-line platinum-based (cisplatin or carboplatin) chemotherapy was conducted. Clinical evaluation of neuropathy (using the CTCAE v4.03 for peripheral sensory neuropathy) was performed at baseline and before each cycle (four cycles, every three weeks) of platinum-based chemotherapy and at three and six months after treatment initiation. The evaluated single nucleotide polymorphisms (SNPs) were selected based on a review of the existing evidence. The relationship between 34 SNPs in 26 genes and any grade (grade $\geq$ 1) as well as severe (grade $\geq$ 2) CIPN was assessed by using univariate and multivariate logistic regression modelling, assuming both a dominant and recessive model. Patient and disease characteristics, concomitant chemotherapeutic agents, number of administered cycles of chemotherapy and performance status were taken into account as potential confounding factors and/or effect modifiers. The false discovery rate was used for correction in multiple testing based on the Benjamini-Hochberg procedure.

Results: In total, 320 patients were included of which 26.3% (*n* = 84) and 8.1% (*n* = 26) experienced any grade and severe CIPN, respectively. Median age was 65 years and 10% had diabetes. The GG-genotype (rs879207, A>G) of *TRPV1*, a gene expressed in peripheral sensory neurons, was observed in 11.3% (*n* = 36) of the patients and found to be associated with an increased risk of severe neuropathy (OR 5.2, 95% CI 2.1-12.8, adjusted *p*-value 0.012). A quarter (25%, *n* = 9/36) of the patients with the GG-genotype developed severe neuropathy compared to 6% (*n* = 17/282) of the patients with the AG- or AA- genotype. In multivariate logistic regression analysis statistically significant associations between the GG-genotype of rs879207 (ORadj 4.7, 95% CI 1.8-12.3) and between concomitant use of paclitaxel (ORadj 7.2, 95% CI 2.5-21.1) and severe CIPN were observed.

Conclusions: This study shows that patients with the GG-genotype (rs879207) of *TRPV1* have an almost 5-fold higher risk of developing severe neuropathy when treated with platinum-based therapy. Future studies should aim to validate these findings in an independent cohort. In addition, the implementation of these results in clinical practice should be investigated in clinical intervention studies with a special focus on further individualization of platinum-based therapy to prevent the occurrence of neuropathy.

Introduction

Chemotherapy-induced peripheral neuropathy (CIPN), a disorder characterized by damage or dysfunction of the peripheral sensory nerves, is a frequently occurring, disabling and often long-lasting or even irreversible side effect of platinum-based chemotherapy.[1,2] Neuropathy manifests with clinical symptoms such as numbness, prickling or tingling in hands and feet, burning or shooting pain, muscle weakness and loss of taste.[3,4] Patients suffering from paresthesia can experience difficulties in activities of daily living, which affects patients, quality of life to a considerable extent.[5] Frequently, CIPN may necessitate dose reduction, treatment delay, treatment switch or even early treatment termination, which may affect the disease prognosis.[6,7] As described by McWhinney *et al*[8,9], the incidence and severity of neuropathy do not appear to be directly related to the response to platinum-based chemotherapy. For that reason, CIPN should be approached as an avoidable side effect of platinum-based chemotherapy.[8] Currently, no proven preventive strategies for platinum-induced neuropathy are available and clinical management is complicated by the fact that limit treatment options (e.g. duloxetine, gabapentin) are available, with only moderate effects on symptoms relief.[10-12]

A higher cumulative dose of platinum-based chemotherapy increases the risk for CIPN; hence, symptoms of peripheral neuropathy usually occur after the second course of chemotherapy. However, neuropathy may also manifest or worsen 3-6 months after the start of platinum-based chemotherapy.[1,2,13] Patient and treatment characteristics such as pre-existing polyneuropathy, older age, diabetes mellitus, cumulative dose of chemotherapy and excessive alcohol consumption are well-known risk factors for CIPN.[6,12] In addition, genetic variants of genes involved in the development of toxicity may be of interest as predictors of benefit and harm as well. Nowadays, there is growing evidence from preclinical and clinical studies that single nucleotide polymorphisms (SNPs) are associated with susceptibility to platinum-induced peripheral sensory neuropathy.[14] Particularly, genetic variants in organic transporter molecules, DNA repair enzyme genes or genes encoding for metabolic enzymes involved in platinum detoxification are of special interest.[12,15] For example, Cecchin *et al* described the association between neurotoxicity SNPs located in ATP-binding cassette subfamily C (*ABCC*) genes in colorectal cancer patients treated with platinum-based chemotherapy and CIPN.[15] The protein encoded by *ABCC* genes are called multidrug resistance proteins and involved in the transport of substances out of cells, like platinum efflux. Other examples of genes of interest are those coding for enzymes that play an important role in detoxification (glutathione S-transferases) or in nucleotide excision repair pathways (such as *ERCC1, ERCC2*) involved in DNA repair.[16] In addition, genes expressed in peripheral sensory neurons, involved in pain sensation, like transient receptor potential cation channel Subfamily V (*TRPV),* and genes that regulates neurotransmission,

such as calcium/calmodulin-dependent protein kinases (*CAMK*), might be of special interest as well.[16] However, previous studies investigating the contribution of genetic variants are hampered by small sample sizes and differences in clinical evaluations of neuropathy.[16] Moreover, most studies evaluating CIPN are performed in patients with colon carcinoma treated with oxaliplatin.[3,15,17-19] Little is known about genetic predisposition and association with CIPN in cisplatin- and carboplatin-based treatment in patients with non-small cell lung cancer (NSCLC).[20-22]

This study aims to further explore genetic risk factors for CIPN by investigating previously reported genetic associations in a large independent cohort of NSCLC patients treated with platinum-based chemotherapy.

Methods

Study design and patients

This study was performed as part of the PGxLUNG study, in which 350 patients were included. The study design of the PGxLUNG study has been published previously.[23] In brief, patients of the PGxLUNG study were recruited from one academic hospital (University Medical Center Utrecht), two teaching hospitals (St. Antonius Hospital Nieuwegein/Utrecht, Meander Medical Center Amersfoort) and three general hospitals (Diakonessenhuis Utrecht, Groene Hart Ziekenhuis Gouda, Ziekenhuis Rivierenland Tiel), all in the Netherlands, between February 2016 and August 2019. The inclusion criteria for this multicenter prospective follow-up study were as follows: (i) $\geq$ 18 years of age; (ii) radiologically confirmed stage II-IV NSCLC; (iii) planned or initiated first-line treatment with platinum-based (cisplatin or carboplatin) chemotherapy or chemoradiotherapy (according to the contemporary ESMO Clinical Practice Guidelines); and (iv) previously platinum-based chemotherapy-naïve. To avoid confounding by ancestry, patients of non-European ancestry were excluded from the present study. All data were extracted from the hospitals' electronic information systems and managed using REDCap electronic data capture tools.[24]

Ethical considerations

Study procedures were approved by the accredited Medical Research Ethics Committee in Nieuwegein (MEC-U, number R15.056) and implemented in accordance with the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013). The PGxLUNG study was registered on The Netherlands National Trial Register (NTR) on 26 April 2016 (NTR number NL5373610015). All patients provided written informed consent.

Neuropathy phenotype

During treatment with platinum-based therapy the contemporary ESMO Clinical Practice Guidelines for diagnosis, prevention, treatment and follow-up of CIPN were taken into account.[12] Neuropathy was assessed by lung oncologists using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 definition of "Peripheral sensory neuropathy" as categorical variable.[25] Clinical evaluation consisted of asking about typical symptoms of CIPN (such as numbness, prickling or tingling in hands and feet or loss of balance and coordination). When (severe) neuropathy was suspected, further neurological testing by a neurologist was performed at the discretion of the treating physician. Assessment of neuropathy was conducted at baseline and before each cycle (four cycles, every three weeks) of platinum-based chemotherapy and, at three and six months after treatment initiation. The highest CTCAE grade within a patient between treatment initiation and the last day of follow-up was recorded, whereby neuropathy ≥ grade 2 was defined as severe neuropathy. The follow-up period for the assessment of neuropathy was six months after initiation of platinum-based chemotherapy.

Candidate SNPs selection

A systematic search was performed in PubMed on 15 March 2022. The search terms included 'platinum-based chemotherapy', 'pharmacogenetics', 'neurotoxicity', and synonyms for each of these terms as described in Supplementary S1. Only full papers of clinical studies published in English were considered. References of the included studies were screened to identify additional studies. In addition, the online Pharmacogenomics Knowledge Base (PharmGKB) was used to identifying relevant peer-reviewed publications.[26] Genetic variants associated with CIPN caused by cisplatin or carboplatin were included when the clinical annotation levels of evidence were 'moderate' (level 2) or 'high' (level 1). In total, 73 papers were considered (see Supplementary S1). In these studies, CIPN has been graded with different instruments, such as CTCAE for peripheral sensory neuropathy, and self-reported neuropathy has been graded using the CIPN20 questionnaire scores and the scale for chemotherapy-induced long-term neurotoxicity (SCIN).[27] From these publications, a total of 42 SNPs were selected by using a candidate SNPs approach based on the predefined criteria (see Figure S1).

Genotyping and imputation

DNA samples were obtained from EDTA-blood samples using the EZ1 DNA Blood 200 µl kit (Qiagen, Hilden, Germany). DNA isolation was performed according to validated in-house protocols of the Pharmacogenetics, Pharmaceutical and Toxicological Laboratory

(FarmaToxLab) of the Department of Clinical Pharmacy (ISO15189 certified), St. Antonius Hospital Nieuwegein/Utrecht, the Netherlands. SNPs were genotyped by using Kompetitive allele specific PCR (KASP) at LGC Genomics (Hoddesdon, UK) and by using the Infinium Global Screening Array (GSA)-24 Kit (Illumina, San Diego, CA) at Life and Brain (Bonn, Germany). Sample quality control (QC) was performed for the genotyping by using the GSA-24 Kit with the following criteria: sample call rate > 98%, heterozygosity ± 3 SD from the sample's heterozygosity rate mean and pi-hat < 0.2 to eliminate cryptic relatedness. Genetic ethnicity was analyzed using the multidimensional scaling (MDS) approach based on Human Genome 1K data. Standard quality control was applied for pre- and post-genotype imputation data. The following criteria were used for SNPs QC: SNP call rate > 98%, MAF > 0.05 Hardy-Weinberg equilibrium (HWE) $p \geq 0.05$. Imputation using these QC-passed SNPs was conducted on the University of Michigan Imputation Server[28] using the Minimac4 1.2.1, 1000G Phase 3 v5 reference panel, GRCh37/hg19 array build and Eagle v2.4 phasing. Those SNPs with imputation quality (Rsq) > 0.8 and MAF > 0.05 were retained for association analysis. QC was performed using PLINK version 1.9.[29,30] Since for 8 SNPs (rs113807868, rs1799735, rs1263292, rs23885, rs366631, rs56360211, rs77637129, rs830884) pre- and/or post-imputation QC were not met, in total 34 SNPs in 26 genes were included in the current study (see Figure S1)[6,7,9,15,16,18,31-46]. Table S2 shows the details (such as rsID, gene, chromosome position and functional consequence) of the selected SNPs and their distribution in the study population. Minor allele frequencies (MAFs) for the investigated SNPs were in-line with those previously reported in Caucasian populations.[47]

Potential confounders / effect modifiers

The following parameters were considered to be potentially confounding and/or effect modifying variables for CIPN: age (≤ 70 years vs > 70 years), gender, Eastern Cooperative Oncology Group (ECOG) performance status[12,48] (ECOG PS 0 vs ≥ 1), diabetes mellitus, Charlson comorbidity index score (2-3 vs 4-5 vs ≥ 6)[12,49], concomitant chemotherapeutic agent (gemcitabine vs paclitaxel vs pemetrexed vs other), platinum-agent (cisplatin vs carboplatin), number of administered cycles of platinum-based chemotherapy, renal function (eGFR using CKD-EPI formula[50], < 60 ml/min/1.73 m^2 versus ≥ 60 ml/min/1.73 m^2), body mass index (BMI)[51] (< 18.5 kg/m^2 vs 18.5-< 25 kg/m^2 vs 25-< 30 kg/m^2 vs ≥ 30 kg/m^2) and tobacco use (current smoker vs former smoker vs non-smoker vs unknown).[12]

Data analysis

Standard summary statistics were used to describe the sample data set by using SPSS version 26.0 (IBM SPSS Statistics). The strength of the association between genetic variants

and CIPN was assessed in univariate and multivariate settings with logistic regression modelling and expressed as odds ratios (OR) with corresponding 95% confidence intervals (CI). Associations of the individual SNPs with the neuropathy phenotype were tested in both a dominant and recessive model. The Pearson chi-square test or Fisher's Exact test (in case the cell count in any of the tables was < 5) (for categorical independent variable) was used. The false discovery rate (FDR), set at 5%, was used for correction in multiple testing based on the Benjamini-Hochberg procedure. Covariates used in the multivariate analysis were selected based on statistical significance (*p*-value < 0.10) in univariate logistic regression analysis. In addition, based on earlier described clinical significance, the number of administered cycles of platinum-based therapy was added to the multivariate model. Adjusted OR (ORadj) were calculated and a *p*-value < 0.05 (2-sided), was considered statistically significant. For the SNP with the strongest evidence for association with CIPN, the number needed to genotype (NNG) was calculated (based on the formula described by Tonk *et al*)[52], to estimate the efficiency of genotyping to prevent one patient from having an adverse effect. In addition, the number needed to treat (NNT) was calculated to express the number of patients with the risk genotype who need an intervention to prevent one patient from having an adverse event.[52]

Results

Population characteristics

In total, 320 patients with previously untreated NSCLC, receiving platinum-based chemotherapy between April 2011 and July 2019, of the PGxLUNG study cohort (*n* = 350)[23] were included in the current analyses (30 patients excluded: 17 patients were not of European ancestry, 11 patients did not meet pre- or post-imputation QC, 2 patients died before first clinical evaluation of neuropathy at week 3). Demographic and clinical characteristics stratified by (severe) CIPN status are shown in Table 1. Median age was 65 years and 10% had diabetes. Patients received a median of three cycles (IQR 3-4) of platinum-based chemotherapy.

Incidence of CIPN

At treatment initiation, none of the patients were suffering from pre-existing peripheral neuropathy. In total, 26.3% (*n* = 84) patients were affected by some degree of peripheral neuropathy as assessed by the CTCAE criteria during the six months follow-up after treatment initiation. For 18.1% (*n* = 58) of the patients, grade 1 toxicity was the highest CTCAE grade recorded during follow-up. Severe neuropathy was found in 8.1% (*n* = 26) patients, with grade 2 or grade 3 toxicity presented in 7.5% (*n* = 24) and 0.6% (*n* = 2) patients,

respectively. Table S3 shows the distribution of the outcome at the different follow-up moments. The highest number of patients (*n* = 36) with any grade neuropathy was assessed after administration of two cycles of platinum-based therapy. The highest number of cases (*n* = 12) of severe neuropathy was assessed three months after treatment initiation.

Association analysis: clinical characteristics and neuropathy

Univariate analysis showed a statistically significant association between ECOG PS at treatment initiation and neurotoxicity (ECOG≥ 1, OR 0.5, 95% CI 0.3-0.9), as shown in Table 1. Patients treated with carboplatin/paclitaxel were at higher risk for developing both any grade (OR 8.9, 95% CI 3.3-23.7) as well as severe (OR 7.6, 95% CI 2.7-21.2) neuropathy.

Table 1. Demographic, clinical characteristics and treatment characteristics: univariate analysis of (severe) neuropathy

Characteristics	Total	Neuropathy[a] (≥ grade 1)			Neuropathy[a] (≥ grade 2)		
		No	Yes	Crude OR (95% CI)	No	Yes	Crude OR (95% CI)
Patients, *n* (%)	320 (100)	236 (73.7)	84 (26.3)	-	294 (91.9)	26 (8.1)	-
Gender, *n* (%)							
Male	179 (55.9)	136 (57.6)	43 (51.2)	Ref.	166 (56.5)	13 (50.0)	Ref.
Female	141 (44.1)	100 (42.4)	41 (48.8)	1.3 (0.8-2.1)	128 (43.5)	13 (50.0)	1.3 (0.6-2.9)
Age at treatment initiation							
Years, mean ± SD	65.1 ± 9.3	65.0 ± 9.5	65.3 ± 8.6	1.0 (1.0-1.0)	65.1 ± 9.3	64.9 ± 8.5	1.0 (1.0-1.0)
≤ 70 years, *n* (%)	213 (66.6)	153 (64.8)	60 (71.4)	Ref.	193 (65.6)	20 (76.9)	Ref.
> 70 years, *n* (%)	107 (33.4)	83 (35.2)	24 (28.6)	0.7 (0.4-1.3)	101 (34.4)	6 (23.1)	0.6 (0.2-1.5)
ECOG PS at treatment initiation, *n* (%)							
0	127 (39.7)	85 (36.0)	42 (50.0)	Ref.	114 (38.8)	13 (50.0)	Ref.
≥ 1	143 (44.7)	114 (48.3)	29 (34.5)	0.5 (0.3-0.9)*	132 (44.9)	11 (42.3)	0.7 (0.3-1.7)
Unknown	50 (15.6)	37 (15.7)	13 (15.5)	0.7 (0.3-1.5)	48 (16.3)	2 (7.7)	0.4 (0.1-1.7)
Diabetes mellitus, *n* (%)							
No	288 (90.0)	213 (90.3)	75 (89.3)	Ref.	265 (90.1)	23 (88.5)	Ref.
Yes	32 (10.0)	23 (9.7)	9 (10.7)	1.1 (0.5-2.5)	29 (9.9)	3 (11.5)	1.2 (0.3-4.2)

Characteristics	Total	Neuropathy[a] (≥ grade 1)			Neuropathy[a] (≥ grade 2)		
		No	Yes	Crude OR (95% CI)	No	Yes	Crude OR (95% CI)
Charlson comorbidity index[b], *n* (%)							
2-3	108 (33.8)	77 (32.6)	31 (36.9)	Ref.	98 (33.3)	10 (38.5)	Ref.
4-5	105 (32.8)	82 (34.8)	23 (27.4)	0.7 (0.4-1.3)	98 (33.3)	7 (26.9)	0.7 (0.3-1.9)
≥ 6	107 (33.4)	77 (32.6)	30 (35.7)	1.0 (0.5-1.8)	98 (33.3)	9 (34.6)	0.9 (0.4-2.3)
Chemotherapeutic agents, first cycle, *n* (%)							
Pemetrexed	198 (61.8)	150 (63.6)	48 (57.2)	Ref.	185 (62.9)	13 (50.0)	Ref.
Gemcitabine	84 (26.3)	68 (28.8)	16 (19.0)	0.7 (0.4-1.4)	79 (26.9)	5 (19.2)	0.9 (0.3-2.6)
Paclitaxel	23 (7.2)	6 (2.5)	17 (20.2)	8.9 (3.3-23.7)*	15 (5.1)	8 (30.8)	7.6 (2.7-21.2)*
Other/unknown	15 (4.7)	12 (5.1)	3 (3.6)	0.8 (0.2-2.9)	15 (5.1)	0 (0)	0.9 (0.2-3.5)
Platinum-based chemotherapy, *n* (%)							
Carboplatin-based	171 (53.4)	131 (55.5)	40 (47.6)	Ref.	159 (54.1)	12 (46.2)	Ref.
Cisplatin-based	104 (32.5)	74 (31.4)	30 (35.7)	1.3 (0.8-2.3)	94 (32.0)	10 (38.5)	1.4 (0.6-3.4)
Switch cis>carbo during treatment	45 (14.1)	31 (13.1)	14 (16.7)	1.5 (0.7-3.1)	41 (13.9)	4 (15.4)	1.3 (0.4-4.2)
Cycles of platinum-based therapy, *n* (%)							
1	11 (3.4)	8 (3.4)	3 (3.6)	Ref.	10 (3.4)	1 (3.8)	Ref.
2	35 (10.9)	31 (13.1)	4 (4.8)	0.3 (0.1-1.9)	34 (11.5)	1 (3.8)	0.3 (0.0-5.1)
3	116 (36.6)	95 (40.3)	21 (25.0)	0.6 (0.1-2.4)	109 (37.1)	7 (26.9)	0.6 (0.1-5.8)
4	158 (49.4)	102 (43.2)	56 (66.7)	1.5 (0.4-5.7)	141 (48.0)	17 (65.5)	1.2 (0.2-10.0)
Renal function, baseline eGFR (CKD-EPI)							
eGFR (mL/min/1.73 m^2), mean ± SD	83 ± 13	83 ± 14	83 ± 12	1.0 (1.0-1.0)	83 ± 13	80 ± 13	1.0 (1.0-1.0)
≥ 60 ml/min/1.73 m^2, *n* (%)	294 (91.9)	214 (90.7)	80 (95.2)	Ref.	270 (91.8)	24 (92.3)	Ref.
< 60 ml/min/1.73 m^2, *n* (%)	26 (8.1)	22 (9.3)	4 (4.8)	0.5 (0.2-1.5)	24 (8.2)	2 (7.7)	0.9 (0.2-4.2)

Characteristics	Total	Neuropathy[a] (≥ grade 1)			Neuropathy[a] (≥ grade 2)		
		No	Yes	Crude OR (95% CI)	No	Yes	Crude OR (95% CI)
BMI (kg/m^2), *n* (%)							
18.5 - < 25 (normal weight)	131 (40.9)	101 (42.8)	30 (35.7)	Ref.	122 (41.5)	9 (34.6)	Ref.
< 18.5 (underweight)	12 (3.8)	9 (3.8)	3 (3.6)	1.1 (0.3-4.4)	11 (3.7)	1 (3.8)	1.2 (0.1-10.7)
25 - < 30 (overweight)	126 (39.4)	87 (36.9)	39 (46.4)	1.5 (0.9-2.6)	113 (38.4)	13 (50.0)	1.6 (0.6-3.8)
≥ 30 (obese)	51 (15.9)	39 (16.5)	12 (14.3)	1.0 (0.5-2.2)	48 (16.3)	3 (11.5)	0.9 (0.2-3.3)
Smoking status							
Current smoker	76 (23.8)	56 (23.7)	20 (23.7)	Ref.	71 (24.1)	5 (19.2)	Ref.
Former smoker	215 (67.1)	159 (67.4)	56 (66.7)	1.0 (0.3-3.6)	196 (66.7)	19 (73.1)	1.0 (0.1-9.4)
Non-smoker	15 (4.7)	11 (4.7)	4 (4.8)	1.0 (0.5-1.8)	14 (4.8)	1 (3.8)	1.4 (0.5-3.8)
Unknown	14 (4.4)	10 (4.2)	4 (4.8)	1.1 (0.3-4.0)	13 (4.4)	1 (3.8)	1.1 (0.1-10.1)

Abbreviations: BMI, body mass index; CI, confidence interval; CTCAE, Common Terminology Criteria for Adverse Events; ECOG PS, Eastern Cooperative Oncology Group Performance Status; eGFR, estimated glomerular filtration rate; OR, odds ratio; SD, standard deviation.

[a] CTCAE version 4.03 grade for peripheral sensory neuropathy between chemotherapy initiation and the last day of follow-up (six months). Clinical evaluation of neuropathy was conducted at baseline and before each cycle of platinum-based chemotherapy and, at three and six months after treatment initiation.

[b] Charlson comorbidity index score provides a simple means to quantify the effect of comorbid illnesses, including cardiovascular diseases, chronic obstructive pulmonary disease, liver disease and diabetes mellitus among others and, accounts for the aggregate effect of multiple concurrent diseases. A higher score indicates more comorbidities.

* *p*-value < 0.05 based on independent t-test (for continuous independent variable) and Pearson chi-square test or Fisher's Exact test (in case the cell count in any of the tables was < 5) (for categorical independent variable).

Association analysis: genetic variants and neuropathy

To validate previously reported associations between SNPs with some aspect of CIPN, 34 selected SNPs in 26 genes were examined with the association of CTCAE for peripheral sensory neuropathy. Univariate analysis of the individual SNPs showed unadjusted statically significant associations between six SNPs and neuropathy (see Table 2 and Table S4). After multiple testing correction, the GG-genotype (rs879207, A>G) of *TRPV1*, a gene expressed in peripheral sensory neurons observed in 11.3% of the patients, was found to be associated with an increased risk of severe neuropathy (OR 5.2, 95% CI 2.1-

12.8, FDR adjusted *p*-value 0.012). A quarter (25%, *n* = 9/36) of the patients with the GG-genotype developed severe neuropathy compared to 6% (*n* = 17/282) of patients with the AG- or AA- genotype. Within the patients with the GG-genotype, patients treated with paclitaxel (*n* = 5) experienced severe neuropathy in 80% of cases (see Table S5).

Multivariate analysis: clinical and genetic characteristics and neuropathy

Multivariate analysis of genetic and clinical characteristics was performed as shown in Table 3**,** taking into account the GG-genotype (rs879207, A>G) of *TRPV1,* the number of administered cycles of platinum-based chemotherapy, ECOG PS and concomitant chemotherapeutic agents*.* Statistically significant association between the GG-genotype of rs879207 (ORadj 4.7, 95% CI 1.8-12.3) and between concomitant use of paclitaxel (ORadj 7.2, 95% CI 2.5-21.1) and severe CIPN were observed.

Population impact measures

The NNG and NNT for rs879207, with the GG-genotype defined as the risk genotype, were 62.2 and 7.0 for any grade neuropathy and 47.1 and 5.3 for severe neuropathy respectively. Within patients treated with carboplatin/paclitaxel (*n* = 23), the NNG and NNT were 13.8 and 3.0 for any grade neuropathy and 8.0 and 1.7 for severe neuropathy respectively.

Table 2. Associations of SNPs with neuropathy

Gene	rsID	Relevance to platinum agents / neurotoxicity	Model	Neuropathy/patient, %	Neuropathy endpoint	Crude OR (95% CI)	Adjusted *p*-value
ABCA1	rs2230806	Drug transporters (membrane efflux proteins)	Recessive	HT + WT (82/291), 28.2% HM (2/28), 7.1%	Any grade	Ref. 0.2 (0.1-0.9)	0.51
	rs1885301		Recessive	HT + WT (77/265), 29.1% HM (7/55), 12.7%	Any grade	Ref. 0.4 (0.2-0.8)	0.53
ABCC2	rs3740066		Dominant	HT + HM (39/186), 21.0% WT (45/134), 33.6%	Any grade	Ref. 0.5 (0.3-0.9)	0.42
	rs4148396		Recessive	HT + WT (74/261), 28.4% HM (8/55), 14.5%	Any grade	Ref. 0.4 (0.2-0.9)	0.44
CAMK2N1	rs12023000	Protein kinase in neurons, regulates neurotransmission	Recessive	HT + WT (24/317), 7.6% HM (2/3), 66.7%	Severe	Ref. 24.4 (2.1-279.1)	0.18
TRPV1	rs879207	Expressed in peripheral sensory neurons involved in pain sensation	Recessive	HT + WT (17/282), 6.0% HM (9/36), 25.0%	Severe	Ref. 5.2 (2.1-12.8)	0.012*

Abbreviations: *ABCA1* or *C2*, ATP-binding cassette subfamily A, member 1 or subfamily C, member 2; *CAMK2N1*, calcium/calmodulin-dependent protein kinase II inhibitor 1; HM, homozygous minor allele; HT, heterozygous minor allele; OR, odds ratio; *TRPV1*, transient receptor potential cation channel subfamily V member 1; WT, wild type homozygous major allele.

* False discovery rate adjusted *p*-value < 0.05

Table 3. Multivariate analysis of (severe) neuropathy

Characteristics	*n* (%)	Neuropathy any grade (≥ grade 1)		Severe neuropathy (≥ grade 2)	
		Univariate analysis[a] crude OR (95% CI)	Multivariate analysis[b] adjusted OR[c] (95% CI)	Univariate analysis[a] crude OR (95% CI)	Multivariate analysis[b] adjusted OR[c] (95% CI)
Total	320 (100)	-	-	-	-
TRPV1 (rs879207)					
HT+WT (*AG+AA*)	282 (88.1)	Ref.	Ref.	Ref.	Ref.
HM (*GG*)	36 (11.3)	2.0 (1.0-4.1)	1.9 (0.8-4.2)	5.2 (2.1-12.8)*	4.7 (1.8-12.3)*
ECOG PS at start chemotherapy					
0	127 (39.7)	Ref.	Ref.	Ref.	Ref.
≥ 1	143 (44.7)	0.5 (0.3-0.9)*	0.5 (0.3-0.9)*	0.7 (0.3-1.7)	0.8 (0.3-2.0)
Unknown	50 (15.6)	0.7 (0.3-1.5)	0.7 (0.3-1.5)	0.4 (0.1-1.7)	0.5 (0.1-2.3)
Cycles of platinum-based therapy					
1	11 (3.4)	Ref.	Ref.	Ref.	Ref.
2	35 (10.9)	0.3 (0.1-1.9)	0.7 (0.1-4.6)	0.3 (0.0-5.1)	0.8 (0.0-19.7)
3	116 (36.6)	0.6 (0.1-2.4)	1.1 (0.2-5.6)	0.6 (0.1-5.8)	1.6 (0.1-21.0)
4	158 (49.4)	1.5 (0.4-5.7)	2.0 (0.4-10.5)	1.2 (0.2-10.0)	2.1 (0.2-25.1)
Chemotherapeutic agents, first cycle					
Pemetrexed	198 (61.8)	Ref.	Ref.	Ref.	Ref.
Gemcitabine	84 (26.3)	0.7 (0.4-1.4)	0.8 (0.4-1.7)	0.9 (0.3-2.6)	1.0 (0.4-3.0)
Paclitaxel	23 (7.2)	8.9 (3.3-23.7)*	7.5 (2.6-21.4)*	7.6 (2.7-21.2)*	7.2 (2.5-21.1)*
Other/unknown	15 (4.7)	0.8 (0.2-2.9)	0.9 (0.3-3.6)	0.9 (0.2-3.5)	-

Abbreviations: CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status; HM, homozygous minor allele; HT, heterozygous minor allele; OR, odds ratio; *TRPV1*, transient receptor potential cation channel subfamily V member 1; WT, wild type (homozygote major allele).

[a] Univariate logistic regression analysis.

[b] Multivariate logistic regression analysis (Backward: wald).

[c] Adjusted odds ratio: adjusted for concomitant chemotherapeutic agent, number of administered cycles of platinum-based therapy, ECOG PS and *TRPV1* genotype in multivariate logistic regression analysis.

* p-value < 0.05

Discussion

Main findings

The present study demonstrates that NSCLC patients with the GG-genotype (rs879207) of *TRPV1* are at a nearly 5-fold higher risk of developing severe neuropathy when treated with platinum-based therapy. Although significant associations were found between SNPs in *ABCA1* (rs2230806), *ABCC2 (*rs1885301, rs3740066, rs4148396) and *CAMK2N1* (rs12023000) and CIPN in univariate analyses, none of these SNPs were associated with neuropathy in multivariate setting. TRPV1 receptors are predominantly found in the nociceptive neurons of the peripheral nervous system and are involved in the transmission and modulation of pain.[53] Previously, the association between genetic predisposition of *TRPV1* and CIPN was described in a cohort of ovarian cancer patients treated with carboplatin combined with paclitaxel or docetaxel.[9] In this case-control study, patients with the AG-genotype of *TRPV1* (rs879207) had a 1.6-fold higher risk to develop CIPN CTCAE grade ≥ 2 as compared to non-carriers of the G-allele, while statistical significance was not reached for the comparison between patients with the AA- versus the GG-genotype. Notably, the treatment protocols and study population differed between the studies, which may have affected the risk to develop peripheral neuropathy. Although a relatively low number of the patients received paclitaxel (n = 23), the results of our study pointed out that the neurotoxicity was most frequent in those receiving the combination of carboplatin and paclitaxel.

Furthermore, patients with lower ECOG PS had a higher risk for developing neuropathy. This might be explained by the fact that clinicians tend to prescribe less intensive treatment regimens to patients with an impaired condition.

Strengths and limitations

As a major strength of the current study, CIPN was investigated in a large independent cohort with a complete and detailed database of prospectively collected data. As a result, the quantification of the associations between CIPN, genetic variants and clinical and treatment characteristics was possible. The present study has some limitations. First, we analyzed populations of European descent only. However, the GG-genotype (rs879207) of *TRPV1* is common, not only in the European population (MAF = 0.32) but also in the global population (MAF = 0.31). [54] For that reason, it is plausible that the results of the current study can be extrapolated to other populations and are most likely highly relevant for a large number of patients. Second, although the widely-used and internationally validated CTCAE grading tool for CIPN was used, there are some concerns regarding this approach,

such as the occurrence of inter-observer bias.[55] However, no substantial differences in the incidence of CIPN between patients recruited in the six different hospitals was found. Nevertheless, in general, clinicians tend to underestimate the incidence or severity of neuropathy. This may be partly caused by the fact that early symptoms are often very subtle and can easily be unnoticed if not specifically asked for.[12,27,56,57] Consequently, due to possible underreporting or underestimating of neurotoxicity by clinicians, the actual association between the GG-genotype (rs879207) of *TRPV1* and CIPN might be even stronger than has been demonstrated in our study.[58,59]

Potential clinical relevance

Since recovery of CIPN is, in general, merely partial with residual symptoms in most patients, the quality of life can be reduced considerably.[5] The only proven effective measure for CIPN consists of lowering treatment intensity; therefore, the occurrence of severe neuropathy will frequently result in clinical interventions such as a dose reduction of up to 75% or early discontinuation of treatment. However, lowering treatment intensity might compromise its efficacy. Based on the results of our cohort, out of every nine patients who are genotyped, one will carry the GG-genotype of rs879207. Since our data demonstrated that carrying two copies of the minor G allele contributes to susceptibility for neuropathy, these patients are likely to benefit from further individualization of therapy. Thus, further individualization of therapy may be beneficial for at least 10% of the patients of European ancestry treated with platinum-based therapy; screening patients for the *TRPV1* (rs879207) GG-genotype could have a relevant impact on clinical practice. In addition, with a NNG of 8, we demonstrated that interventions such as dose adjustments might be considered for 12.5% of patients treated with carboplatin/paclitaxel in order to prevent severe neuropathy. Since for advanced NSCLC patients treated with cisplatin- or carboplatin-based therapy equivalent overall survival and response rates are reported[60], the choice of the platinum-agent should be based on expected side effects as well as the patient's comorbidities and preferences.

Future research

In accordance with McWhinney-Glass *et al*[9], we demonstrated an association between the *TRPV1* (rs879207) GG-genotype and CIPN. Therefore, it would be of great importance to investigate this newly discovered association in an independent cohort of patients with different malignancies treated with cisplatin- or carboplatin-based therapies. In addition, further stratification according to the concomitant chemotherapeutic agent would be

informative. While functional understanding of *TRPV1* is desired, the validation of our results could pave the way for a clinical intervention study. To accurately determine whether patients with the GG-genotype of (rs879207) will benefit more from an individualized regimen, a randomized controlled trial should, preferably, be performed. In this trial, the choice of the platinum-agent should take into account the *TRPV1* (rs879207) genotype with both treatment effectiveness and (neuro)toxicity as a primary endpoint.

Conclusions

This study shows that patients with the GG-genotype (rs879207) of *TRPV1* have an almost 5-fold higher risk severe neuropathy when treated with platinum-based therapy. Future studies should aim to validate these likely clinically significant findings in an independent cohort. In addition, the implementation of these results in clinical practice should be investigated in clinical intervention studies with a special focus on further individualization of platinum-based therapy to prevent the occurrence of neuropathy.

Declarations

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Authors' contributions

Conceptualization, CJ, GH, TE and VD; methodology, CJ, GH, TE and VD; formal analysis, CJ; supervision, GH, TE and VD; writing original draft, CJ; writing, review and editing, CJ, GH, SH, FM, AL, AH, JB, TE and VD. The final version of the manuscript was seen and approved by all authors.

Competing interests

The authors declare that they have no competing interests.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

The protocol of the PGxLUNG study complied with the Good Clinical Practice guidelines and the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013), and was approved by the accredited Medical Research Ethics Committee in Nieuwegein (MEC-U, number R15.056).

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Informed consent

All patients provided written informed consent.

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Supplementary materials

Supplement S1. Search strategy and candidate SNPs selection;

Figure S1. Flowchart of candidate SNPs selection;

Table S1. Details and distribution of the candidate SNPs (*n* = 34);

Table S2. Distribution of outcomes;

Table S3. Univariate analysis of SNPs and (severe) peripheral sensory neuropathy;

Table S4. Univariate and multivariate analysis of *TRPV1* genotype and concomitant therapy with paclitaxel and (severe) neuropathy.

S1. Search strategy and candidate SNPs selection

S1.1. Search strategy

A systematic search was performed on 15 March 2022. The search terms in PubMed included 'platinum-based chemotherapy' (exposure), 'pharmacogenetics' (determinant), 'neurotoxicity' (outcome), and synonyms for each of these terms. Query: ("Cisplatin"[Mesh] OR "Cisplatin"[tiab] OR "Carboplatin"[Mesh] OR "Carboplatin"[tiab] OR "CDDP"[tiab] OR ("platinum"[tiab] AND "chemotherap*"[tiab])) AND ("Polymorphism, Genetic"[Mesh] OR (("gene"[tiab] OR "genes"[tiab] OR "genetic*"[tiab]) AND "polymorphism*"[tiab]) OR "pharmacogenomic*"[tiab] OR "SNP"[tiab] OR "SNPs"[tiab] OR "Precision Medicine"[Mesh] OR "Precision Medicine"[tiab] OR "personalized medicine"[tiab]) AND ("neurotoxic*"[tiab] OR "neuropath*"[tiab]). In total 56 publications were identified. The online Pharmacogenomics Knowledge Base (PharmGKB) was used to identifying relevant peer-reviewed publications.[26] Genetic variants associated with CIPN caused by cisplatin or carboplatin were included when the clinical annotation levels of evidence was at least 'moderate' (level 2B). No additional SNPs were added as a result of the PharmGKB search.

S1.2. Screening of publications

All publications were screened for eligibility, inclusion criteria were as follows: 1) publication in English language, 2) full-text available, 3) clinical data, 4) endpoint chemotherapy-induced peripheral neuropathy (CIPN), related to platinum-based chemotherapy. In addition, the references of the included studies were screened to identify additional studies. As shown in Figure S1, a total of 73 publications (56 from PubMed search, 17 from references screening) were considered.

S1.3. Candidate SNPs selection

In the current candidate SNPs selection only single nucleotide polymorphisms (SNPs) were included (no other genetic variants such as insertions, gene deletions or variations in copy numbers were selected). Inclusion criteria for SNPs were as follows: 1) SNP was statistical significantly associated with some aspect of CIPN related to platinum-based chemotherapy, 2) rsID of the SNP was published. A total of 42 SNPs associated with susceptibility to CIPN were selected through this candidate SNPs approach.

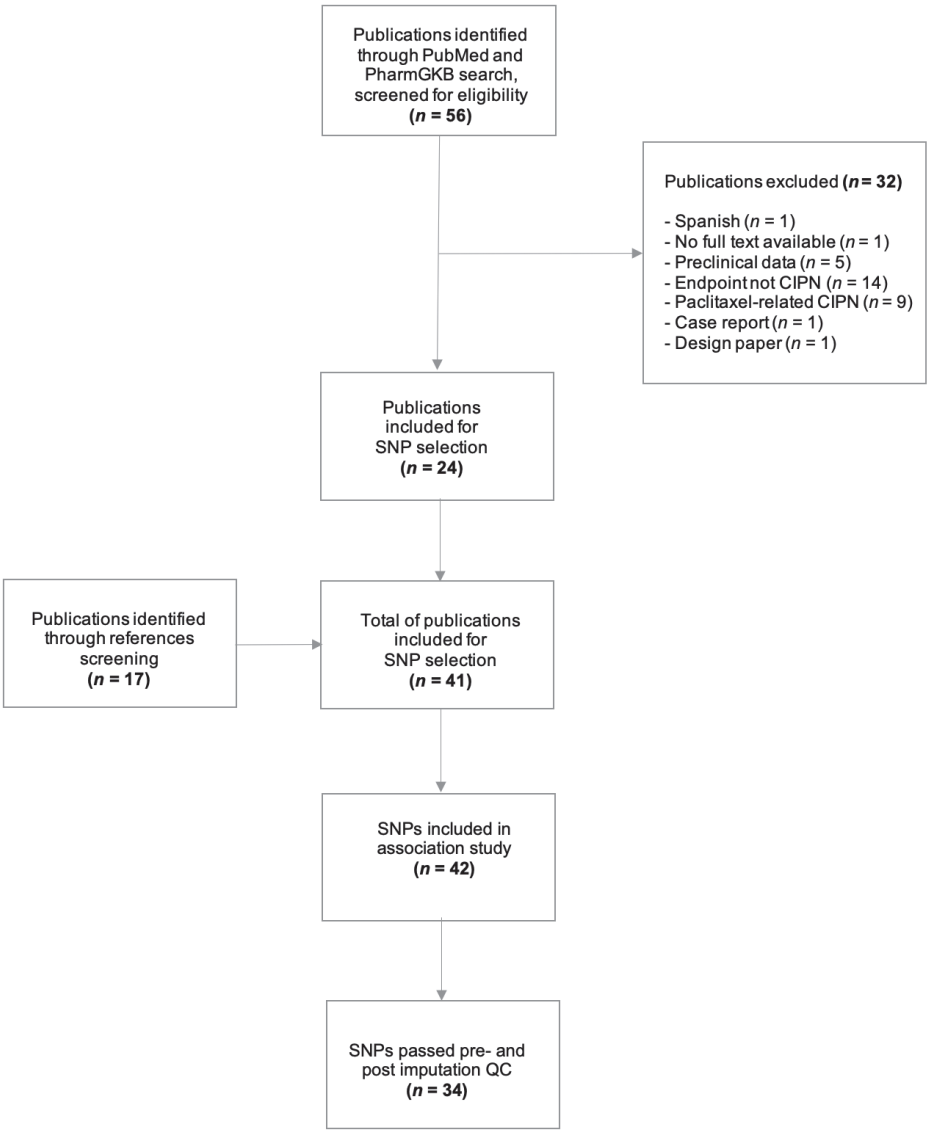

Figure S1. Flowchart of candidate SNPs selection.

Table S1. Details and distribution of the candidate SNPs (*n* = 34)

Reference (year)	Relevance to platinum agents / neurotoxicity	Gene	SNP (rsID)	Chromosome position (GRCh37)	WT (%)	HT (%)	HM (%)	MAF	Missing (%)
Hasmats (2012)	Drug transporters (membrane efflux proteins)	*ABCA1*	rs2230806	9:107620867	51.2	39.7	8.8	0.29	0.3
Ferracini (2021)		*ABCB1*	rs1045642	7:87138645	26.9	49.7	22.5	0.48	0.9
Cecchin (2013)		*ABCC1*	rs2074087	16:16184232	2.2	24.4	73.4	0.14	0
Cecchin (2013)		*ABCC2*	rs1885301	10:101541053	17.2	45.6	37.2	0.40	0
			rs3740066	10:101604207	41.9	45.3	12.8	0.35	0
			rs4148396	10:101591944	37.5	44.1	17.2	0.40	1.3
			rs717620	10:101542578	64.3	31.6	4.1	0.20	0
Johnson (2015)		*ABCC4*	rs1729786	13:95823239	11.6	18.4	1.9	0.35	68.1
Custodio (2014), Lamba (2014)		*ABCG2*	rs13120400	4:89033527	6.9	37.2	52.5	0.26	3.4
			rs3114018	4:89064581	17.2	52.5	29.4	0.44	0.9
Won (2012)	Drug metabolism and bioactivation	*ACYP2*	rs843748	2:54502912	24.7	49.7	25.0	0.50	0.6
Gamelin (2007)	Detoxification enzyme	*AGXT*	rs34116584	2:241808314	63.4	32.2	4.4	0.21	0
			rs4426527	2:241817516	62.5	33.1	4.4	0.21	0
McWhinney-Glass (2013)	Apoptosis-related protein in nerve tissues	*BCL2*	rs2849380	18:60979360	67.2	30.3	2.5	0.18	0
Won (2012)		*BTG4*	rs4936453	11:111300782	10.3	45.0	44.7	0.50	0
Avan (2015)	Protein kinase in neurons, regulates neurotransmission	*CAMK2N1*	rs12023000	1:20789645	84.7	14.4	0.9	0.08	0
Custodio (2014)	Cell cycle progression	*CCNH*	rs2230641	5:86695274	63.4	32.5	4.1	0.20	0
Inada (2010), Kim (2009)	DNA repair mechanisms	*ERCC1*	rs11615	19:45923653	41.2	47.2	11.6	0.35	0
			rs3212986	19:45912736	4.4	32.2	60.9	0.21	2.5
Lamba (2014)		*ERCC2*	rs13181	19:45854919	10.3	51.9	37.8	0.36	0
Won (2012)		*FARS2*	rs17140129	6:5298362	70.3	25.3	4.4	0.17	0
			rs6924717	6:5304851	68.0	26.3	4.1	0.18	1.6

Reference (year)	Relevance to platinum agents / neurotoxicity	Gene	SNP (rsID)	Chromosome position (GRCh37)	WT (%)	HT (%)	HM (%)	MAF	Missing (%)
Hong (2011), Chen (2010), Inada (2010), McLeod (2010), Lecomte (2006), Kumamoto (2013), Li (2010), Oldenburg (2007), Goekkurt (2009), Katayanagi (2019), Lecomte (2006), Liu (2013)	Detoxification enzyme	*GSTP1*	rs1695	11:67352689	38.1	47.5	14.1	0.38	0.3
Johnson (2015), Thomaier (2021)		*GPX7*	rs3753753	1:53069514	9.7	35.9	36.9	0.34	17.5
Antonacopoulou (2010)	Cell adhesion and cell surface-mediated signaling	*ITGB3*	rs5918	17:45360730	2.5	27.5	70.0	0.16	0
Won (2012)		*LOC105374610*	rs797519	13:51231132	2.2	55.6	2.2	0.50	40.0
Won (2012)		*LOC107986555*	rs2338	6:1573613	6.6	37.5	55.3	0.26	0.6
McWhinney-Glass (2013)		*OPRM1*	rs544093	6:154457493	1.6	17.2	81.3	0.10	0
Argyriou (2013)	Voltage-gated sodium channels	*SCN4A*	rs2302237	17:62048707	37.5	42.2	10.3	0.35	10.0
McWhinney-Glass (2013), Thomaier (2021)		*SOX10*	rs139887	22:38371396	38.1	42.2	19.7	0.41	0
Won (2012)		*TAC1*	rs10486003	7:97229778	81.0	18.1	0.9	0.10	0
McWhinney-Glass (2013)	Expressed in peripheral sensory neurons involved in pain sensation	*TRPV1*	rs879207	17:3466596	47.5	40.6	11.3	0.32	0.6
Lamba (2014)		*XPC*	rs2228001	3:14187449	36.9	47.5	15.0	0.39	0.6
Lamba (2014)		*	rs1347851	12:90566978	2.5	24.1	40.0	0.22	33.4

Abbreviations: HM, homozygous variant (homozygote minor allele); HT, heterozygous variant; NA, not available; MAF, minor allele frequency; WT, wild type (homozygote major allele). * SNPs not in a gene, selected from genome-wide association study.

ABCA1 or *B1/C1/C2/C4/G2*, ATP-binding cassette subfamily A, member 1 or B1/C1/C2/C4/G2; *ACYP2*, acylphosphatase 2, muscle type; *AGXT*; alanine-glyoxylate and serine-pyruvate aminotransferase; *BACH2*, BTB domain and CNC homolog 2; *BCL2*, B-cell lymphoma 2; *BTG4*, B-cell translocation gene 4; *CAMK2N1*, calcium/calmodulin-dependent protein kinase II inhibitor 1; *CCNH*, cyclin H; *ERCC1* or *2*,Excision repair cross-complementing; *FARS2*, phenylalanyl-tRNA synthetase 2; *GSTP1*, glutathione S-transferase pi 1; *GPX7*, glutathione peroxidase 7; *ITGB3*, integrin subunit beta 3; *OPRM1*, opioid receptor mu 1; *SCN4A*, sodium voltage-gated channel alpha subunit 4; *SOX10*, SRY-box transcription factor 10; *TAC1*, tachykinin precursor 1; *TRPV1*, transient receptor potential cation channel subfamily V member 1; *XPC*, xeroderma pigmentosum complementation group C.

Table S2. Distribution of outcomes

Neurotoxicity[a]	Follow-up 3 weeks *n* (%)	Follow-up 6 weeks *n* (%)	Follow-up 9 weeks *n* (%)	Follow-up 3 months *n* (%)	Follow-up 6 months *n* (%)	Highest grade during follow-up *n* (%)
Alive	320 (100)	317 (99.1)	314 (98.1)	306 (95.6)	281 (87.8)	-
Grade 0 (no neuropathy)[a]	298 (93.1)	275 (85.9)	264 (82.5)	246 (76.9)	232 (72.5)	236 (73.7)
Grade 1	21 (6.6)	27 (8.4)	23 (7.2)	19 (5.9)	14 (4,4)	58 (18.1)
Grade 2	1 (0.3)	8 (2.5)	5 (1.6)	10 (3.1)	8 (2.5)	24 (7.5)
Grade 3	0 (0)	1 (0.3)	1 (0.3)	2 (0.6)	1 (0.3)	2 (0.6)
Any grade (grade ≥ 1 AKI-CTCAE)	22 (6.9)	36 (11.3)	29 (9.1)	31 (9.7)	23 (7.2)	84 (26.3)
Severe (grade ≥ 2)	1 (0.3)	9 (2.8)	6 (1.9)	12 (3.8)	9 (2.8)	26 (8.1)
Missing	0 (0)	6 (1.9)	21 (6.6)	29 (9.1)	26 (8.1)	-

[a] Neuropathy was assessed by lung oncologists using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 definition of "Peripheral sensory neuropathy".

Table S3. Univariate analysis of SNPs and (severe) peripheral sensory neuropathy

rsID	Model	Variants	Incidence any grade (≥ grade 1) neuropathy	Univariate logistic regression analysis any grade (CTCAE ≥ 1) neuropathy crude OR (95% CI)	Incidence severe (≥ grade 2) neuropathy	Univariate logistic regression analysis severe (CTCAE ≥ 2) neuropathy crude OR (95% CI)
Total	-	-	26.3 (84/320)	-	8.1 (26/320)	-
rs1045642	Dominant	WT	25.6 (22/86)	Ref.	5.8 (5/86)	Ref.
		HM + HT	26.8 (62/231)	1.1 (0.6-1.9)	9.1 (21/231)	1.6 (0.6-4.4)
	Recessive	HT + WT	25.3 (62/245)	Ref.	7.8 (19/245)	Ref.
		HM	30.6 (22/72)	1.3 (0.7-2.3)	9.7 (7/72)	1.3 (0.5-3.2)
rs10486003	Dominant	WT	25.9 (67/259)	Ref.	7.7 (20/259)	Ref.
		HM + HT	27.9 (17/61)	1.1 (0.6-2.0)	9.8 (6/61)	1.3 (0.5-3.4)
	Recessive	HT + WT	26.2 (83/317)	Ref.	8.2 (26/317)	Ref.
		HM	33.3 (1/3)	1.4 (0.1-15.8)	0 (0/3)	-
rs11615	Dominant	WT	25.0 (33/132)	Ref.	8.3 (11/132)	Ref.
		HM + HT	27.1 (51/188)	1.1 (0.7-1.9)	8.0 (15/188)	1.0 (0.4-2.2)
	Recessive	HT + WT	26.5 (75/283)	Ref.	8.1 (23/283)	Ref.
		HM	24.3 (9/37)	0.9 (0.4-2.0)	8.1 (3/37)	1.0 (0.3-3.5)

rsID	Model	Variants	Incidence any grade (≥ grade 1) neuropathy	Univariate logistic regression analysis any grade (CTCAE ≥ 1) neuropathy crude OR (95% CI)	Incidence severe (≥ grade 2) neuropathy	Univariate logistic regression analysis severe (CTCAE ≥ 2) neuropathy crude OR (95% CI)
rs12023000	Dominant	WT	26.2 (71/271)	Ref.	7.4 (20/271)	Ref.
		HM + HT	26.5 (13/49)	1.0 (0.5-2.0)	12.2 (6/49)	1.8 (0.7-4.6)
	Recessive	HT + WT	25.9 (82/317)	Ref.	7.6 (24/317)	Ref.
		HM	66.7 (2/3)	5.7 (0.5-64.1)	66.7 (2/3)	24.4 (2.1-279.1)*
rs13120400	Dominant	WT	27.4 (46/168)	Ref.	8.3 (14/168)	Ref.
		HM + HT	25.5 (36/141)	0.9 (0.6-1.5)	8.5 (12/141)	1.0 (0.5-2.3)
	Recessive	HT + WT	27.5 (79/287)	Ref.	8.4 (24/287)	Ref.
		HM	13.6 (3/22)	0.4 (0.1-1.4)	9.1 (2/22)	1.1 (0.2-5.0)
rs13181	Dominant	WT	25.6 (31/121)	Ref.	10.7 (13/121)	Ref.
		HM + HT	26.6 (53/199)	1.1 (0.6-1.8)	6.5 (13/199)	0.6 (0.27-1.3)
	Recessive	HT + WT	25.4 (73/287)	Ref.	8.0 (23/287)	Ref.
		HM	33.3 (11/33)	1.5 (0.7-3.2)	9.1 (3/33)	1.2 (0.3-4.1)
rs1347851	Dominant	WT	22.7 (29/128)	Ref.	6.3 (8/128)	Ref.
		HM + HT	28.2 (24/85)	1.3 (0.7-2.5)	11.8 (10/85)	2.0 (0.8-5.3)
	Recessive	HT + WT	24.9 (51/205)	Ref.	8.3 (17/205)	Ref.
		HM	25.0 (2/8)	1.0 (0.2-5.1)	12.5 (1/8)	1.6 (0.2-13.6)
rs139887	Dominant	WT	25.4 (31/122)	Ref.	5.7 (7/122)	Ref.
		HM + HT	26.8 (53/198)	1.1 (0.6-1.8)	9.6 (19/198)	1.7 (0.7-4.3)
	Recessive	HT + WT	28.4 (73/257)	Ref.	8.6 (22/257)	Ref.
		HM	17.5 (11/63)	0.5 (0.3-1.1)	6.3 (4/63)	0.7 (0.2-2.2)
rs1695	Dominant	WT	25.4 (31/122)	Ref.	7.4 (9/122)	Ref.
		HM + HT	26.9 (53/197)	1.1 (0.7-1.8)	8.6 (17/197)	1.2 (0.5-2.8)
	Recessive	HT + WT	25.5 (70/274)	Ref.	7.7 (21/274)	Ref.
		HM	31.1 (14/45)	1.3 (0.7-2.6)	11.1 (5/45)	1.5 (0.5-4.2)
rs17140129	Dominant	WT	23.6 (53/225)	Ref.	7.6 (17/225)	Ref.
		HM + HT	32.6 (31/95)	1.6 (0.9-2.7)	9.5 (9/95)	1.3 (0.6-3.0)
	Recessive	HT + WT	26.1 (80/306)	Ref.	8.5 (26/306)	Ref.
		HM	28.6 (4/14)	1.1 (0.4-3.7)	0 (0/14)	-

rsID	Model	Variants	Incidence any grade (≥ grade 1) neuropathy	Univariate logistic regression analysis any grade (CTCAE ≥ 1) neuropathy crude OR (95% CI)	Incidence severe (≥ grade 2) neuropathy	Univariate logistic regression analysis severe (CTCAE ≥ 2) neuropathy crude OR (95% CI)
rs1729786	Dominant	WT	21.6 (8/37)	Ref.	5.4 (2/37)	Ref.
		HM + HT	24.6 (16/65)	1.2 (0.5-3.1)	12.3 (8/65)	2.5 (0.5-12.2)
	Recessive	HT + WT	25.0 (24/96)	Ref.	10.4 (10/96)	Ref.
		HM	0 (0/6)	-	0 (0/6)	-
rs1885301	Dominant	WT	26.9 (32/119)	Ref.	8.4 (10/119)	Ref.
		HM + HT	25.9 (52/201)	1.0 (0.6-1.6)	8.0 (16/201)	0.9 (0.4-2.2)
	Recessive	HT + WT	29.1 (77/265)	Ref.	8.7 (23/265)	Ref.
		HM	12.7 (7/55)	0.4 (0.2-0.8)*	5.5 (3/55)	0.6 (0.2-2.1)
rs2074087	Dominant	WT	25.5 (60/235)	Ref.	8.5 (20/235)	Ref.
		HM + HT	28.2 (24/85)	1.2 (0.7-2.0)	7.1 (6/85)	0.8 (0.3-2.1)
	Recessive	HT + WT	26.2 (82/313)	Ref.	8.0 (25/313)	Ref.
		HM	28.6 (2/7)	1.1 (0.2-5.9)	14.3 (1/7)	1.9 (0.2-16.6)
rs2228001	Dominant	WT	26.1 (31/119)	Ref.	10.9 (13/119)	Ref.
		HM + HT	26.4 (53/201)	1.0 (0.6-1.7)	6.5 (13/201)	0.6 (0.3-1.3)
	Recessive	HT + WT	27.9 (76/272)	Ref.	8.8 (24/272)	Ref.
		HM	16.7 (8/48)	0.5 (0.2-1.2)	4.2 (2/48)	0.5 (0.1-2.0)
rs2230641	Dominant	WT	28.6 (58/203)	Ref.	8.4 (17/203)	Ref.
		HM + HT	22.2 (26/117)	0.7 (0.4-1.2)	7.7 (9/117)	0.9 (0.4-2.1)
	Recessive	HT + WT	26.7 (82/307)	Ref.	7.8 (24/307)	Ref.
		HM	15.4 (2/13)	0.5 (0.1-2.3)	15.4 (2/13)	2.1 (0.5-10.2)
rs2230806	Dominant	WT	23.8 (39/164)	Ref.	8.5 (14/164)	Ref.
		HM + HT	29.0 (45/155)	1.3 (0.8-2.2)	7.7 (12/155)	0.9 (0.4-2.0)
	Recessive	HT + WT	28.2 (82/291)	Ref.	8.6 (25/291)	Ref.
		HM	7.1 (2/28)	0.2 (0.1-0.9)*	3.6 (1/28)	0.4 (0.1-3.0)
rs2302237	Dominant	WT	26.7 (32/120)	Ref.	5.0 (6/120)	Ref.
		HM + HT	23.8 (40/168)	0.9 (0.5-1.5)	9.5 (16/168)	2.0 (0.8-5.3)
	Recessive	HT + WT	25.1 (64/255)	Ref.	7.1 (18/255)	Ref.
		HM	24.2 (8/33)	1.0 (0.4-2.2)	12.1 (4/33)	1.8 (0.6-5.7)

rsID	Model	Variants	Incidence any grade (≥ grade 1) neuropathy	Univariate logistic regression analysis any grade (CTCAE ≥ 1) neuropathy crude OR (95% CI)	Incidence severe (≥ grade 2) neuropathy	Univariate logistic regression analysis severe (CTCAE ≥ 2) neuropathy crude OR (95% CI)
rs2338	Dominant	WT	27.7 (49/177)	Ref.	9.0 (16/177)	Ref.
		HM + HT	24.1 (34/141)	0.8 (0.5-1.4)	6.4 (9/141)	0.7 (0.3-1.6)
	Recessive	HT + WT	26.6 (79/297)	Ref.	8.9 (24/297)	Ref.
		HM	19.0 (4/21)	0.7 (0.2-2.0	4.8 (1/21)	0.6 (0.1-4.4)
rs2849380	Dominant	WT	26.5 (57/215)	Ref.	9.3 (20/215)	Ref.
		HM + HT	25.7 (27/105)	1.0 (0.6-1.6)	5.7 (6/105)	0.6 (0.2-1.5)
	Recessive	HT + WT	26.6 (83/312)	Ref.	8.0 (25/312)	Ref.
		HM	12.5 (1/8)	0.4 (0.1-3.3)	12.5 (1/8)	1.6 (0.2-13.9)
rs3114018	Dominant	WT	33.0 (31/94)	Ref.	9.6 (9/94)	Ref.
		HM + HT	23.3 (52/223)	0.6 (0.4-1.1)	7.6 (17/223)	0.8 (0.3-1.8)
	Recessive	HT + WT	25.6 (67/262)	Ref.	8.0 (21/262)	Ref.
		HM	29.1 (16/55)	1.2 (0.6-2.3)	9.1 (5/55)	1.2 (0.4-3.2)
rs3212986	Dominant	WT	26.2 (51/195)	Ref.	9.2 (18/195)	Ref.
		HM + HT	26.5 (31/117)	1.0 (0.6-1.7)	6.8 (8/117)	0.7 (0.3-1.7)
	Recessive	HT + WT	26.2 (78/298)	Ref.	8.1 (24/298)	Ref.
		HM	28.6 (4/14)	2.0 (0.9-4.1)	14.3 (2/14)	1.9 (0.4-9.0)
rs34116584	Dominant	WT	25.6 (52/203)	Ref.	6.4 (13/203)	Ref.
		HM + HT	27.4 (32/117)	1.1 (0.7-1.8)	11.1 (13/117)	1.8 (0.8-4.1)
	Recessive	HT + WT	25.8 (79/306)	Ref.	6.9 (24/306)	Ref.
		HM	35.7 (5/14)	0.4 (0.2-1.1)	14.3 (2/14)	0.6 (0.1-2.4)
rs3740066	Dominant	WT	33.6 (45/134)	Ref.	11.2 (15/134)	Ref.
		HM + HT	21.0 (39/186)	0.5 (0.3-0.8)*	5.9 (11/186)	0.5 (0.2-1.1)
	Recessive	HT + WT	28.0 (78/279)	Ref.	8.6 (24/279)	Ref.
		HM	14.6 (6/41)	0.4 (0.2-1.1)	4.9 (2/41)	0.6 (0.1-2.4)
rs3753753	Dominant	WT	31.4 (37/118)	Ref.	9.3 (11/118)	Ref.
		HM + HT	23.3 (34/146)	0.7 (0.4-1.2)	6.8 (10/146)	0.7 (0.3-1.8)
	Recessive	HT + WT	28.8 (67/233)	Ref.	9.0 (21/233)	Ref.
		HM	12.9 (4/31)	0.4 (0.1-1.1)	0 (0/31)	-

rsID	Model	Variants	Incidence any grade (≥ grade 1) neuropathy	Univariate logistic regression analysis any grade (CTCAE ≥ 1) neuropathy crude OR (95% CI)	Incidence severe (≥ grade 2) neuropathy	Univariate logistic regression analysis severe (CTCAE ≥ 2) neuropathy crude OR (95% CI)
rs4148396	Dominant	WT	30.8 (37/120)	Ref.	9.2 (11/120)	Ref.
		HM + HT	23.0 (45/196)	0.7 (0.4-1.1)	7.1 (14/196)	0.8 (0.3-1.7)
	Recessive	HT + WT	28.4 (74/261)	Ref.	8.4 (22/261)	Ref.
		HM	14.5 (8/55)	0.4 (0.2-0.9)*	5.5 (3/55)	0.6 (0.2-2.2)
rs4388268	Dominant	WT	24.4 (51/209)	Ref.	9.1 (19/209)	Ref.
		HM + HT	30.4 (31/102)	1.4 (0.8-2.3)	5.9 (6/102)	0.6 (0.2-1.6)
	Recessive	HT + WT	26.0 (79/304)	Ref.	8.2 (25/304)	Ref.
		HM	42.9 (3/7)	2.1 (0.5-9.8)	0 (0/7)	-
rs4426527	Dominant	WT	25.0 (50/200)	Ref.	6.5 (13/200)	Ref.
		HM + HT	28.3 (34/120)	1.2 (0.7-2.0)	10.8 (13/120)	1.8 (0.8-3.9)
	Recessive	HT + WT	25.8 (79/306)	Ref.	7.8 (24/306)	Ref.
		HM	35.7 (5/14)	1.6 (0.5-4.9)	14.3 (2/14)	2.0 (0.4-9.3)
rs4936453	Dominant	WT	26.6 (38/143)	Ref.	7.0 (10/143)	Ref.
		HM + HT	26.0 (46/177)	1.0 (0.6-1.6)	9.0 (16/177)	1.3 (0.6-3.0)
	Recessive	HT + WT	26.1 (75/287)	Ref.	8.4 (24/287)	Ref.
		HM	27.3 (9/33)	1.1 (0.5-2.4)	6.1 (2/33)	0.7 (0.2-3.1)
rs544093	Dominant	WT	27.7 (72/260)	Ref.	8.8 (23/260)	Ref.
		HM + HT	20.0 (12/60)	0.7 (0.3-1.3)	5.0 (3/60)	0.5 (0.2-1.9)
	Recessive	HT + WT	26.7 (84/315)	Ref.	8.3 (26/315)	Ref.
		HM	0 (0/5)	-	0 (0/5)	-
rs5918	Dominant	WT	25.4 (57/224)	Ref.	7.6 (17/224)	Ref.
		HM + HT	28.1 (27/96)	1.2 (0.7-2.0)	9.4 (9/96)	1.3 (0.5-2.9)
	Recessive	HT + WT	26.3 (82/312)	Ref.	8.3 (26/312)	Ref.
		HM	25.0 (2/8)	0.9 (0.2-4.7)	0 (0/8)	-
rs6924717	Dominant	WT	26.5 (58/219)	Ref.	7.3 (16/219)	Ref.
		HM + HT	27.1 (26/96)	1.0 (0.6-1.8)	10.4 (10/96)	1.5 (0.6-3.4)
	Recessive	HT + WT	27.1 (82/303)	Ref.	8.6 (26/303)	Ref.
		HM	16.7 (2/12)	0.5 (0.1-2.5)	0 (0/12)	-

rsID	Model	Variants	Incidence any grade (≥ grade 1) neuropathy	Univariate logistic regression analysis any grade (CTCAE ≥ 1) neuropathy crude OR (95% CI)	Incidence severe (≥ grade 2) neuropathy	Univariate logistic regression analysis severe (CTCAE ≥ 2) neuropathy crude OR (95% CI)
rs717620	Dominant	WT	29.6 (61/206)	Ref.	8.7 (18/206)	Ref.
		HM + HT	20.2 (23/114)	0.6 (0.4-1.0)	7.0 (8/114)	0.8 (0.3-1.9)
	Recessive	HT + WT	27.0 (83/307)	Ref.	8.1 (25/307)	Ref.
		HM	7.7 (1/13)	0.2 (0.0-1.8)	7.7 (1/13)	0.9 (0.1-7.5)
rs797519	Dominant	WT	14.3 (1/7)	Ref.	0 (0/7)	Ref.
		HM + HT	28.6 (53/185)	2.4 (0.3-20.5)	7.6 (14/185)	-
	Recessive	HT + WT	28.1 (52/185)	Ref.	7.0 (13/185)	Ref.
		HM	28.6 (2/7)	1.0 (0.2-5.4)	14.2 (1/7)	2.2 (0.3-19.7)
rs843748	Dominant	WT	21.5 (17/79)	Ref.	10.1 (8/79)	Ref.
		HM + HT	27.6 (66/239)	1.4 (0.8-2.6)	7.5 (18/239)	0.7 (0.3-1.7)
	Recessive	HT + WT	22.5 (18/80)	Ref.	5.0 (4/80)	Ref.
		HM	27.3 (65/238)	0.8 (0.4-1.4)	9.2 (22/238)	0.5 (0.2-1.6)
rs879207	Dominant	WT	25.0 (38/152)	Ref.	7.2 (11/152)	Ref.
		HM + HT	27.1 (45/166)	1.1 (0.7-1.8)	9.0 (15/166)	1.3 (0.6-2.9)
	Recessive	HT + WT	24.5 (69/282)	Ref.	6.0 (17/282)	Ref.
		HM	38.9 (14/36)	2.0 (1.0-4.1)	25.0 (9/36)	5.2 (2.1-12.8)*

Abbreviations: CI, confidence interval; CTCAE, Common Terminology Criteria for Adverse Events; OR, Odds ratio; HM, homozygous variant (homozygote minor allele); HT, heterozygous variant; WT, wild type (homozygote major allele).

* p-value < 0.05

Table S4. Univariate and multivariate analysis of *TRPV1* genotype and concomitant therapy with paclitaxel and (severe) neuropathy

	Incidence any grade (≥ grade 1) neuropathy	Univariate analysis[a] crude OR (95% CI)	Multivariate analysis[b] adjusted OR (95% CI)	Incidence severe (≥ grade 2) neuropathy	Univariate analysis[a] crude OR (95% CI)	Multivariate analysis[b] adjusted OR (95% CI)
HT+WT (*AG+AA*), no paclitaxel	21.6% (57/264)	Ref.	Ref.	4.9% (13/264)	Ref.	Ref.
HM (*GG),* no paclitaxel	29.0% (9/31)	1.5 (0.7-3.4)	1.5 (0.7-3.5)	16.1% (5/31)	3.7 (1.2-11.2)*	3.7 (1.2-11.5)*
HT+WT (*AG+AA*), paclitaxel	66.7% (12/18)	7.3 (2.6-20.2)*	6.1 (2.1-17.6)*	22.2% (4/18)	5.5 (1.6-19.1)*	5.0 (1.4-18.2)*
HM (*GG),* paclitaxel	100% (5/5)	-	-	80% (4/5)	77.2 (8.1-740.9)*	70.5 (5.9-837.7)*

Abbreviations: CI, confidence interval; HM, homozygous minor allele; HT, heterozygous major allele; OR, odds ratio; *TRPV1*, transient receptor potential cation channel subfamily V member 1; WT, wild type, homozygous major allele.

[a] Univariate logistic regression analysis.

[b] Multivariate logistic regression analysis (Backward: wald). Adjusted odds ratio: adjusted for the number of administered cycles of platinum-based therapy and ECOG PS in multivariate logistic regression analysis.

* p-value < 0.05

Chapter 3

Anthropometric and serum biomarkers for platinum-based therapy-related response and toxicity

Chapter 3.1

Association between skeletal muscle measures and chemotherapy-induced toxicity in patients with NSCLC treated with first-line platinum-based chemotherapy

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Abstract

Background: Chemotherapy-induced toxicities frequently occur in non-small cell lung cancer (NSCLC) patients treated with platinum-based chemotherapy. Low skeletal muscle mass (SMM) has been associated with a higher incidence of toxicities for several types of cancers and cytostatics. The aim of this study was to evaluate the association between skeletal muscle measures and chemotherapy-induced toxicity in a large cohort of NSCLC patients.

Methods: A multicentre prospective follow-up study (PGxLUNG, NTR number NL5373610015) in NSCLC patients was conducted. Included were patients diagnosed with NSCLC (stage II–IV) treated with first-line platinum-based (cisplatin or carboplatin) chemotherapy of whom pretreatment imaging was available. Skeletal muscle area (SMA) segmentation was performed on abdominal imaging at the level of the third lumbar vertebra (L3). SMA at the level of L3 was corrected for squared height (m^2) to yield the lumbar skeletal muscle mass index (LSMI). Skeletal muscle density (SMD) was calculated as the mean Hounsfield Unit (HU) of the segmented SMA. SMM and SMD were categorized as low, intermediate, and high, based on LSMI and mean HU tertiles, respectively. Chemotherapy-induced toxicity was scored using CTCAE v4.03 and categorized into haematological (anaemia, leukocytopenia, neutropenia, and thrombocytopenia), non-haematological (nephrotoxicity, neurotoxicity, and esophagitis), and dose-limiting toxicity (DLT) (treatment switch, delay, de-escalation, discontinuation, or hospitalization). The relationship between SMM, SMD, and toxicities was assessed with logistic regression modelling taking into account potential confounders like gender and body mass index (BMI).

Results: In total, 297 patients (male *n* = 167, median age 64 years) were included. Haematological toxicity grade 3/4 was experienced in 36.6% (*n* = 108) of the patients, 24.6% (*n* = 73) experienced any non-haematological toxicity grade ≥ 2, and 55.6% (*n* = 165) any DLT. Multivariate logistic regression analysis showed that low SMM (ORadj 2.41, 95% CI 1.31–4.45, *p* = 0.005) and age at diagnosis > 65 years (ORadj 1.76, 95% CI 1.07–2.90, *p* = 0.025) were statistically significantly associated with overall haematological toxicity grade 3/4. No statistically significant associations were found between low SMM or low SMD and non-haematological toxicities. Low SMM (ORadj 2.23, 95% CI 1.23–4.04, *p* = 0.008) and high SMD (ORadj 0.41, 95% CI 0.23–0.74, *p* = 0.003) were statistically significantly associated with a higher respectively lower risk of DLT.

Conclusions: Non-small cell lung cancer patients with pretreatment low SMM are at significant higher risk for haematological toxicities grade 3/4 and DLT. NSCLC patients with high SMD are at significant lower risk for DLT. Further studies should be aimed to investigate whether platinum dosing based on skeletal muscle measurements and/or improvement of pretreatment SMM/SMD could reduce the risk of toxicity without compromising efficacy.

Introduction

Lung cancer is worldwide the leading cause of cancer-related deaths.[1] Although immune therapy changed the therapeutic landscape, platinum-based chemotherapy (including cisplatin or carboplatin) is still considered as the standard first-line therapy for the vast majority of patients. Nevertheless, the degree and impact of the efficacy and toxicity of chemotherapy differ remarkably among patients.[2] Although platinum-based therapy can be effective in treating lung cancer, chemotherapy-induced toxicity is common and can lead to treatment discontinuation or hospitalization. In addition, dose-limiting toxicity (DLT) may influence disease progression because patients receive suboptimal treatment (i.e., in terms of therapeutic regimen, timing, and dose), which may negatively impact both prognosis and quality of life. Over the past years, a relationship has been observed between low skeletal muscle mass (SMM) and poor treatment outcomes in lung cancer.[3-7] Besides, several studies in different types of cancers have shown that low SMM leads to significant risk for chemotherapy-related toxicities and DLTs.[8-12] An explanation for the relationship between low SMM and toxicity might be altered pharmacokinetics because hydrophilic drugs, such as platinum agents, mainly distribute in the lean body mass (LBM) of which SMM is the largest contributor.[13] Consequently, it can be hypothesized that patients with low SMM will have higher blood levels of chemotherapeutic agents, resulting in an increased risk of chemotherapy-induced toxicity. In addition, pretreatment low SMM was demonstrated to be independently associated with frailty in multiple studies in patients with head and neck cancer.[14-15] Given the potential association between SMM and the occurrence of chemotherapy-induced toxicities[12], information about SMM values of individual patients can possibly help physicians identify patients at risk for poor treatment tolerability.[16] For lung cancer patients, recently, Halvorson *et al*. performed a study in patients with limited small cell lung cancer (SCLC) and found that patients who received a high dose of cisplatin per kilogram LBM had more often haematological toxicity and neutropenic infections.[17] In a study performed by Srdic *et al*. in non-small cell lung cancer (NSCLC) patients treated with platinum-based chemotherapy, no association was found between skeletal muscle measurements and chemotherapy-induced toxicity.[18] However, only 55 patients met the inclusion criteria for muscle mass measurements. This low number of included patients may have contributed to the fact that in this study no association was found between skeletal muscle measurements and chemotherapy-induced toxicity. Therefore, the present study aimed to evaluate the association between SMM, SMD and chemotherapy-induced toxicity in a multicentre prospective follow-up study of a large cohort of NSCLC patients receiving first-line platinum-based chemotherapy.

Materials and methods

Setting, study design, and study population

This study was performed as part of the PGxLUNG study, in which 350 patients were included. The study design of the PGxLUNG study has been published previously.[19] In brief, patients of the PGxLUNG study were recruited from an academic hospital (University Medical Center Utrecht), two teaching hospitals (St. Antonius Hospital Nieuwegein/Utrecht and Meander Medical Center Amersfoort), and three general hospitals (Diakonessenhuis Utrecht, Groene Hart Ziekenhuis Gouda, and Ziekenhuis Rivierenland Tiel), all in the Netherlands, between February 2016 and August 2019. The inclusion criteria for this multicentre prospective follow-up study were as follows: (i) ≥ 18 years of age; (ii) radiologically confirmed stage II-IV NSCLC; (iii) planned or initiated first-line treatment with platinum-based (cisplatin or carboplatin) chemotherapy or chemoradiotherapy (according to the contemporary ESMO Clinical Practice Guidelines); and (iv) previously platinum-based chemotherapy-naïve. Patients of the PGxLUNG cohort of whom a pretreatment abdominal imaging was available were included for the present study.

Ethical considerations

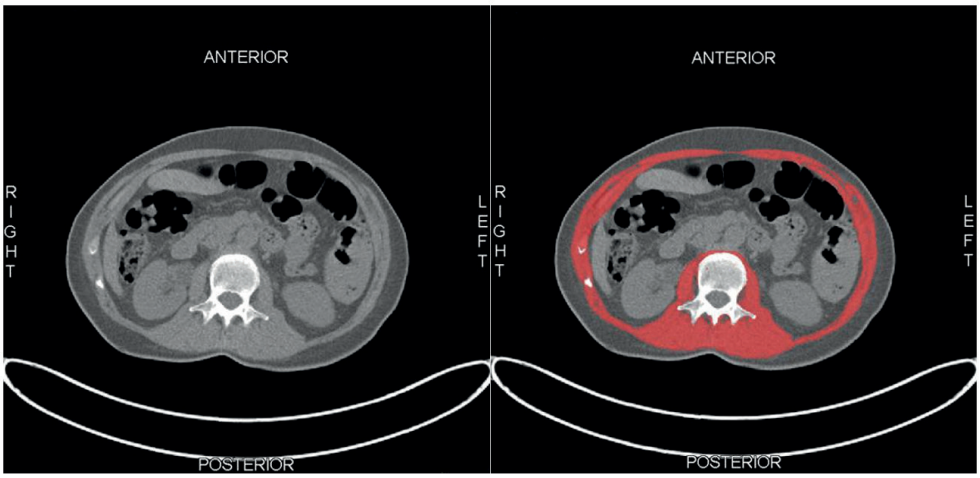
The study was approved by the accredited Medical Research Ethics Committee in Nieuwegein (MEC-U, number R15.056), and the study procedures were implemented in accordance with the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013). The PGxLUNG study was registered on the Netherlands National Trial Register (NTR) on 26 April 2016 (NTR number NL5373610015). All patients provided written informed consent.

Image analysis and anthropometric measurements

The skeletal muscle area at the level of the third lumbar vertebra (L3) has shown excellent correlation with whole body skeletal muscle mass as measured with abdominal imaging (considered as the golden standard).[20]

Segmentation of SMM was manually performed using Slice-o-matic version 5.0 (Tomovision, Canada), using a muscle-specific Hounsfield Unit (HU) range between -29 and +150. SMM was measured on pretreatment abdominal computed tomography (CT) imaging [as part of whole body positron emission tomography-CT imaging], which were routinely acquired for diagnostic workup. At the level of L3 on a single axial-slice, the area of the psoas, erector spinae, quadratus lumborum, transversus abdominis,

external and internal obliques, and rectus abdominis muscles were segmented, and this yielded the total skeletal muscle area (SMA) (Figure 1). SMA was divided by squared height (m^2) to obtain the lumbar skeletal muscle mass index (LSMI). The mean HU of the segmented SMA was retrieved and represents the skeletal muscle density (SMD) as surrogate measure of muscle quality.[21] Because contrast may influence the mean HU (higher HU), SMD was not calculated for patients who received pretreatment contrast enhanced CT. All scans were assessed by one trained individual (N.C.).



A B

Figure 1. Example of segmentation of skeletal muscle tissue at the level of the third lumbar vertebra (L3). **A.** Unsegmented skeletal muscle tissue. **B.** Segmented skeletal muscle tissue (red).

Chemotherapy-induced toxicities

Registration of chemotherapy-induced toxicities [using the Common Terminology Criteria for Adverse Events (CTCAE, version 4.03) or predefined definitions] took place throughout all cycles of platinum-based chemotherapy, and at 3, 6, and 12 months after treatment initiation. Endpoints were chemotherapy-induced toxicities, defined as haematological, non-haematological, and/or dose-limiting toxicities. Haematological toxicities, including anaemia (haemoglobin < 7.0 mmol), leukocytopenia (leukocytes < $4.0 \cdot 10^9$/L, neutropenia (neutrophils < $1.6 \cdot 10^9$/L), and thrombocytopenia < $150 \cdot 10^9$/L), were assessed by recording the nadir blood counts. Blood counts were performed at prespecified timepoints; prior to each cycle and at 3, 6, and 12 months after treatment initiation. Additional counts between follow-up moments were performed at the discretion of the treating physician. Blood counts were scored according to the CTCAE version 4.03. Haematological

toxicities CTCAE grade 3/4 were considered as severe toxicities. Non-haematological toxicities comprised nephrotoxicity, neurotoxicity, and esophagitis, assessed by lung oncologists using CTCAE version 4.03. Non-haematological toxicities CTCAE grade 2 or higher were considered as severe toxicities. DLT was defined as 'switching treatment' (cisplatin to carboplatin), 'treatment delay' (≥ 7 days from initially planned), 'treatment de-escalation' (dose reduction ≥ 25% of platinum agent), early treatment termination, and hospitalization ≥ 1 day, all due to chemotherapy-induced side effects. Registration of chemotherapy-induced toxicities and blood counts took place throughout all cycles of platinum-based chemotherapy. The follow-up period of haematological toxicities and DLT was 3 months after chemotherapy initiation, as these toxicities are expected to occur soon after and are related to chemotherapy administration. For non-haematological toxicities and treatment-related hospitalization, the follow-up period was 12 months after chemotherapy initiation, as these toxicities may also occur after a longer period of time after end of treatment.

Potential confounders and/or effect modifiers

The following parameters were considered to be potentially confounding and/or effect modifying variables for chemotherapy-induced toxicities: gender, age (≤ 65 years vs. > 65 years), weight, body surface area (BSA) (Dubois method)[22], co-morbidities (Charlson comorbidity index score[23], 2–3 vs. 4–5 vs. ≥ 6), Eastern Cooperative Oncology Group (ECOG) performance status[24] (ECOG PS 0–1 vs. ≥ 2), absolute dose of platinum agent [carboplatin (mg), cisplatin (mg/BSA)], renal function (eGFR using CKD-EPI formula[25], < 60 ml/min/1.73 m^2 vs. ≥ 60 ml/min/1.73 m^2), serum albumin level (< 37.5 g/L vs. ≥ 37.5 g/L)[18], and body mass index (BMI) (< 18.5 kg/m^2 vs. 18.5 to < 25 kg/m^2 vs. 25 to < 30 kg/m^2 vs. ≥ 30 kg/m^2).[26]

Data analysis

All data were extracted from the hospitals' electronic information system which contain patients' medical records and managed using REDCap electronic data capture tools.[27] Standard summary statistics were used to describe the sample data set by using SPSS version 26.0 (IBM SPSS Statistics) and visualized using GraphPad Prism version 8.3. The strength of the association between skeletal muscle measures (SMM and SMD) and chemotherapy-induced toxicity was assessed in univariate and multivariate settings with logistic regression modelling and expressed as odds ratios (OR) with corresponding 95% confidence intervals (CI). Covariates used in the multivariate analysis were

those aforementioned potential confounders and/or effect modifiers with statistical significance ($p < 0.10$) in univariate logistic regression analysis or with clinical significance based on previous studies. In the multivariate analysis a p-value < 0.05 (2-sided), was considered statistically significant. Because cut-off values for skeletal muscle measures are lacking, patients were stratified into three equal groups by SMM and SMD status. Patients were categorized into low SMM, intermediate SMM and high SMM for the first, second and third tertile of LSMI, respectively. For SMD, patients were categorized into low SMD, intermediate SMD and high SMD for the first, second and third tertile of the mean HU, respectively. Sarcopenic obesity was defined as the presence of both low SMM and obesity (≥ 30 kg/m^2).

Results

Population characteristics

In total, 297 patients of the PGxLUNG cohort (n = 350) with previously untreated NSCLC, receiving at least one cycle of platinum-based chemotherapy between April 2011 and July 2019, were included. Data on SMM/SMD were not available for 51 patients (pretreatment abdominal imaging not available), and two patients died before the first clinical evaluation. In addition, 13 patients underwent contrast-enhanced pretreatment imaging; consequently, HU values of these patients could not be used to quantify muscle quality (SMD). The clinical characteristics of the patients are summarized in Table 1.

Table 1. Demographic and clinical characteristics (*n* = 297)

Characteristics	***n* (%)**
Male, *n* (%)	167 (56.2)
Age at diagnosis in years, mean ± SD	64.3 ± 9.5
> 65 years, *n* (%)	155 (52.2)
Charlson comorbidity index[a], *n* (%)	
2–3	100 (33.7)
4–5	98 (33.0)
≥ 6	99 (33.3)
Performance status, *n* (%)	
ECOG 0	115 (38.7)
ECOG 1	133 (44.8)
ECOG ≥ 2	8 (2.7)
Unknown	41 (13.8)
Disease stage, *n* (%)	
IIA	6 (2.0)
IIB	27 (9.1)
IIIA	58 (19.5)
IIIB	72 (24.2)
IV	134 (45.1)
Tumour histology, *n* (%)	
Squamous	72 (24.2)
Non-squamous	186 (62.6)
Large Cell	6 (2.0)
Combined or unspecified	33 (11.1)
Smoking status, *n* (%)	
Never	14 (4.7)
Current/former	270 (90.9)
Unknown	13 (4.4)
Treatment intention, *n* (%)	
Curative/adjuvant	152 (51.2)
Palliative	145 (48.8)
Radiotherapy (RT) regimen, *n* (%)	
No thoracic RT	162 (54.5)
Concurrent thoracic RT	50 (16.8)
Sequential thoracic RT	85 (28.7)
Carboplatin-based chemotherapy, *n* (%)	205 (69.0)
Number of cycles, median (IQR)	3 (2-4)
Cumulative dose (mg), median (IQR)	1780 (1125-2280)
Cisplatin-based chemotherapy, *n* (%)	133 (44.8)
Number of cycles, median (IQR)	3 (2-4)
Cumulative dose (mg/m^2), median (IQR)	225 (150-277)
Renal function, eGFR, CKD-EPI	
Median mL/min/1.73 m^2, (IQR)	89 (76-90)
< 60 ml/min/1.73 m^2, *n* (%)	24 (8.1)
Serum albumin (g/L), median (IQR)	40.1 (36.7-42.5)
< 37.5 (g/L), *n* (%)	81 (27.3)
≥ 37.5 (g/L), *n* (%)	190 (64.0)
Unknown	26 (8.7)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; eGFR, estimated glomerular filtration rate; IQR, interquartile range; RT, radiotherapy; SD, standard deviation.

[a] Charlson comorbidity index score provides a simple means to quantify the effect of comorbid illnesses, including cardiovascular diseases, chronic obstructive pulmonary disease, liver disease and diabetes mellitus among others and, accounts for the aggregate effect of multiple concurrent diseases. A higher score indicates more comorbidities.

Median time between the pretreatment imaging and start of the first cycle of platinum-based chemotherapy was 41 days (IQR 27–69). The median number of cycles of platinum-based chemotherapy was three (IQR 2–4). Median sum (IQR) of cisplatin dose/BSA for low, intermediate, and high SMM was 225 mg/m^2 (80–276), 225 mg/m^2 (152–279), and 223 mg/m^2 (154–279); median sum (IQR) of cisplatin dose/BSA for low, intermediate, and high SMD was 208 mg/m^2 (149–277), 219 mg/m^2 (94–278), and 226 mg/m^2 (171–292), respectively. Median sum (IQR) of absolute carboplatin dose for low, intermediate, and high SMM was 1650 mg (1082–2190), 1868 mg (1025–2410), and 1850 mg (1460–2410), for low, intermediate, and high SMD it was 1738 mg (1086–2250), 1750 mg (1120–2210), and 2063 mg (1450–2600), respectively.

Image analysis and anthropometric measurements

Mean weight was 77 kilograms (kg) (IQR 65-88 kg). The majority of patients had normal weight (40.1%) or overweight (39.1%), as indicated by a body mass index (BMI) of 18.5 to < 25 kg/m^2 and 25 to < 30 kg/m^2, respectively. A median BSA of 1.91 m^2 (IQR 1.75-2.05 m^2) was found. In total nine patients (3%) suffered from sarcopenic obesity. Female patients were overrepresented in the low SMM group (73 females (73.7%) vs. 26 males (26.3%), $p < 0.001$).

Skeletal muscle mass/skeletal muscle density status and chemotherapy-induced toxicity

In the Supplementary materials, Table S1, chemotherapy-induced toxicities stratified by the SMM status (Table S1A) and SMD status (Table S1B) are shown. Haematological toxicities during platinum-based chemotherapy were very common, as 90.2% of the patients developed any kind of haematological toxicity grade ≥ 1. As shown in Figure 2A, overall haematological toxicity grade 3/4 occurred significantly more often in patients with low SMM (48.5%) compared to patients with intermediate (28.6%) or high SMM (32.7%). Besides, patients with low SMM had a statistically significant lower Hb nadir (5.7 mmol/L, IQR 5.2-6.5) compared with patients with intermediate (6.0 mmol/L, IQR 5.4-6.8) or high (6.5 mmol/L, IQR 5.8-7.3) SMM (Table S1A). In addition, low SMD status was associated with statistically significant lower Hb, leukocytes, and thrombocytes nadirs, as shown in Table S1B. No significant associations were found between SMM or SMD status and overall non-haematological toxicity grade ≥ 2 (Figure 2B, E). The distribution by severity of chemotherapy-induced (non-)haematological toxicities (as scored by the CTCAE), stratified by SMM and SMD, are available in Figures S1 and S2).

In total, 55.6% of the patients developed any DLT, and for 32.7% of the patients unplanned treatment-related hospitalization was necessary. Patients with low SMM tended to develop DLT (64.6%) more frequently compared to patients with intermediate (48.5%) or high (53.5%) SMM (Figure 2C). SMD was found statistically significant associated with treatment-related hospitalization (Table S2B, low SMD 44.2%, intermediate SMD 33.7% versus high SMD 21.3%, respectively) as well as with overall DLT (low SMD 64.2%, intermediate SMD 62.1% versus high SMD 39.4%, respectively (Figure 2F)). No statistically significant associations were found between sarcopenic obesity status and chemotherapy-induced toxicities.

Multivariate analysis

Table 2A shows the univariate and multivariate logistic regression analysis for the association with overall haematological toxicity grade 3/4. In univariate analysis, low SMM status (OR 2.35, 95% CI 1.31-4.24; p = 0.004) and age at diagnosis > 65 years (OR 1.73, 95% CI 1.07-2.80; p = 0.026) were statistically significantly associated with increased risk of haematological toxicity grade 3/4. Although BMI was not statistically associated with haematological toxicities in univariate analysis, BMI was added in the multivariate analysis based on the well-known correlation with SMM. As shown in Table 2, low SMM status (ORadj 2.41, 95% CI 1.31-4.45; p = 0.005) and age at diagnosis > 65 years (ORadj 1.76, 95% CI 1.07-2.90; p = 0.025) were confirmed in multivariate logistic regression analysis to be significantly associated with chemotherapy-induced overall haematological toxicity grade 3/4, while BMI status was not significantly associated. Low SMM (ORadj 2.23, 95% CI 1.23-4.04; p = 0.008) and high SMD (ORadj 0.41, 95% CI 0.23-0.74; p = 0.003) were significantly associated with overall DLT (Table 3).

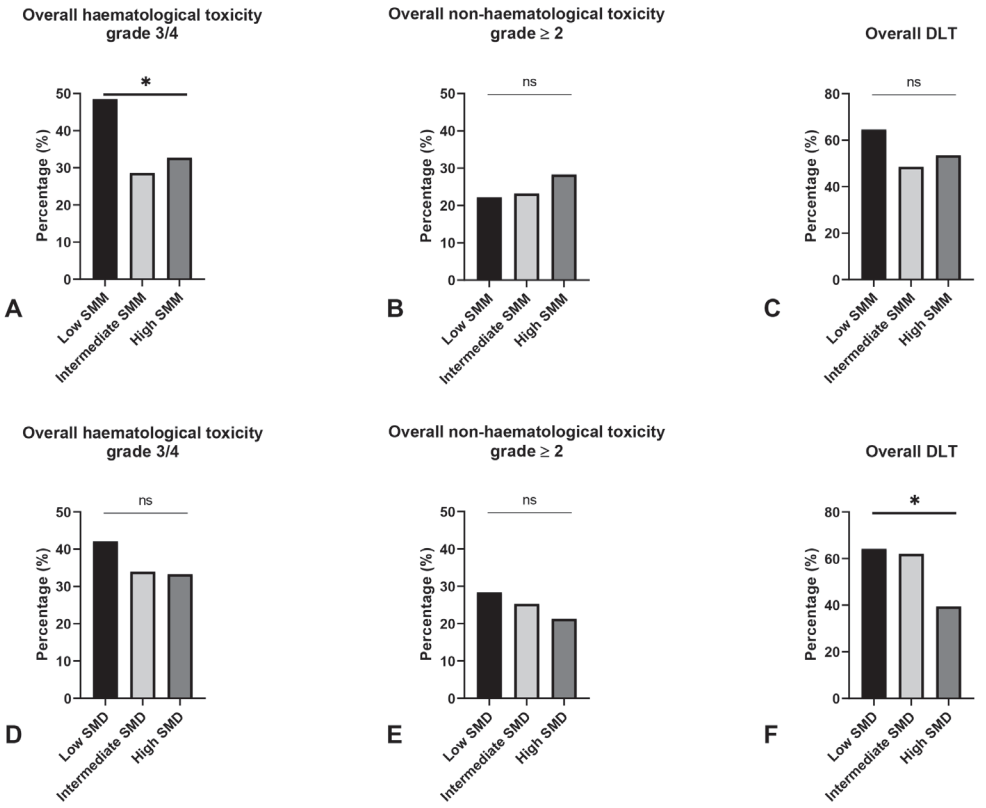


Figure 2. Chemotherapy-induced toxicity stratified by SMM and SMD status. Percentage of chemotherapy-induced toxicity stratified by low, intermediate and high SMM and SMD status using the Pearson chi-square test or Fisher's Exact Test (in case the cell count in any of the tables was < 5). * $p < 0.05$. Composite endpoints: overall haematological toxicity grade 3/4 scored using CTCAE: anaemia OR leukocytopenia OR neutropenia OR thrombocytopenia; overall non-haematological toxicity CTCAE grade ≥ 2 scored using CTCAE: nephrotoxicity OR neurotoxicity OR esophagitis; overall dose-limiting toxicity: switching treatment (cisplatin to carboplatin) OR treatment delay (≥ 7 days) OR treatment de-escalation (≥ 25%) OR treatment termination OR treatment-related hospitalization. **A.** Overall haematological toxicity stratified by SMM status. **B.** Overall non-haematological toxicity stratified by SMM status. **C.** Overall DLT stratified by SMM status. **D.** Overall haematological toxicity stratified by SMD status. **E.** Overall non-haematological toxicity stratified by SMD status. **F.** Overall DLT stratified by SMD status. Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; DLT, dose-limiting toxicity; ns, not statistically significant; SMD, skeletal muscle density; SMM, skeletal muscle mass.

Table 2. Univariate and multivariate analysis of overall haematological toxicity grade 3/4

Characteristics	*n* = 295	Univariate analysis[a]	Multivariate analysis[b] SMM	Multivariate analysis[b] SMD
		Crude OR (95% CI)	Adjusted OR[c] (95% CI)	Adjusted OR[d] (95% CI)
SMM status				
Intermediate	99	Ref.	Ref.	Ref.
Low	98	2.35 (1.31-4.24)	2.41 (1.31-4.45)*	2.38 (1.25-4.50)*
High	98	1.21 (0.66-2.23)	1.18 (0.63-2.18)	1.19 (0.63-2.25)
SMD status				
Intermediate	94	Ref.		Ref.
Low	95	1.41 (0.78-2.54)		1.16 (0.61-2.18)
High	93	0.97 (0.53-1.78)		1.16 (0.61-2.20)
Gender				
Male	165	Ref.		
Female	130	1.46 (0.91-2.35)		
Age at diagnosis in years				
≤ 65 years	140	Ref.	Ref.	Ref.
> 65 years	155	1.73 (1.07-2.80)	1.76 (1.07-2.90)*	1.73 (1.02-2.94)*
ECOG PS				
0	115	Ref.		
1	133	1.40 (0.82-2.40)		
≥ 2	8	2.49 (0.59-10.53)		
BMI (kg/m^2)				
18.5-< 25	118	Ref.	Ref.	Ref.
< 18.5	11	0.57 (0.14-2.25)	0.38 (0.09-1.56)	0.54 (0.12-2.36)
25-< 30	116	0.80 (0.47-1.35)	0.86 (0.49-1.52)	0.81 (0.46-1.48)
≥ 30	50	0.85 (0.43-1.69)	0.96 (0.46-1.99)	0.97 (0.45-2.10)
Charlson comorbidity index[e]				
2–3	99	Ref.		
4–5	97	0.99 (0.56-1.76)		
> 6	99	0.70 (0.39-1.26)		
Renal function (ml/min/1.73 m^2)				
≥ 60	273	Ref.		
< 60	22	0.80 (0.31-2.02)		
Serum albumin (g/L)				
≥ 37.5	190	Ref.		
< 37.5	81	1.46 (0.85-2.49)		
BSA (m^2)	295	0.47 (0.16-1.41)		
Weight (kg)	295	0.99 (0.98-1.01)		

Abbreviations: BMI, body mass index; BSA, body surface area; ECOG PS, Eastern Cooperative Oncology Group Performance Status; OR, odds ratio; SD, standard deviation; SMD, skeletal muscle density; SMM, skeletal muscle mass.

[a] Univariate logistic regression analysis.
[b] Multivariate logistic regression analysis (Backward: wald).
[c] Adjusted odds ratio: adjusted for SMM status, age at diagnosis and BMI in multivariate logistic regression analysis.
[d] Adjusted odds ratio: adjusted for SMD status, SMM status, age at diagnosis and BMI in multivariate logistic regression analysis.
[e] Charlson comorbidity index score provides a simple means to quantify the effect of comorbid illnesses, including cardiovascular diseases, chronic obstructive pulmonary disease, liver disease and diabetes mellitus among others and accounts for the aggregate effect of multiple concurrent diseases. A higher score indicates more comorbidities.
* p-value < 0.05

Table 3. Univariate and multivariate analysis of overall dose-limiting toxicity

Characteristics	*n* = 297	Univariate analysis[a]	Multivariate analysis[b] SMM	Multivariate analysis[b] SMD
		Crude OR (95% CI)	Adjusted OR[c] (95% CI)	Adjusted OR[d] (95% CI)
SMM status				
Intermediate	99	Ref.	Ref.	Ref.
Low	99	1.94 (1.10-3.44)	2.23 (1.23-4.04)*	2.11 (1.12-3.98)*
High	99	1.22 (0.70-2.14)	1.16 (0.66-2.03)	1.25 (0.69-2.25)
SMD status				
Intermediate	95	Ref.		Ref.
Low	95	1.10 (0.61-1.97)		0.94 (0.50-1.75)
High	94	0.40 (0.22-0.71)		0.41 (0.23-0.74)*
Gender				
Male	167	Ref.		
Female	130	1.17 (0.74-1.85)		
Age at diagnosis in years				
≤ 65 years	142	Ref.		
> 65 years	155	1.11 (0.70-1.75)		
ECOG PS				
0	115	Ref.		
1	133	1.22 (0.74-2.01)		
≥ 2	8	1.48 (0.34-6.47)		
BMI (kg/m^2)				
18.5-< 25	119	Ref.	Ref.	Ref.
< 18.5	11	0.74 (0.21-2.56)	0.56 (0.16-2.01)	0.56 (0.13-2.32)
25-< 30	116	1.17 (0.70-1.96)	1.36 (0.79-2.32)	1.25 (0.71-2.19)
≥ 30	51	1.38 (0.71-2.69)	1.66 (0.83-3.33)	1.44 (0.68-3.06)
Charlson comorbidity index[e]				
2–3	100	Ref.		
4–5	98	1.23 (0.70-2.16)		
> 6	99	1.25 (0.72-2.19)		
Renal function (ml/min/1.73 m^2)				
≥ 60	273	Ref.		
< 60	24	2.05 (0.82-5.12)		
Serum albumin (g/L)				
≥ 37.5	190	Ref.		
< 37.5	81	1.48 (0.87-2.52)		
BSA (m^2)	297	1.16 (0.41-3.30)		
Weight (kg)	297	1.00 (0.99-1.02)		

Abbreviations: BMI, body mass index; BSA, body surface area; ECOG PS, Eastern Cooperative Oncology Group Performance Status; OR, odds ratio; SD, standard deviation; SMD, skeletal muscle density; SMM, skeletal muscle mass.

[a] Univariate logistic regression analysis.
[b] Multivariate logistic regression analysis (Backward: wald).
[c] Adjusted odds ratio: adjusted for SMM status and BMI in multivariate logistic regression analysis.
[d] Adjusted odds ratio: adjusted for SMD status, SMM status and BMI in multivariate logistic regression analysis.
[e] Charlson comorbidity index score provides a simple means to quantify the effect of comorbid illnesses, including cardiovascular diseases, chronic obstructive pulmonary disease, liver disease and diabetes mellitus among others and accounts for the aggregate effect of multiple concurrent diseases. A higher score indicates more comorbidities.
* p-value < 0.05

Discussion

Main findings

Chemotherapy-induced toxicity frequently occurs in NSCLC patients treated with platinum-based chemotherapy. Previous studies have shown that low SMM is associated with chemotherapy-induced toxicity, across chemotherapeutic regimens and cancer types.[12] Although some studies[16,28] have described the prognostic value of body composition in patients with NSCLC treated with chemotherapy, data on treatment tolerability [in terms of (non-)haematological and dose-limiting toxicity] and the association with skeletal muscle measures in large cohorts are lacking. To the best of our knowledge, this is the largest clinical study that evaluates the association between pretreatment skeletal muscle measurements and chemotherapy-induced toxicity in NSCLC patients treated with platinum-based chemotherapy. The present prospective follow-up study demonstrated that low SMM increased the risk of severe haematological toxicity nearly 2.5-fold. In addition, low SMM and high SMD were significantly associated with a 2-fold higher and 2.5-fold lower risk of DLT, respectively.

The differences in incidence of chemotherapy-induced toxicity among patients with various skeletal muscle status may be explained by the correlation between SMM and anthropometric measurements (such as BMI, weight, and BSA) which might predict the pharmacokinetics of platinum-agents. However, in our cohort, no correlation between chemotherapy-induced toxicity and BMI, weight, and/or BSA was found, while SMM and SMD were associated with severe haematological and dose-limiting toxicity. In addition, patients with low SMM were generally more likely to receive a lower cumulative cisplatin and carboplatin dose compared with patients with intermediate or high SMM, which is a potential validation of the need for dose reduction or different treatment regimens in patients with low SMM compared with patients with intermediate/high SMM. In a recent study among 151 patients with solid tumours treated with capecitabine (a hydrophilic chemotherapeutic agent), no alterations in pharmacokinetics of capecitabine and the active and toxic metabolite 5-FU were observed in patients with low SMM.[29] The previously identified increased toxicity and decreased survival in patients with low SMM could therefore not be explained by changes in pharmacokinetic characteristics of capecitabine and its metabolites.[29] In addition, according to a pharmacokinetic study, in 184 oesophageal cancer patients treated with paclitaxel, skeletal muscle measures were not superior to BSA in predicting pharmacokinetics of paclitaxel and did not have any added value to BSA.[30]

An additional explanation for a higher incidence of chemotherapy-induced toxicity in patients with low SMM is the correlation between low SMM and frailty, which has been observed in multiple studies performed in patients with head and neck cancer.[14,15] In addition, Portal *et al*. also described low L3 skeletal muscle measures as surrogate marker for frailty, which can support the prognostication process of NSCLC patients.[31] In clinical practice, a frailty assessment is based on clinical characteristics like overall performance status and co-morbidity indices. However, it has been shown that clinicians tend to overestimate a patient's physical fitness.[32,33] Moreover, the present study could not establish an association between ECOG performance status or the Charlson comorbidity index score and chemotherapy-induced toxicity. So it seems plausible that objective skeletal muscle measures may support predicting treatment tolerability and clinical decision making. Besides the role of SMM in chemotherapy-induced toxicity, a chemotherapeutic agent itself, like cisplatin, can cause muscle wasting by activating a wide range of mechanisms, like inducing nuclear factor-*kB* signalling.[34,35] Consequently, SMM and SMD may further decrease during treatment, thereby negatively affecting chemotherapy tolerability leading to suboptimal treatment. The muscle wasting effect may be further increased due to the combination of different chemotherapeutic agents, which represent the standard treatment regimen for NSCLC patients. Hence, the effect of these different combinations on muscle wasting should be further elucidated.

Strengths and limitations

The present study has several strengths. First, to the best of our knowledge, this is the largest prospective follow-up study exploring the association between skeletal muscle measures and chemotherapy-induced toxicity of NSCLC patients receiving first-line platinum-based chemotherapy. Second, the variables collected in our cohort were based on real-world clinical data. Therefore, the results of this study reflect the actual clinical setting, which strengthens the possibility of extrapolating our findings.

The present analysis has some limitations. First, because population specific cut-off values for skeletal muscle measures in NSCLC patients are lacking, patients were stratified into three equal groups by SMM and SMD status. Consequently, comparing our results with studies using different cut-off values is complicated. However, a strong association between skeletal muscle measurements and chemotherapy-induced toxicity was found in our cohort. Second, in the present study, changes in body composition during chemotherapy were not taken into account, because repeated measures were lacking. Because early loss of SMM during first-line chemotherapy may be a poor

prognostic factor in stage IV NSCLC patients[16], muscle wasting during chemotherapy may also act as an effect modifier for chemotherapy-induced toxicity. Third, no data were available regarding recent weight loss before start of chemotherapy, COPD, and cardiovascular disease status, which may all act as confounders or effect modifiers for SMM/SMD status. Nevertheless, surrogate markers for nutritional status (serum albumin level) and co-morbidity (Charlson comorbidity index score) were used. In addition, because the number of available blood counts in between follow-up moments differs among patients, the nadir values may be lower than reported for our study patients. This might be an explanation for the fact that no association was found between low skeletal muscle measures and neutropenia.

Potential clinical relevance

In clinical practice, chemotherapy-induced toxicity will frequently result into clinical interventions such as delaying chemotherapy, dose adjustment, or treatment switch, all affecting treatment effectiveness adversely. The present results indicate an association between low SMM and the incidence of chemotherapy-induced toxicity in NSCLC patients treated with first-line platinum-based chemotherapy. Therefore, pretreatment skeletal muscle measurements may be useful to select patients at higher risk for chemotherapy-induced toxicity. In addition, dose-adjustments based on image analysis could result in better treatment tolerance in patients with low SMM, which is especially relevant in a palliative setting. In contrast, patients with high SMM or high SMD may benefit from a higher dose of chemotherapy, thereby improving treatment effectiveness. Hence, pretreatment evaluation of SMM and SMD, as well as repeated measures during treatment, may provide opportunities for tailored therapy and could have a significant impact on clinical care.

Future research

Based on our results, future studies should focus on finding the optimal cut-off values to differentiate NSCLC patients with and without low SMM and low SMD. SMD represents the muscle lipid content and is a marker of muscle quality, whereas SMM represents muscle quantity. In literature, SMM is investigated more often in patients with cancer than SMD due to current technological possibilities of muscle segmentation. For SMM segmentation, there is not any confounding effect of scanning with or without contrast enhancement. However, for SMD, it is still a debate whether scanning with contrast enhancement may influence the measurement of muscle lipid content. Because SMD is

measured based on the mean HU, the HU makes up the grayscale in medical CT imaging, which may be influenced by contrast application.[36] Nevertheless, it is likely that SMD has the potential to be a better marker of muscle fitness than SMM because it describes the muscle quality rather than the quantity, and quality may be better related to functional status than quantity. Indeed, Williams *et al*. found that SMD was better related to frailty than SMM in older patients with cancer.[37] Further research is needed to investigate a robust measurement of SMD in patients with cancer. Consequently, it will be possible to select patients who are at a higher risk for chemotherapy-induced toxicity. Therefore, a clinical study that investigates chemotherapy doses based on skeletal muscle measurements would be an important next step. To determine accurately whether patients with low SMM and low SMD will benefit more from dose reduction, ideally a randomized controlled trial should be performed. In such a phase 3 trial, dose adjustments based on skeletal muscle measures (e.g. a starting dose of cisplatin in a range of 60–90 mg/m^2) should be compared with a fixed cisplatin starting dose of 75 mg/m^2. To ensure that in patients with dose adjustments based on low SMM status treatment effectiveness is not reduced, endpoints should be focused on both toxicity and treatment response (in terms of radiological response, progression free survival, and/or overall survival).

In addition, future research should be focused on the quantification of pretreatment L3 skeletal muscle mass in patients diagnosed with NSCLC and its association with frailty. Subsequently, impact analysis of the implementation of routine skeletal muscle measurements on clinical decision-making should be of special interest. Currently, manual segmentation of skeletal muscle mass requires multiple steps and is time-consuming, which may limit its use in routine clinical practice. However, an automated method for accurate and reproducible segmentation of skeletal muscle area at L3, as recently described by Amarasinghe *et al*., radically increases the prospect of implementation routine determination of skeletal muscle measures in clinical practice.[38]

Besides, research should indicate whether patients will profit from improved physical fitness and higher SMM status (prehabilitation) before chemotherapy, in line with preoperative physical exercise interventions.[39]

Conclusions

In conclusion, this prospective follow-up study suggests that NSCLC patients with pretreatment low SMM are at a significantly higher risk for developing chemotherapy-induced severe haematological toxicity and DLT. NSCLC patients with high SMD are at significant lower risk for DLT. Future studies have to reveal whether skeletal muscle measurements have a higher correlation with the pharmacokinetics of chemotherapeutics than the current dosing strategies based on weight or BSA and to reveal the association with frailty. Such results may provide an explanation for increased toxicity in patients with low SMM. Moreover, research should be focused on whether chemotherapy dosing based on SMM could reduce the risk of chemotherapy-induced toxicity without compromising effectiveness. Future studies should also investigate whether improvement of pretreatment SMM/SMD could reduce the risk of chemotherapy-induced toxicity.

Declarations

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Authors' contributions

Conceptualization, CJ, NC, GH, TE, RB and VD; methodology, CJ, NC, GH, AH, TE, RB and VD; formal analysis, CJ and NC; supervision, GH, RB, TE, VD; writing original draft, CJ and NC; writing, review and editing, CJ, NC, GH, SH, FM, AL, AH, JB, PJ, LD, AH, TE, RB and VD; All authors have read and agreed to the published version of the manuscript. CJ and NC share first authorship since these authors contributed equally to this work.

The authors of this manuscript certify that they comply with the ethical guidelines for authorship and publishing in the Journal of Cachexia, Sarcopenia and Muscle.[40]

Competing interests

The authors declare that they have no competing interests.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

The protocol of the PGxLUNG study complied with the Good Clinical Practice guidelines and the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013), and was approved by the accredited Medical Research Ethics Committee in Nieuwegein (MEC-U, number R15.056).

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Informed consent

All patients provided written informed consent.

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Supplementary materials

Table S1A. Chemotherapy-induced toxicity stratified by SMM status;

Table S1B. Chemotherapy-induced toxicity stratified by SMD status;

Figure S1. Chemotherapy-induced haematological toxicity stratified by SMM and SMD status;

Figure S2. Chemotherapy-induced non-haematological toxicity stratified by SMM and SMD status.

Table S1A. Chemotherapy-induced toxicity stratified by SMM status

Chemotherapy-induced toxicity	*n*	All patients	*n*	Low SMM	*n*	Intermediate SMM	*n*	High SMM	*p*-value*
Haematological toxicities									
Nadir Hb (mmol/L), median (IQR)	295	6.0 (5.5-6.9)	99	5.7 (5.2-6.5)	98	6.0 (5.4-6.8)	98	6.5 (5.8-7.3)	< 0.001*
Anaemia, any grade, *n* (%)	295	225 (76.3)	99	86 (86.9)	98	75 (75.8)	98	64 (64.6)	0.002*
Anaemia, grade 3/4, *n* (%)	295	27 (9.2)	99	17 (17.2)	98	7 (7.1)	98	3 (3.1)	0.002*
Nadir leukocytes (· 10^9/L), median (IQR)	295	3.3 (2.4-4.5)	99	3.1 (2.1-4.3)	98	3.6 (2.4-4.7)	98	3.4 (2.4-4.6)	0.30
Leukocytopenia, any grade, *n* (%)	295	195 (66.1)	99	67 (67.7)	98	61 (62.2)	98	67 (68.4)	0.61
Leukocytopenia, grade 3/4, *n* (%)	295	48 (16.3)	99	20 (20.2)	99	13 (13.1)	99	15 (15.2)	0.38
Nadir neutrophils (· 10^9/L), median (IQR)	205	1.5 (0.8-2.4)	71	1.2 (0.6-2.4)	63	1.7 (0.7-2.6)	71	1.3 (0.9-2.0)	0.30
Neutropenia, any grade, *n* (%)	205	110 (53.7)	71	42 (59.2)	63	25 (39.7)	71	43 (60.6)	0.03*
Neutropenia, grade 3/4, *n* (%)	205	65 (31.7)	71	25 (35.2)	63	17 (27.0)	71	23 (32.4)	0.59
Nadir thrombocytes (· 10^9/L), median (IQR)	295	134 (75-203)	99	127 (70-192)	98	149 (92-227)	98	129 (73-195)	0.08
Thrombocytopenia, any grade, *n* (%)	295	166 (55.9)	99	60 (60.6)	98	48 (49.0)	98	58 (59.2)	0.22
Thrombocytopenia, grade 3/4, *n* (%)	295	42 (14.2)	99	19 (19.2)	98	13 (13.3)	98	10 (10.2)	0.19
Overall haematological toxicity[a], any grade, *n* (%)	295	266 (90.2)	99	92 (92.9)	98	87 (88.8)	98	87 (88.8)	0.41
Overall haematological toxicity[a], grade 3/4, *n* (%)	295	108 (36.6)	99	48 (48.5)	98	28 (28.6)	98	32 (32.7)	0.007*
Non-haematological toxicities									
Nephrotoxicity, any grade, *n* (%)	297	84 (28.3)	99	27 (27.3)	99	30 (30.3)	99	27 (27.3)	0.86
Nephrotoxicity, grade ≥ 2, *n* (%)	297	15 (5.1)	99	5 (5.1)	99	4 (4.0)	99	6 (6.1)	0.81
Neurotoxicity, any grade, *n* (%)	297	84 (28.3)	99	29 (29.3)	99	30 (30.3)	99	25 (25.3)	0.71
Neurotoxicity, grade ≥ 2, *n* (%)	297	27 (9.1)	99	11 (11.1)	99	9 (9.1)	99	7 (7.1)	0.61
Esophagitis, any grade, *n* (%)	297	91 (30.6)	99	30 (30.3)	99	28 (28.3)	99	33 (33.3)	0.74
Esophagitis, grade ≥ 2, *n* (%)	297	36 (12.1)	99	10 (10.1)	99	10 (10.1)	99	16 (16.2)	0.32
Overall non-haem.toxicity[a], any grade, *n* (%)	297	188 (63.3)	99	68 (68.7)	99	60 (60.6)	99	60 (60.6)	0.40
Overall non-haem.toxicity[a], grade ≥ 2, *n* (%)	297	73 (24.6)	99	22 (22.2)	99	23 (23.2)	99	28 (28.3)	0.57
Dose-limiting toxicities, *n* (%)									
Switching treatment (cisplatin to carboplatin)	133	30 (22.6)	45	11 (24.4)	44	11 (25.0)	44	8 (18.2)	0.70
Treatment delay (≥ 7 days)	297	49 (16.5)	99	20 (20.2)	99	13 (13.1)	99	16 (16.2)	0.41
Treatment de-escalation (≥ 25%)	297	37 (12.5)	99	17 (17.2)	99	8 (8.1)	99	12 (12.1)	0.15
Treatment termination	297	28 (9.4)	99	6 (6.1)	99	11 (11.1)	99	11 (11.1)	0.37
Treatment-related hospitalization	297	97 (32.7)	99	41 (41.4)	99	30 (30.3)	99	26 (26.3)	0.063
Overall dose-limiting toxicity[a]	297	165 (55.6)	99	64 (64.6)	99	48 (48.5)	99	53 (53.5)	0.065

Abbreviations: Hb, haemoglobin; IQR, interquartile range; SMM, skeletal muscle mass.

[a] Composite endpoints: overall non-haematological toxicity: nephrotoxicity OR neurotoxicity OR esophagitis; overall haematological toxicity: anaemia OR leukocytopenia OR neutropenia OR thrombocytopenia; overall dose-limiting toxicity: switching treatment (cisplatin to carboplatin) OR treatment delay (≥ 7 days) OR treatment de-escalation (≥ 25%) OR treatment termination OR treatment-related hospitalization.

* *p*-value < 0.05 based on Kruskal Wallis Test (for continuous independent variable) and Pearson chi-square test or Fisher's Exact Test (in case the cell count in any of the tables was < 5) (for categorical independent variable).

Table S1B. Chemotherapy-induced toxicity stratified by SMD status

Chemotherapy-induced toxicity	*n*	All patients	*n*	Low SMM	*n*	Intermediate SMM	*n*	High SMM	*p*-value*
Haematological toxicities									
Nadir Hb (mmol/L), median (IQR)	282	6.1 (5.5-6.9)	95	5.8 (5.2-6.8)	94	6.2 (5.7-7.0)	93	6.1 (5.5-7.3)	0.012*
Anaemia, any grade, *n* (%)	282	215 (76.2)	95	79 (83.2)	94	71 (75.5)	93	65 (69.9)	0.10
Anaemia, grade 3/4, *n* (%)	282	26 (9.2)	95	12 (12.6)	94	8 (8.5)	93	6 (6.5)	0.33
Nadir leukocytes (· 10^9/L), median (IQR)	282	3.4 (2.4-4.5)	95	3.0 (2.0-4.1)	94	3.5 (2.5-4.7)	93	3.5 (2.6-4.7)	0.041*
Leukocytopenia, any grade, *n* (%)	282	185 (65.6)	95	69 (72.6)	94	58 (61.7)	93	58 (62.4)	0.21
Leukocytopenia, grade 3/4, *n* (%)	282	48 (17.0)	95	22 (23.2)	94	11 (11.6)	93	15 (16.0)	0.10
Nadir neutrophils (· 10^9/L), median (IQR)	193	1.5 (0.7-2.4)	60	1.3 (0.6-2.6)	69	1.5 (0.9-2.4)	64	1.6 (0.8-2.4)	0.69
Neutropenia, any grade, *n* (%)	193	102 (52.8)	60	39 (65.0)	69	35 (50.7)	64	28 (43.8)	0.05
Neutropenia, grade 3/4, *n* (%)	193	61 (31.6)	60	22 (36.7)	69	19 (27.5)	64	20 (31.3)	0.54
Nadir thrombocytes (· 10^9/L), median (IQR)	282	134 (75-203)	95	100 (53-174)	94	150 (96-227)	93	144 (82-225)	< 0.001*
Thrombocytopenia, any grade, *n* (%)	282	157 (55.7)	95	67 (70.5)	94	44 (46.8)	93	46 (49.5)	0.001*
Thrombocytopenia, grade 3/4, *n* (%)	282	42 (14.9)	95	23 (24.2)	94	8 (8.5)	93	11 (11.8)	0.006*
Overall haematological toxicity[a], any grade, *n* (%)	282	253 (89.7)	95	89 (93.7)	94	85 (90.4)	93	79 (84.9)	0.15
Overall haematological toxicity[a], grade 3/4, *n* (%)	282	103 (36.5)	95	40 (42.1)	94	32 (34.0)	93	31 (33.3)	0.38
Non-haematological toxicities									
Nephrotoxicity, any grade, *n* (%)	284	78 (27.5)	95	29 (30.5)	95	25 (26.3)	94	24 (25.5)	0.71
Nephrotoxicity, grade ≥ 2, *n* (%)	284	13 (4.6)	95	7 (7.4)	95	4 (4.2)	94	2 (2.1)	0.22
Neurotoxicity, any grade, *n* (%)	284	83 (29.2)	95	26 (27.4)	95	30 (31.6)	94	27 (28.7)	0.81
Neurotoxicity, grade ≥ 2, *n* (%)	284	27 (9.5)	95	11 (11.6)	95	8 (8.4)	94	8 (8.4)	0.70
Esophagitis, any grade, *n* (%)	284	88 (31.0)	95	33 (34.7)	95	26 (27.4)	94	29 (30.9)	0.55
Esophagitis, grade ≥ 2, *n* (%)	284	36 (12.7)	95	14 (14.7)	95	12 (12.6)	94	10 (10.6)	0.70
Overall non-haem.toxicity[a], any grade, *n* (%)	284	178 (62.7)	95	59 (62.1)	95	63 (66.3)	94	56 (59.6)	0.63
Overall non-haem.toxicity[a], grade ≥ 2, *n* (%)	284	71 (25.0)	95	27 (28.4)	95	24 (25.3)	94	20 (21.3)	0.52
Dose-limiting toxicities, *n* (%)									
Switching treatment (cisplatin to carboplatin)	127	29 (22.8)	43	10 (27.8)	43	12 (30.0)	41	7 (13.7)	0.13
Treatment delay (≥ 7 days)	284	47 (16.5)	95	15 (15.8)	95	17 (17.9)	95	15 (16.0)	0.91
Treatment de-escalation (≥ 25%)	284	35 (12.3)	95	16 (16.8)	95	10 (10.5)	95	9 (9.6)	0.26
Treatment termination	284	24 (8.5)	95	9 (9.5)	95	11 (11.6)	95	4 (4.3)	0.18
Treatment-related hospitalization	284	94 (33.1)	95	42 (44.2)	95	32 (33.7)	95	20 (21.3)	0.004*
Overall dose-limiting toxicity[a]	284	157 (55.3)	95	61 (64.2)	95	59 (62.1)	95	37 (39.4)	0.001*

Abbreviations: Hb, haemoglobin; IQR, interquartile range; SMD, skeletal muscle density.

[a] Composite endpoints: overall non-haematological toxicity: nephrotoxicity OR neurotoxicity OR esophagitis; overall haematological toxicity: anaemia OR leukocytopenia OR neutropenia OR thrombocytopenia; overall dose-limiting toxicity: switching treatment (cisplatin to carboplatin) OR treatment delay (≥ 7 days) OR treatment de-escalation (≥ 25%) OR treatment termination OR treatment-related hospitalization.

* *p*-value < 0.05 based on Kruskal Wallis Test (for continuous independent variable) and Pearson chi-square test or Fisher's Exact Test (in case the cell count in any of the tables was < 5) (for categorical independent variable).

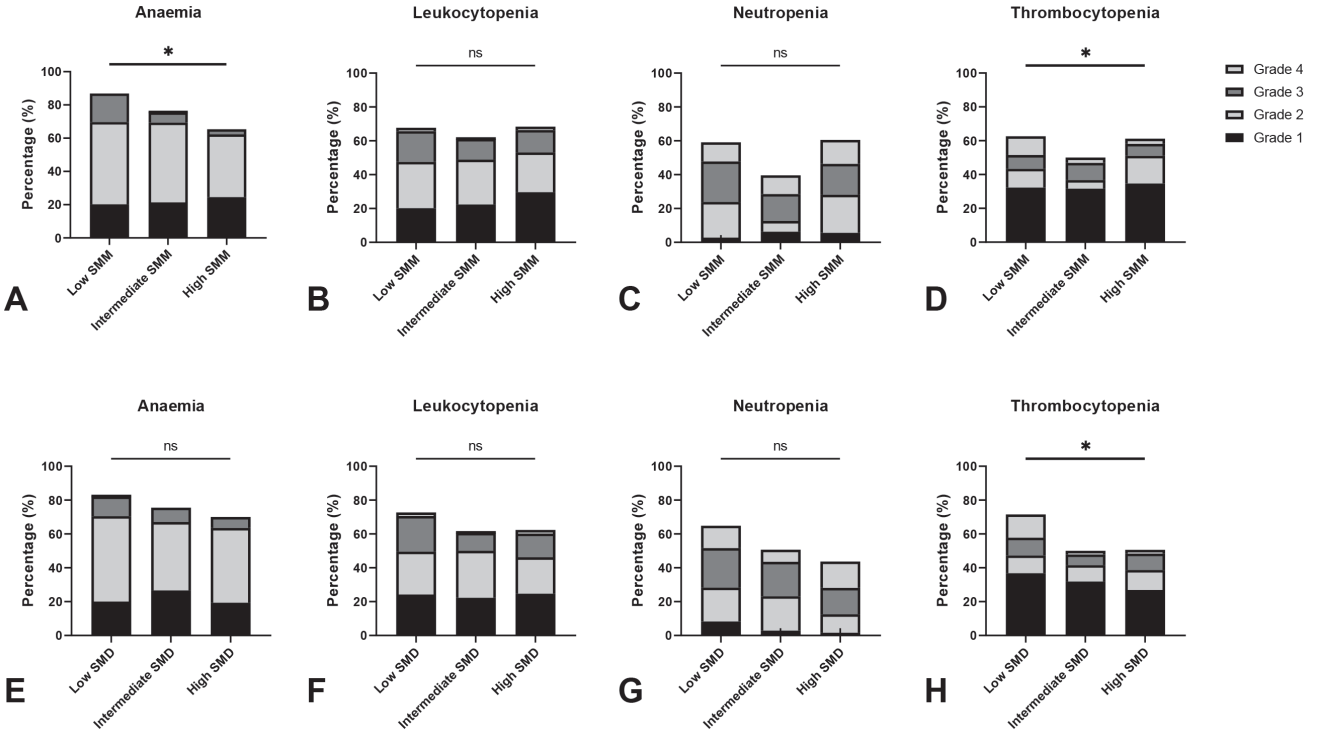

Figure S1. Chemotherapy-induced haematological toxicity stratified by SMM and SMD status. Percentage of chemotherapy-induced haematological toxicity scored using CTCAE, stratified by low, intermediate and high SMM and SMD status using the Pearson chi-square test or Fisher's Exact Test (in case the cell count in any of the tables was < 5). * $p < 0.05$. **A.** Anaemia stratified by SMM status. **B.** Leukocytopenia stratified by SMM status. **C.** Neutropenia stratified by SMM status. **D.** Thrombocytopenia stratified by SMM status. **E.** Anaemia stratified by SMD status. **F.** Leukocytopenia stratified by SMD status. **G.** Neutropenia stratified by SMD status. **H.** Thrombocytopenia stratified by SMD status. Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; ns, not statistically significant; SMD, skeletal muscle density; SMM, skeletal muscle mass.

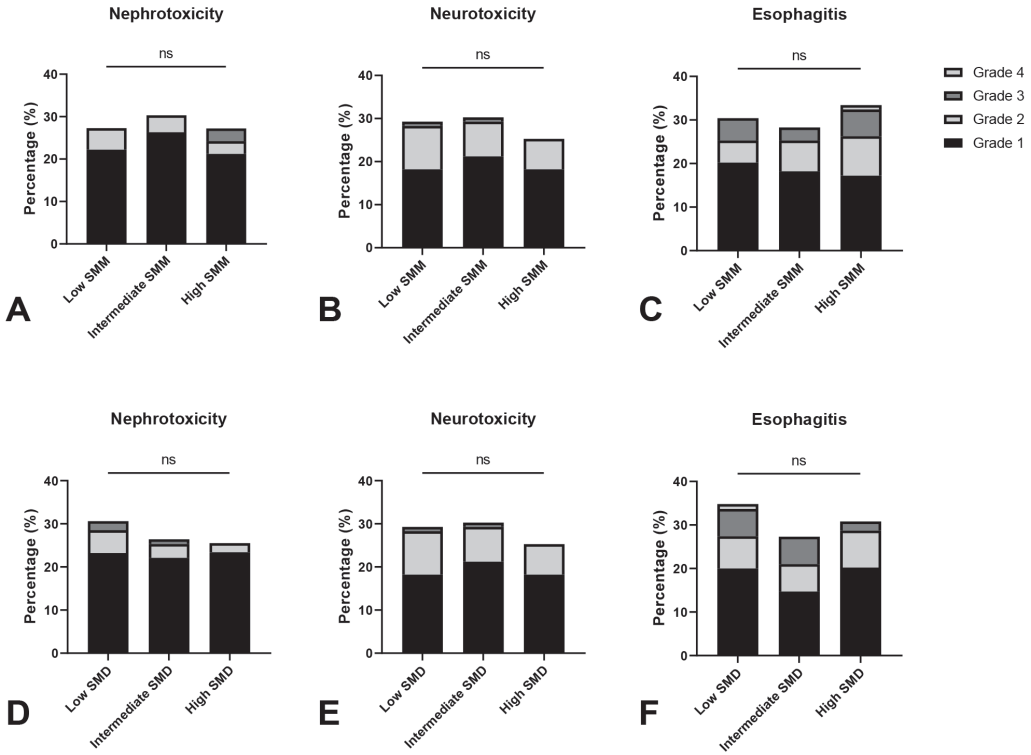

Figure S2. Chemotherapy-induced non-haematological toxicity stratified by SMM and SMD status. Percentage of chemotherapy-induced non-haematological toxicity scored using CTCAE, stratified by low, intermediate and high SMM and SMD status using the Pearson chi-square test or Fisher's Exact Test (in case the cell count in any of the tables was < 5). * $p < 0.05$. **A.** Nephrotoxicity stratified by SMM status. **B.** Neurotoxicity stratified by SMM status. **C.** Esophagitis stratified by SMM status. **D.** Nephrotoxicity stratified by SMD status. **E.** Neurotoxicity stratified by SMD status. **F.** Esophagitis stratified by SMD status. Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; ns, not statistically significant; SMD, skeletal muscle density; SMM, skeletal muscle mass.

Chapter 3.2

Association between serum biomarkers CEA and LDH and response in advanced (non-)small cell lung cancer patients treated with platinum-based chemotherapy

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Abstract

Background: In addition to radiological evaluation, biomarkers may be useful in providing early information on the response to treatment, and supporting clinical decision-making. The objective of this study was to investigate carcinoembryonic antigen (CEA) and lactate dehydrogenase (LDH) as biomarkers for early assessment of response in patients with advanced (non-)small cell lung cancer ((N)SCLC) treated with platinum-based chemotherapy.

Methods: A retrospective follow-up study was conducted from 2012 to 2017 among 593 consecutive patients with advanced (N)SCLC treated with first-line platinum-based chemotherapy in a large teaching hospital in the Netherlands. Pretreatment biomarker levels and changes from pretreatment levels were studied for association with radiologic response (partial response [PR] or complete response [CR], according to RECIST 1.1) using multivariate logistic regression, and with overall survival using COX proportional hazard modeling. Patient and disease characteristics such as age and disease stage were taken into account as potential confounding factors.

Results: Decreases in CEA and LDH (≥ 20%), particularly early in treatment, were significantly associated with better radiological response. Increases in these biomarkers (≥ 20%) and high pretreatment LDH levels (≥ 247 U/L) were significantly associated with lower overall survival.

Conclusions: Our results support determination of CEA and LDH levels for earlier assessment of response to platinum-based chemotherapy in patients with advanced (N)SCLC. Hence, routine determination and evaluation of CEA and LDH levels, prior to each cycle of platinum-based chemotherapy in advanced (N)SCLC, should be considered as part of daily clinical practice.

Introduction

Platinum-based chemotherapy, often combined with immunotherapy in current practice, is the most frequently applied first-line treatment for patients with advanced (non-)small cell lung cancer ((N)SCLC) without an epidermal growth factor receptor (*EGFR*) mutation or anaplastic lymphoma kinase (*ALK*) rearrangement.[1,2] However, the added value of chemotherapy is limited compared with best supportive care, given the median survival benefit of less than three months and the substantial impact of chemotherapy-induced toxicity on quality of life.[3-5] Since clearly not all patients will benefit from systemic chemotherapy, early evaluation of response to treatment is of great relevance. The measurement of treatment response by radiological evaluation, takes place after two and four cycles of platinum-based treatment.[6] Thus, a first evaluation is feasible six and 12 weeks after treatment initiation. Serum biomarkers predicting response earlier in treatment would be useful in addition to standard clinical imaging methods.

Carcinoembryonic antigen (CEA), a glycoprotein involved in the modulation of cellular processes, cell-cell recognition and cell adhesion, is used worldwide as a biomarker in several malignancies.[7] Data from a few studies have suggested that pretreatment CEA levels and changes from pretreatment levels during treatment are indicative of treatment response in lung cancer.[8-10] However, these results were obtained from small cohorts of patients which differ largely e.g. in terms of stages of disease. Another biomarker used in the follow-up of cancer treatment is lactate dehydrogenase (LDH), an enzyme that plays an essential role in anaerobic glycolysis and induces cell proliferation. As higher LDH levels are associated with the promotion of tumor invasion and metastases, high LDH levels indicate poor overall survival in (N)SCLC.[11-13]

Current clinical guidelines regarding the monitoring of treatment in advanced lung cancer do not recommend the routine determination of biomarkers.[6] To evaluate CEA and LDH levels in relation to treatment response, a retrospective follow-up study in a large cohort of patients with advanced (N)SCLC receiving first-line platinum-based chemotherapy, was conducted.

Methods

Study population

This retrospective follow-up study with prospectively collected data was conducted in a teaching hospital in the Netherlands (St. Antonius Hospital, Nieuwegein/Utrecht) in which approximately 200 patients are newly-diagnosed with (N)SCLC yearly. Consecutive

patients with pathology proven advanced (N)SCLC (stage IIIA, IIIB, or IV, according to tumor node metastasis [TNM] version 7) who started with first-line platinum-based (cisplatin or carboplatin) chemotherapy according to the ESMO Clinical Practice Guidelines between 01 January 2012 and 31 December 2017 were eligible.[6,14] Patients diagnosed with mesothelioma, patients who underwent lobectomy with adjuvant chemotherapy in stage IIIA, and patients with missing pretreatment levels of both CEA and LDH were excluded. Serum CEA and LDH levels were determined to a maximum of one month prior to start chemotherapy, and prior to each platinum-based chemotherapy cycle, which is part of the hospital's standard of care for the entire population of (N)SCLC patients. The study was conducted in accordance with the guidelines for the REporting of tumor MARKer studies (REMARK).[15] All data were extracted from the hospital's electronic medical record system.

Ethical considerations

The study protocol complied with the Good Clinical Practice Guidelines and the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013). The hospital's accredited Medical Ethics Committee assessed the study protocol and concluded that the Human Subjects Act (Dutch legislation: WMO) did not apply to this study. Consequently, the committee officially stated to having no objection to the conduct of the study followed by the board of directors of our hospital giving written permission for the conduct of the study. All patients gave permission for the use for research purposes of (coded) data collected as part of regular patient care. The inclusion in the study did not change patients' care they received or additional interventions such as blood sampling.

Assessment of treatment response

Treatment response was assessed radiologically and in terms of survival. Radiological response to treatment was measured after two and four chemotherapy cycles (at six and 12 weeks after treatment initiation, respectively) by computed tomography (CT) scan, fluorine-18 deoxyglucose positron emission tomography (FDG-PET) and/or magnetic resonance imaging (MRI) and assessed by pulmonary physicians specialized in pulmonary oncology. Response was categorized as progressive disease (PD), stable disease (SD), partial response (PR) or complete response (CR), according to the World Health Organization (WHO) Response Evaluation Criteria in Solid Tumors (RECIST 1.1).[16] Pretreatment tumor assessment was performed by chest CT imaging. For this study, overall response rate was used and patients were classified as either "responder" (PR or CR) or "non-responder" (PD or SD) to therapy, at six and 12 weeks after platinum-based chemotherapy initiation.

Individual patient overall survival time was defined as the time difference between the date of pretreatment biomarker measurement until death. The last extraction of data from the medical records was performed on 31 January 2019. Patients who were alive had their data censored at the last date of contact, as reported in the medical record.

Analysis of CEA and LDH

Measurements of CEA and LDH were performed by the Department of Clinical Chemistry of the St. Antonius Hospital in Nieuwegein/Utrecht, The Netherlands, using standardized diagnostic methods on an automated Cobas 6000 platform (Roche Diagnostics, Mannheim, Germany). CEA levels were measured using an electrochemiluminescence immunoassay (Roche Diagnostics). LDH measurements were performed using the IFCC-recommended enzymatic assay of Roche Diagnostics (LDHI2). Internal and external (interlaboratory comparisons) quality control procedures were in place. For internal quality control procedures, two levels of Liquichek Unassayed Chemistry Control (for LDH) and Liquicheck Immunoassay plus (for CEA) were used (Bio-Rad, Hercules, CA, USA) daily. Analytical performance based on the external quality control system for LDH was as follows; bias of 3.5% and a, precision of 4.3%, yielding a total measurement uncertainty of 12.1%. For CEA, the bias was 0.2% and the precision 5.7%, with a total measurement uncertainty of 11.6%.

Potential confounding variables

The following parameters were considered to be potentially confounding variables: gender, age at diagnosis, Eastern Cooperative Oncology Group (ECOG) performance status (on a 5-point scale, with higher scores indicating increasing disability)[17], histological tumor type (NSCLC squamous cell, NSCLC non-squamous and SCLC), disease stage, number of cycles of first-line platinum-based chemotherapy, smoking status, pretreatment LDH level, and manifestation of metastases in the central nervous system (CNS). CNS metastases (at diagnosis or within 30 days after diagnosis) were determined by CT or MRI scan.

Data analysis

Statistical analysis was performed using SPSS version 25.0 (IBM SPSS Statistics), R version 3.2.1 (www.r-project.org), and GraphPad Prism 8.0.1. Standard summary statistics were used to describe the sample data set. High pretreatment biomarker level was defined as any value above the local upper limit of normal, i.e., CEA levels $\geq$5.0 µg/L for non-

smokers, ≥10.0 µg/L for smokers and LDH levels ≥247 U/L. Changes in biomarker levels from pretreatment levels were calculated at three, six, nine and 12 weeks. To differentiate patients with and without biomarker change, and to indicate whether levels decreased or increased, the population was divided into three categories: "decreased" (biomarker level decrease ≥20%), "unchanged" (biomarker level decrease <20% or biomarker level increase <20%) and "increased" (biomarker level increase ≥20%), based on earlier published cut-off values for biomarker response.[9]

The strength of the association between biomarker levels (i.e., pretreatment levels and changes from pretreatment levels during treatment) and radiological response was estimated using logistic regression and expressed as odds ratios (OR) with 95% confidence intervals (CI). Median overall survival was plotted in Kaplan-Meier curves and groups were compared by using the log rank test. Hazard ratios (HR) with 95% CI were calculated with Cox proportional hazard modeling. The multivariate setting of both logistic regression and Cox proportional hazard regression was used to take all potential confounders into account and to calculate adjusted OR (ORadj) and adjusted HR (HRadj). Age, ECOG PS and LDH pretreatment level were categorized into two groups (≤65 and >65 years, ECOG PS 0–1 and ≥2, and LDH <247 U/L and ≥247 U/L, respectively), and included in multivariate analysis.

Results

Patients characteristics

A total of 593 consecutive patients with previously untreated advanced (N)SCLC, receiving platinum-based chemotherapy, between 01 January 2012 and 31 December 2017 were retrospectively screened for inclusion. In total 486 patients were included (107 patients were excluded: 104 patients underwent lobectomy; two patients were diagnosed with mesothelioma and one patient had missing pretreatment CEA and LDH levels).

The majority of the study population was male (55.1%), and the median age at diagnosis was 64 years (range: 33–84 years) (Table 1). The population included 138 patients (28.4%) diagnosed with SCLC and 348 (71.6%) with NSCLC, of which 235 (67.5%) had the non-squamous histologic subtype. At diagnosis, 67 patients (13.7%) had manifestation of metastases in the CNS. In total, 432 (88.8%) were active smokers or had smoked in the past. Before treatment initiation, the vast majority of patients (90.4%) had an ECOG PS score of 0 or 1. All patients received at least one cycle of first-line platinum-based chemotherapy, 376 (77.4%) patients received three or four cycles until 12 weeks after treatment initiation. High pretreatment CEA and LDH levels were found in 254 (52.3%) and 232 (47.7%) patients, respectively.

Table 1. Patient characteristics

Characteristics	***n* (%)**
Number of patients	486 (100)
Gender (male)	268 (55.1)
Age at diagnosis (years)	
Median (range)	64 (33–84)
> 65 years	188 (38.7)
Tumour histology	
NSCLC	348 (71.6)
Non-squamous	235 (67.5)
Squamous	82 (23.6)
Large Cell	23 (6.6)
Combined or unspecified	8 (2.3)
SCLC	138 (28.4)
Disease stage	
IIIA	94 (19.3)
IIIB	87 (17.9)
IV	305 (62.8)
CNS metastases (at diagnosis)	67 (13.7)
Cycles of of chemotherapy	
1	40 (8.2)
2	70 (14.4)
3	151 (31.1)
4	225 (46.3)
Performance status	
ECOG 0	126 (26.0)
ECOG 1	313 (64.4)
ECOG ≥ 2	40 (8.2)
Unknown	7 (1.4)
Smoking status	
Never	44 (9.1)
Active	177 (36.4)
Former	255 (52.4)
Unknown	10 (2.1)
CEA pretreatment levels (µg/L)	
Available levels	454 (93.4)
Median (IQR)	6.5 (2.7–28)
High (≥ 5.0 µg/L (non-smokers), ≥ 10 µg/L (smokers))	254 (52.3)
LDH pretreatment levels (U/L)	
Available levels	486 (100)
Median (IQR)	244 (202–317)
High (≥ 247 U/L)	232 (47.7)

Abbreviations: CEA, carcinoembryonic antigen; CNS, central nervous system; ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

Radiological response

At six and 12weeks after platinum-based chemotherapy initiation, 240 (49.4%) respectively 188 (38.7%) patients showed radiological response (PR or CR). Radiological evaluation revealed statistically significant (p <0.001) differences in response between tumor histology at week 6 (NSCLC 41.1% vs. SCLC 70.3%) and week 12 (NSCLC 30.7% vs. SCLC 58.7%). Stratified analysis of histology subtypes for the association between pretreatment biomarker levels and changes from pretreatment levels and radiological response did not show differences (data not shown). In addition, the number of cycles of platinum-based chemotherapy was significantly associated with radiological response at week 6, but not at week 12 (Table S1).

As shown in Tables 2 and 3, high pretreatment CEA levels and high LDH levels were not associated with radiological response. Multivariate analyses demonstrated, particularly in early stage of treatment, significant associations between CEA decreases and favorable response. Significant associations were found between CEA decrease at week 3 and radiological response (CR and PR) at week 6 (ORadj 2.27, 95% CI: 1.28–4.03), and between CEA decrease at week 6 and better response at week 6 (ORadj 2.38, 95% CI: 1.36–4.17). Also CEA decrease at week 3 and favorable response at week 12 were associated (ORadj 2.09, 95% CI: 1.14–3.83). Significant associations were found between LDH decrease at week 3 and response at week 6 (ORadj 1.72, 95% CI: 1.02–2.88) and LDH decrease at week 6 and response at week 6 (ORadj 1.82, 95% CI: 1.07–3.09).

Survival analysis

Median follow-up duration from pretreatment biomarker measurement was 11.4 months (interquartile range [IQR] 5.5–20.3 months) with a median overall survival for the total cohort of 12.2 months (95% CI: 10.4–14.0). ECOG PS, disease stage, number of cycles of first-line platinum-based chemotherapy, and pretreatment LDH level were significantly associated with overall survival (Figure 1, Table S2).

No statistically significant differences in overall survival between patients with NSCLC and SCLC were found (12.5 vs. 10.6 months respectively). In addition, stratified analysis of histology subtypes for the association between pretreatment biomarker levels and changes from pretreatment levels and overall survival did not show differences (data not shown). As shown in Table 4, multivariate analyses demonstrated that CEA increases at week 3 (HRadj 1.70, 95% CI: 1.27–2.27) and week 6 (HRadj 1.44, 95% CI: 1.07–1.95), were negatively associated with overall survival. High pretreatment LDH level (HRadj 1.42, 95% CI: 1.15–1.76), LDH increases at week 3 (HRadj 1.62, 95% CI: 1.18–2.22), week 6 (HRadj 1.47, 95% CI: 1.08–2.00) and week 12 (HRadj 1.71, 95% CI: 1.15–2.54) were associated with reduced overall survival (Figure 1, Table 5).

Table 2. Association between CEA levels and radiological response

Radiological response (PR or CR)		Week 6			Week 12	
		Univariate analysis	Multivariate analysis[a]		Univariate analysis	Multivariate analysis[a]
Biomarker levels CEA	***n***	**Crude OR (95% CI)**	**Adjusted OR (95% CI)**	***n***	**Crude OR (95% CI)**	**Adjusted OR (95% CI)**
Low pretreatment < 5.0 µg/L (non-smokers) < 10 µg/L (smokers)	182	Ref.	Ref.	165	Ref.	Ref.
High pretreatment ≥ 5.0 µg/L (non-smokers) ≥ 10 µg/L (smokers)	233	0.72 (0.48–1.06)	0.68 (0.43–1.07)	211	0.90 (0.60–1.36)	0.92 (0.57–1.49)
Week 0 and 3						
Unchanged < 20% decreased / < 20% increased	210	Ref.	Ref.	189	Ref.	Ref.
Increased ≥ 20%	90	1.50 (0.91–2.46)	1.54 (0.90–2.65)	80	1.16 (0.69–1.97)	1.21 (0.66–2.23)
Decreased ≥ 20%	86	2.50 (1.47–4.24)	2.27 (1.28–4.03)	83	2.51 (1.48–4.29)	2.09 (1.14–3.83)
Week 0 and 6						
Unchanged < 20% decreased / < 20% increased	133	Ref.	Ref.	126	Ref.	Ref.
Increased ≥ 20%	113	1.05 (0.63–1.73)	1.11 (0.64–1.93)	102	0.74 (0.44–1.27)	0.78 (0.43–1.43)
Decreased ≥ 20%	121	2.23 (1.35–3.71)	2.38 (1.36–4.17)	112	1.93 (1.15–3.24)	1.79 (1.00–3.20)
Week 0 and 9						
Unchanged < 20% decreased / < 20% increased	-	-	-	85	Ref.	Ref.
Increased ≥ 20%	-	-	-	75	0.72 (0.39–1.35)	0.80 (0.40–1.62)
Decreased ≥ 20%	-	-	-	145	1.23 (0.72–2.10)	1.18 (0.64–2.16)
Week 0 and 12						
Unchanged < 20% decreased / < 20% increased	-	-	-	69	Ref.	Ref.
Increased ≥ 20%	-	-	-	63	0.81 (0.41–1.62)	0.81 (0.36–1.82)
Decreased ≥ 20%	-	-	-	113	1.51 (0.83–2.76)	1.36 (0.68–2.71)

[a] Multivariate analysis adjusted for gender, age, ECOG PS, histological subtype (NSCLC squamous, NSCLC non-squamous, SCLC), cancer stage, number of cycles of first-line of chemotherapy, CNS metastasis, smoking history and pretreatment LDH level in multivariate logistic regression.

Table 3. Association between LDH levels and radiological response

Radiological response (PR or CR)		Week 6			Week 12	
		Univariate analysis	Multivariate analysis[a]		Univariate analysis	Multivariate analysis[a]
Biomarker levels LDH	***n***	**Crude OR (95% CI)**	**Adjusted OR (95% CI)**	***n***	**Crude OR (95% CI)**	**Adjusted OR (95% CI)**
Low pretreatment < 247 U/L	234	Ref.	Ref.	215	Ref.	Ref.
High pretreatment ≥ 247 U/L	211	1.12 (0.77–1.63)	1.04 (0.69–1.58)	189	1.04 (0.71–1.54)	0.93 (0.59–1.45)
Week 0 and 3						
Unchanged < 20% decreased / < 20% increased	249	Ref.	Ref.	229	Ref.	Ref.
Increased ≥ 20%	58	1.15 (0.65–2.04)	1.40 (0.75–2.62)	52	0.86 (0.47–1.58)	1.12 (0.57–2.24)
Decreased ≥ 20%	130	2.10 (1.35–3.26)	1.72 (1.02–2.88)	115	1.48 (0.95–2.33)	1.07 (0.61–1.85)
Week 0 and 6						
Unchanged < 20% decreased / < 20% increased	210	Ref.	Ref.	189	Ref.	Ref.
Increased ≥ 20%	72	1.14 (0.67–1.95)	1.25 (0.69–2.25)	63	1.04 (0.59–1.86)	1.00 (0.53–1.90)
Decreased ≥ 20%	143	2.26 (1.46–3.51)	1.82 (1.07–3.09)	135	1.74 (1.11–2.72)	1.24 (0.70–2.17)
Week 0 and 9						
Unchanged < 20% decreased / < 20% increased	-	-	-	152	Ref.	Ref.
Increased ≥ 20%	-	-	-	61	1.48 (0.82–2.70)	2.06 (1.05–4.05)
Decreased ≥ 20%	-	-	-	140	2.23 (1.40–3.57)	1.68 (0.92–3.06)
Week 0 and 12						
Unchanged < 20% decreased / < 20% increased	-	-	-	140	Ref.	Ref.
Increased ≥ 20%	-	-	-	48	0.57 (0.29–1.15)	0.66 (0.31–1.43)
Decreased ≥ 20%	-	-	-	103	1.83 (1.09–3.06)	1.43 (0.70–2.92)

[a] Multivariate analysis adjusted for gender, age, ECOG PS, histological subtype (NSCLC squamous, NSCLC non-squamous, SCLC), cancer stage, number of cycles of first-line of chemotherapy, CNS metastasis, smoking history and pretreatment LDH level in multivariate logistic regression.

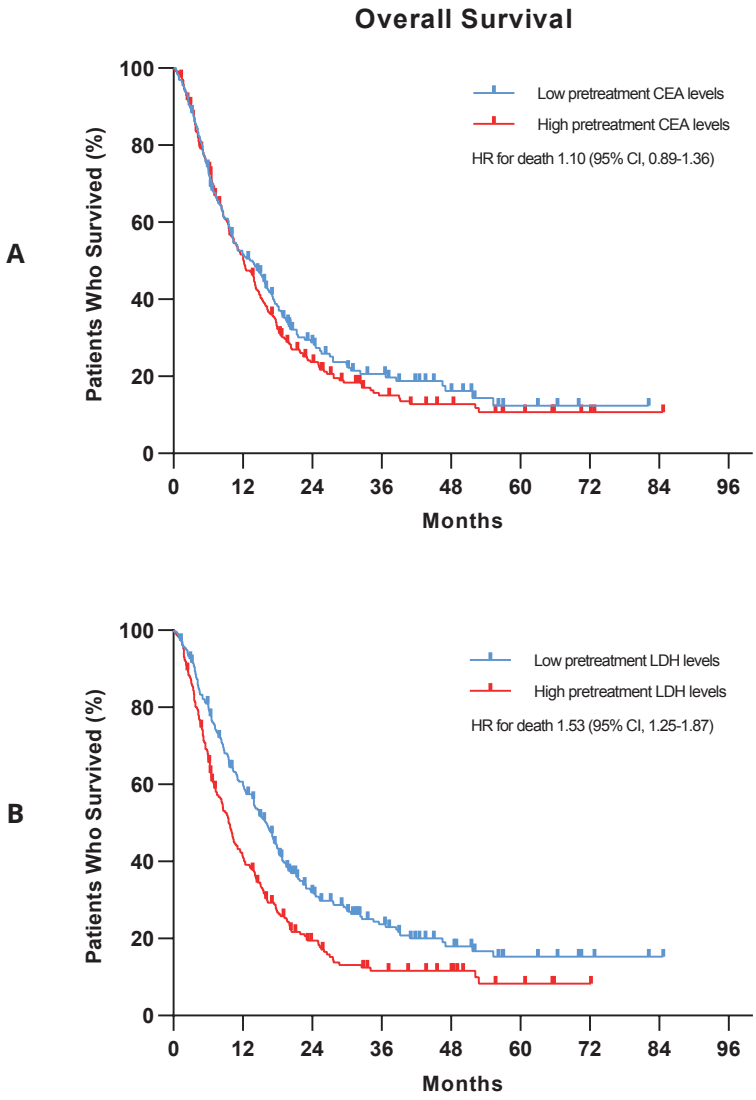

Figure 1. Overall survival. Kaplan-Meier plots illustrate overall survival according to pretreatment CEA and LDH serum levels. Blue lines indicate patients with low pretreatment biomarker levels and red lines indicate those with high levels. **A.** Pretreatment CEA levels; high pretreatment CEA levels defined as ≥ 5.0 µg/L (non-smokers) and ≥ 10 µg/L (smokers); low pretreatment CEA levels defined as < 5.0 µg/L (non-smokers) and < 10 µg/L (smokers). **B.** Pretreatment LDH levels; high pretreatment LDH levels defined as ≥ 247 U/L; low pretreatment LDH levels defined as < 247 U/L. Overall survival is calculated in months after pretreatment biomarker measurement until death. Hazard ratios were calculated in univariate setting with Cox proportional hazard modeling. Abbreviations: CEA, carcinoembryonic antigen; CI, confidence interval; HR, hazard ratio; LDH, lactate dehydrogenase.

Table 4. Association between CEA levels and overall survival

Overall survival			**Univariate analysis**	**Multivariate analysis[a]**
Variable	***n***	**Median[b] (months) (95% CI)**	**Hazard ratio (95% CI)**	**Hazard ratio (95% CI)**
Total cohort	486	12.2 (10.4-14.0)	-	-
Biomarker levels CEA				
Low pretreatment < 5.0 µg/L (non-smokers) < 10.0 µg/L (smokers)	200	13.2 (9.8-16.6)	Ref.	Ref.
High pretreatment ≥ 5.0 µg/L (non-smokers) ≥ 10.0 µg/L (smokers)	254	12.1 (10.1-14.1)	1.10 (0.89-1.36)	1.07 (0.85-1.35)
Week 0 and 3				
Unchanged < 20% decreased / < 20% increased	219	14.8 (12.8-16.8)	Ref.	Ref.
Increased ≥ 20%	96	8.1 (5.7-10.5)	1.65 (1.26-2.16)	1.70 (1.27-2.27)
Decreased ≥ 20%	91	14.5 (11.5-17.5)	1.00 (0.76-1.32)	0.91 (0.68-1.22)
Week 0 and 6				
Unchanged < 20% decreased / < 20% increased	137	15.6 (13.0-18.2)	Ref.	Ref.
Increased ≥ 20%	115	8.6 (6.6-10.6)	1.51 (1.13-2.00)	1.44 (1.07-1.95)
Decreased ≥ 20%	124	16.4 (13.6-19.2)	0.96 (0.73-1.27)	0.86 (0.64-1.16)
Week 0 and 9				
Unchanged < 20% decreased / < 20% increased	93	15.6 (12.3-18.9)	Ref.	Ref.
Increased ≥ 20%	80	9.5 (7.2-11.8)	1.51 (1.07-2.13)	1.38 (0.95-2.00)
Decreased ≥ 20%	154	17.1 (15.4-18.8)	0.95 (0.71-1.29)	0.89 (0.64-1.24)
Week 0 and 12				
Unchanged < 20% decreased / < 20% increased	73	15.3 (13.6-17.0)	Ref.	Ref.
Increased ≥ 20%	65	10.8 (7.1-14.5)	1.07 (0.73-1.59)	0.91 (0.59-1.42)
Decreased ≥ 20%	118	15.4 (13.0-17.8)	1.00 (0.72-1.40)	0.93 (0.65-1.33)

[a] Multivariate analysis adjusted for gender, age, ECOG PS, histological subtype (NSCLC squamous, NSCLC non-squamous, SCLC), cancer stage, number of cycles of first-line platinum–based chemotherapy, CNS metastasis, smoking history and pretreatment LDH level.

[b] Medians were calculated using the Kaplan-Meier method. Hazard ratios were calculated in univariate and multivariate setting with COX proportional hazard modeling.

Table 5. Association between LDH levels and overall survival

Overall survival			**Univariate analysis**	**Multivariate analysis[a]**
Variable	***n***	**Median[b] (months) (95% CI)**	**Hazard ratio (95% CI)**	**Hazard ratio (95% CI)**
Total cohort	486	12.2 (10.4-14.0)	-	-
Biomarker levels LDH				
Low pretreatment < 247 U/L	254	16.0 (14.0-18.0)	Ref.	Ref.
High pretreatment ≥ 247 U/L	232	9.5 (8.2-10.8)	1.53 (1.25-1.87)	1.42 (1.15-1.76)
Week 0 and 3				
Unchanged < 20% decreased / < 20% increased	268	15.6 (13.4-17.8)	Ref.	Ref.
Increased ≥ 20%	80	6.7 (3.6-9.8)	1.87 (1.39-2.52)	1.62 (1.18-2.22)
Decreased ≥ 20%	136	10.1 (8.2-12.0)	1.39 (1.10-1.76)	1.01 (0.78-1.32)
Week 0 and 6				
Unchanged < 20% decreased / < 20% increased	215	15.3 (12.9-17.7)	Ref.	Ref.
Increased ≥ 20%	78	9.7 (6.3-13.1)	1.42 (1.06-1.90)	1.47 (1.08-2.00)
Decreased ≥ 20%	148	11.9 (8.8-15.0)	1.20 (0.95-1.52)	0.83 (0.62-1.09)
Week 0 and 9				
Unchanged < 20% decreased / < 20% increased	161	16.7 (14.5-18.9)	Ref.	Ref.
Increased ≥ 20%	73	13.9 (9.7-18.1)	1.16 (0.83-1.60)	1.11 (0.78-1.59)
Decreased ≥ 20%	151	12.6 (9.5-15.7)	1.26 (0.98-1.63)	1.05 (0.78-1.42)
Week 0 and 12				
Unchanged < 20% decreased / < 20% increased	148	16.7 (14.1-19.3)	Ref.	Ref.
Increased ≥ 20%	51	13.8 (8.7-18.9)	1.46 (1.01-2.10)	1.71 (1.15-2.54)
Decreased ≥ 20%	108	11.5 (8.5-14.5)	1.54 (1.15-2.05)	1.36 (0.96-1.94)

[a] Multivariate analysis adjusted for gender, age, ECOG PS, histological subtype (NSCLC squamous, NSCLC non-squamous, SCLC), cancer stage, number of cycles of first-line platinum–based chemotherapy, CNS metastasis, smoking history and pretreatment LDH level.

[b] Medians were calculated using the Kaplan-Meier method. Hazard ratios were calculated in univariate and multivariate setting with COX proportional hazard modeling.

Discussion

This study reveals that decreases (≥ 20%) in CEA and LDH levels, especially those early in treatment, are associated with favorable radiological response to platinum-based chemotherapy in previously untreated advanced stage lung cancer. In addition, increases in these biomarkers (≥ 20%) and pretreatment high LDH are associated with lower overall survival. In the current study, biomarker response was divided into three categories, which made it possible to distinguish patients with decreased (≥ 20%) biomarker levels as well as patients with unchanged (< 20% decrease/increase) and increased (≥ 20%) biomarker levels. As compared with a decrease in LDH level, a decrease in CEA level at week 3 was found to be stronger associated with better radiological response at week 6 (1.7- and 2.3-fold higher probability, respectively). Since the association between CEA level decrease with radiological response is already shown after the first cycle of chemotherapy, monitoring of CEA levels seems to be particularly relevant in early stage of treatment. Pretreatment levels of CEA and LDH were not associated with radiological response. However, CEA and LDH increase at week 3, as compared with unchanged or decreased biomarker levels, was associated with a significant 1.7- and 1.6-fold higher probability of reduced overall survival. In addition, a 1.4-fold higher probability of inferior overall survival was found in patients with high pretreatment LDH levels. These results are in line with previously reported data suggesting that LDH serum levels may be useful on predicting clinical outcome in patients treated with first-line chemotherapy for different malignancies.[11–13,18,19] For both biomarkers, changes during treatment were superior to pretreatment biomarker levels in predicting therapy response, advocating biomarker assessment during treatment follow-up. These findings support the results of an earlier published systemic review and meta-analysis.20 According to Holdenrieder and colleagues, changes from pretreatment CEA levels during treatment are indicative of treatment response in NSCLC. However, in our cohort biomarker level measurements were available after the first cycle of platinum-based chemotherapy, while most studies report biomarker levels after the second cycle of chemotherapy. Therefore, detailed information earlier in treatment was provided in our cohort. Besides, due to the use of small study cohorts, the inclusion of patients with different stages of NSCLC and the use of different response classifications, the meta-analysis of Holdenrieder *et al*. was influenced by a high level of between-study heterogeneity.[20]

Clinical implications

Biomarkers of treatment response are particularly relevant early after treatment initiation, even prior to radiological evaluation. Moreover, determination of biomarkers might

be even more useful in the evaluation of patients with a mixed radiological response. Clinicians are also frequently confronted with patients with radiologically confirmed progressive disease accompanied by a beneficial clinical response and performance score or vice versa. In these cases, clinicians and patients are facing the dilemma of treatment (dis)continuation. Therefore, in addition to radiological evaluation, changes in biomarker levels might support the process of evaluating treatment response in the continuous consideration of harm and benefit. Currently, LDH measurement during treatment follow-up is standard clinical care for advanced (N)SCLC.[6] However, recommendations are lacking on how pretreatment LDH levels and changes should be taken into account in the assessment of response to platinum-based chemotherapy. In addition, the results of our study indicate that CEA level changes are strongly associated with therapy response, supporting the recommendation that CEA and LDH assessment should be considered as part of standard of care for patients with previously untreated advanced (N)SCLC treated with platinum-based chemotherapy.

Strengths and limitations

The present study has several strengths. First, the biomarkers examined are routinely determined during treatment follow-up of advanced (N)SCLC patients in our hospital. Therefore, the results of this study reflect the actual clinical setting. Second, the study has a single-center design. Since all patients were recruited in the same teaching hospital, low heterogeneity in clinical practice occurred, and all patients underwent the same treatment regimens. Besides, during the defined time frame, a large cohort of consecutive patients was formed, therefore avoiding selection bias. To our knowledge, this is the largest study conducted to investigate the association between CEA and LDH levels and treatment response in stage III/IV (N)SCLC. Additionally, the results can be implemented immediately into daily clinical practice, since measuring CEA and LDH levels is affordable and easy to perform. The present analysis also has some limitations. First, the time of radiological evaluation was not predefined due to the retrospective nature of the study. CT scans were taken after two and four chemotherapy cycles, performed every six to eight weeks in routine care. Therefore, the first and second CT scan after treatment initiation was defined as radiological response at week 6 and 12, respectively. However, there was minor variation in the time of radiological evaluation. In addition, radiological response was measured by pulmonary physicians specialized in pulmonary oncology according to the RECIST 1.1 criteria. Since misclassification can occur, preferably, two observers should have evaluated the endpoints independently. On the other hand, our results reflect the actual clinical setting, a strength mentioned earlier.

Future research

Based on our results, routine measurement and evaluation of both CEA and LDH levels should be considered as part of treatment evaluation in advanced lung cancer patients. However, to our knowledge, only a few hospitals in the Netherlands evaluate CEA levels during follow-up of advanced (N)SCLC patients. Therefore, impact analysis of the implementation of routine biomarker determination on clinical decision-making should be of special interest. Despite the fact that platinum-based chemotherapy has long been the standard first-line treatment for patients with advanced (N)SCLC, the introduction of immunotherapy recently led to new treatment perspectives and strategies. Today, for patients with programmed cell death ligand 1 (PD-L1) expression ≥ 50% of tumor cells (approximately one-third of patients), immunotherapy or immunotherapy in combination with chemotherapy is the first-line treatment option.[6] For these patients starting with mono immunotherapy, recent studies already suggest the significance of both CEA and LDH for the assessment of treatment response,[21–24] which is in line with the findings presented here. Moreover, current research reveals the additional value of combining immunotherapy with platinum-based chemotherapy as first-line treatment.[25,26] Since patients in our cohort started with first-line treatment between 01 January 2012 and 31 December 2017, the vast majority of our patients was treated with platinum-based chemotherapy. Merely three patients (less than 1%) underwent chemotherapy combined with immunotherapy; hence subgroup analysis was not applicable. As determination of CEA and LDH levels in patients undergoing platinum-based chemotherapy or immunotherapy proved to be relevant in treatment evaluation, it is likely that biomarker determination would also be appropriate in the follow-up of combination therapy. Whether biomarker (changes) can also predict response in (N)SCLC patients undergoing novel targeted or immunotherapies combined with conventional chemotherapy, is an important topic for future research.

In conclusion, the results of this retrospective follow-up study support the determination of both CEA and LDH serum levels for identifying subgroups of platinum-based chemotherapy treated (N)SCLC patients differing in radiological response and overall survival. Hence, routine determination and evaluation of CEA and LDH levels, prior to each cycle of platinum-based chemotherapy in advanced (N)SCLC, should be considered as part of daily clinical practice. Biomarker assessment might be particularly relevant alongside radiological evaluation, in the evaluation of patients with a mixed radiological response or in case of discrepancy between clinical and radiological responses.

Declarations

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Authors' contributions

Conceptualization, CJ, VD and GH; methodology, CJ, VD, JK, HR, TE and GH; formal analysis, CJ and JK; supervision, VD, HR, TE, GH; writing original draft, CJ; writing, review and editing, CJ, VD, JK, HR, TE and GH; All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

The study protocol complied with the Good Clinical Practice Guidelines and the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013). The hospital's accredited Medical Ethics Committee assessed the study protocol and concluded that the Human Subjects Act (Dutch legislation: WMO) did not apply to this study. Consequently, the committee officially stated to having no objection to the conduct of the study followed by the board of directors of our hospital giving written permission for the conduct of the study. All patients gave permission for the use for research purposes of (coded) data collected as part of regular patient care.

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Informed consent

Not applicable.

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Supplementary materials

Table S1. Univariate and multivariate analyses of radiological response, potential confounders;

Table S2. Univariate and multivariate analyses of overall survival, potential confounders.

Table S1. Univariate and multivariate analyses of radiological response, potential confounders

Radiological response (PR or CR)		Week 6			Week 12	
		Univariate analysis	Multivariate analysis[a]		Univariate analysis	Multivariate analysis[a]
Biomarker levels CEA[1]	***n***	**Crude OR (95% CI)**	**Adjusted OR (95% CI)**	***n***	**Crude OR (95% CI)**	**Adjusted OR (95% CI)**
Male	247	Ref.	Ref.	219	Ref.	Ref.
Female	198	1.21 (0.83–1.76)	1.25 (0.82–1.91)	185	1.04 (0.70–1.54)	0.98 (0.62–1.40)
Age ≤ 65 year	272	Ref.	Ref.	251	Ref.	Ref.
Age > 65 year	173	0.95 (0.65–1.40)	0.92 (0.59–1.42)	153	1.17 (0.79–1.76)	1.12 (0.70–1.78)
ECOG PS 0-1	404	Ref.	Ref.	368	Ref.	Ref.
ECOG PS ≥ 2	36	0.96 (0.49–1.91)	0.75 (0.35–1.63)	31	0.22 (0.025–2.00)	0.45 (0.19–1.08)
SCLC	124	Ref.	Ref.	116	Ref.	Ref.
NSCLC squamous	75	0.27 (0.15–0.51)	0.28 (0.15–0.55)	64	0.28 (0.15–0.53)	0.33 (0.16–0.68)
NSCLC non-squamous	219	0.19 (0.12–0.32)	0.20 (0.12–0.34)	200	0.25 (0.16–0.41)	0.24 (0.14–0.42)
NSCLC other	27	0.41 (0.17–0.97)	0.42 (0.17–1.03)	24	0.22 (0.09–0.55)	0.25 (0.09–0.67)
Stage IIIA	83	Ref.	Ref.	76	Ref.	Ref.
Stage IIIB	83	1.05 (0.57–1.93)	1.11 (0.58–2.14)	75	1.66 (0.87–3.17)	1.71 (0.84–3.47)
Stage IV	279	1.05 (0.64–1.71)	1.10 (0.63–1.92)	253	1.36 (0.81–2.30)	1.16 (0.61–2.20)
No CNS metastasis	389	Ref.	Ref.	356	Ref.	Ref.
CNS metastasis	56	0.84 (0.48–1.46)	0.66 (0.35–1.24)	48	0.66 (0.35–1.22)	0.56 (0.27–1.14)
Never smokers	39	Ref.	Ref.	37	Ref.	Ref.
Former smokers	231	0.91 (0.46–1.81)	0.71 (0.34–1.46)	204	1.44 (0.71–2.93)	1.30 (0.59–2.84)
Active smokers	166	0.89 (0.44–1.80)	0.78 (0.37–1.64)	154	1.10 (0.53–2.28)	1.14 (0.51–2.54)
1 cycle of chemo	31	Ref.	Ref.	7	Ref.	Ref.
2 cycles of chemo	414	3.09 (1.39–6.86)	2.70 (1.15–6.32)	52	0.67 (0.11–3.94)	0.82 (0.13–5.35)
3 cycles of chemo	-	-	-	125	1.90 (0.36-10.2)	2.16 (0.37-12.6)
4 cycles of chemo	-	-	-	220	3.06 (0.58-16.1)	3.88 (0.68-22.3)
Low pretreatment LDH levels (< 247 U/L)	234	Ref.	Ref.	216	Ref.	Ref.
High pretreatment LDH levels (≥ 247 U/L)	211	1.12 (0.77–1.63)	1.04 (0.69–1.58)	88	1.04 (0.71–1.54)	0.93 (0.59–1.45)

[a] Multivariate analysis adjusted for gender, age, ECOG PS, histological subtype (NSCLC squamous, NSCLC non-squamous, SCLC), cancer stage, number of cycles of first-line of chemotherapy, CNS metastasis, smoking history and pretreatment LDH level.

Table S2. Univariate and multivariate analyses of overall survival, potential confounders

Overall Survival			**Univariate analysis**	**Multivariate analysis[a]**
Variable	***n***	**Median[b] (months) (95% CI)**	**Hazard ratio (95% CI)**	**Hazard ratio (95% CI)**
Total cohort	486	12.2 (10.4–14.0)	-	-
Patient characteristics				
Male	268	11.6 (9.1–14.1)	Ref.	Ref.
Female	218	13.2 (10.7–15.7)	0.86 (0.70–1.05)	0.84 (0.68–1.04)
Age ≤ 65 year	298	12.3 (10.2–14.4)	Ref.	Ref.
Age > 65 year	188	12.2 (9.1–15.3)	1.12 (0.91–1.37)	1.11 (0.89–1.37)
ECOG PS 0-1	439	13.6 (11.9–15.3)	Ref.	Ref.
ECOG PS ≥ 2	40	7.6 (3.6–11.6)	2.00 (1.43–2.87)	1.68 (1.16–2.44)
SCLC	138	10.6 (7.8–13.4)	Ref.	Ref.
NSCLC squamous	82	12.2 (5.9–18.5)	0.87 (0.63–1.18)	0.96 (0.68–1.36)
NSCLC non-squamous	235	13.8 (11.7–15.9)	0.89 (0.70–1.13)	0.92 (0.71–1.18)
NSCLC other	31	10.0 (3.5–16.5)	0.89 (0.57–1.39)	0.82 (0.51–1.30)
Stage IIIA	94	21.3 (16.8–25.8)	Ref.	Ref.
Stage IIIB	87	17.7 (15.1–20.3)	1.31 (0.92–1.86)	1.69 (1.18–2.44)
Stage IV	305	9.4 (8.0–10.8)	2.16 (1.63–2.86)	2.51 (1.83–3.45)
No CNS metastasis	419	13.6 (11.9–15.3)	Ref.	Ref.
CNS metastasis	67	6.7 (4.1–9.3)	1.52 (1.14–2.02)	1.23 (0.91–1.67)
Never smokers	44	13.9 (11.1–16.7)	Ref.	Ref.
Former smokers	255	11.6 (9.4–13.8)	1.33 (0.92–1.93)	1.16 (0.79–1.70)
Active smokers	177	12.4 (8.8–16.0)	1.27 (0.86–1.86)	1.19 (0.80–1.77)
1 cycle of chemo	40	1.8 (1.5–2.1)	Ref.	Ref.
2 cycles of chemo	70	5.5 (3.5–7.5)	0.56 (0.37–0.84)	0.42 (0.27–0.65)
3 cycles of chemo	151	17.7 (14.2–21.2)	0.21 (0.14–0.30)	0.19 (0.13–0.28)
4 cycles of chemo	225	15.0 (13.7–16.3)	0.23 (0.16–0.33)	0.14 (0.10–0.21)
Low pretreatment LDH levels (< 247 U/L)	254	16.0 (14.0–18.0)	Ref.	Ref.
High pretreatment LDH levels (≥ 247 U/L)	232	9.5 (8.2–10.8)	1.53 (1.25–1.87)	1.42 (1.15–1.76)

[a] Multivariate analysis adjusted for gender, age, ECOG PS, histological subtype (NSCLC squamous, NSCLC non-squamous, SCLC), cancer stage, number of cycles of first-line platinum–based chemotherapy, CNS metastasis, smoking history and pretreatment LDH level.

[b] Medians were calculated using the Kaplan-Meier method. Hazard ratios were calculated in univariate and multivariate setting with COX proportional hazard modeling.

Chapter 4

General Discussion

General Discussion

Platinum-based therapy: towards individualized treatment

Lung cancer remains the leading cause of cancer-related death worldwide with many newly diagnosed patients in the Netherlands every year. Despite the rapid introduction of therapeutic innovations, platinum-based therapy is still a cornerstone of NSCLC treatment. However, platinum-based therapy is frequently accompanied by dose-limiting and severe toxicities. Some patients seem to be more susceptible to developing treatment-related toxicities. However, identifying those patients most at risk is hardly possible. When medical treatment starts, there is an immediate urge to predict and monitor its effect on patients to obtain an optimal balance between response and toxicity, particularly among those diagnosed with incurable and life-shortening diseases such as advanced lung cancer. The primary treatment goals for these patients are stabilizing the disease, symptom palliation, and maintaining or improving quality of life or life prolongation. However, patients sometimes have inaccurate perceptions of their prognoses[1,2], and are willing to be exposed to treatments with uncertain responses, possibly accompanied by (significant) toxicity.[3,4] For patients with a short life expectancy, minimizing the severity of side effects should be one of the primary treatment objectives. In addition, the risk of irreversible side effects, such as invalidating peripheral neuropathy, should also be reduced for patients treated in a curative setting as much as possible.[4] Hence, improved treatment response and toxicity prediction in individual patients, followed by informed decision-making, is warranted.

This thesis' main objective is to provide novel insights into the associations between genetic, anthropometric, and serum biomarkers for platinum-based therapy-related response and toxicity in patients with NSCLC in daily clinical practice. Understanding whether and which biomarkers are related to response and the toxicity of platinum-based therapy could contribute to individualized treatments.

The results of the individual studies presented in this thesis are placed in a broader perspective focusing on two main topics:

- The value of biomarkers for platinum-based therapy-related response and toxicity;
- Translating biomarker evidence from daily clinical practice into real-time evaluation.

The value of biomarkers for platinum-based therapy-related response and toxicity

A main challenge remains, how to stratify patients into groups that will differ substantially in platinum-based therapy-related response and toxicity. This thesis provides several biomarkers that demonstrate an association with reduced response (radiological response and overall survival) or with a higher risk for developing platinum-based therapy-related toxicity. This section reflects on the main findings and clinical implications of the individual studies described in this thesis.

Due to inter-individual variation, a medical treatment's effect can differ between patients from achieving a response to the lack of a response or an undesirable effect in the case of treatment-related toxicities. Pharmacogenomic research has already identified several genes influencing an individual's treatment response.[5,6] In the field of oncology, the application of pharmacogenetics is of great importance since chemotherapeutic agents are characterized by a delicate balance between response and toxicity.[7] As a leading example, dose adjustments of fluoropyrimidine-based therapies due to dihydropyrimidine dehydrogenase (*DPYD*) gene variants can designate those patients who need prior treatment adjustments to lower the risk of developing excessive toxicity and optimize treatment response.[6] Such findings significantly impact clinical practice and facilitate individualized treatment. To investigate the association between biomarkers and the response to and toxicity of platinum-based therapy, the PGxLUNG study (described in Chapter 2.1) was designed as a multicenter prospective follow-up study. In addition, we used a genome-wide approach to investigate the association between genetic variants and cisplatin-induced nephrotoxicity in a large cohort, complemented by a validation study in the PGxLUNG study cohort (Chapter 2.3). To the best of our knowledge, this is the first genome-wide association study (GWAS) to investigate the association between genetic variants and cisplatin-induced nephrotoxicity. Although several limitations must be acknowledged concerning the study design[8], a GWAS enabled us to identify SNPs across the entire genome. A GWAS on cisplatin-induced nephrotoxicity was performed in 608 patients with head-and-neck or esophageal cancer and validated in a cohort of 149 patients with NSCLC. We demonstrated that carrying the minor allele A in rs4388268, a common intronic variant of the *BACH2* gene, was consistently associated with an increased risk of cisplatin-induced nephrotoxicity. Carrying the A allele, which was the case in 35% of the patients in our study, increased the risk of cisplatin-induced nephrotoxicity nearly 4- and 1.7-fold in the discovery and validation cohorts, respectively. Naturally, further research is warranted to unravel the underlying mechanisms explaining the impact of

genetic predisposition of *BACH2* and cisplatin-induced nephrotoxicity, for example, through experimental studies in knock-out mice. Since the *BACH2* gene regulates B cell differentiation and function, it is biologically relevant for the pathogenesis of autoimmune diseases.[9] Although *BACH2* is moderately expressed in distal kidney tubules[10], it does not seem to be directly related to the pharmacokinetics of cisplatin (i.e., drug uptake or elimination). Therefore, the association between the genetic predisposition of *BACH2* and nephrotoxicity is probably not limited to treatment with cisplatin but could also exist with other therapeutic agents. Thus, investigating the association between the genetic predisposition of *BACH2* and nephrotoxicity in patients treated with other therapeutic regimens is highly recommended.

Klumpers *et al*. recently described the results of a GWAS on nephrotoxicity in 195 patients treated using platinum-based therapy.[11] The cohort consisted of patients with a pediatric brain tumor treated with cisplatin- or carboplatin-based regimens and adult patients with head-and-neck tumor treated with cisplatin. All the patients received treatment in the Netherlands or Italy between 2000 and 2017. A GWAS was performed to identify the genetic risk factors for nephrotoxicity by investigating both acute kidney injury and hypomagnesemia. The data was also used to replicate earlier reported genetic associations (described in Chapter 2.3). Although Klumpers *et al.'s* study could not replicate the association between *BACH2* (rs4388268) and the cisplatin-induced decrease in eGFR in adult patients, the effect's direction was similar in both studies. As indicated by the authors, cohort differences regarding disease types, clinical risk factors, and treatment regimens could have been responsible for the discrepancies in the study outcomes. A false-positive finding regarding the genetic variant of *BACH2* (rs4388268) (Chapter 2.3) is unlikely, since the association of our discovery cohort (608 patients) was replicated in a cohort of 149 patients. In addition, false-negative findings in Klumpers *et al.'s* study are far more plausible due to the independent cohort's insufficient power. Therefore, additional studies investigating the association between variants of *BACH2* (rs4388268) and nephrotoxicity are still warranted. In contrast to our expectations, we could not confirm any of the previously reported SNPs associated with nephrotoxicity.[12] Zazuli *et al.* recently performed a systematic review, including studies that used cisplatin-based treatment, had genotyping data available, and evaluated nephrotoxicity as an outcome. The review comprised 28 candidate gene studies investigating over 300 SNPs across 135 genes. However, the candidate gene studies investigating genetic biomarkers for the development of cisplatin-induced nephrotoxicity predominantly demonstrated inconsistent findings, which could have been caused by considerable patient and

treatment heterogeneity and variable study designs. Nevertheless, three genes (*ERCC1* [rs11615 and rs3212986], *ERCC2* [rs13181 and rs1799793] and *SLC22A2* [rs316019]) were found to be associated with cisplatin-induced nephrotoxicity in several studies. As described in Chapter 2.3, applying a candidate gene approach resulted in no significant or suggestive associations between these SNPs and renal toxicity.

We performed a candidate gene study (described in Chapter 2.4), selecting previously associated SNPs with neuropathy to confirm earlier findings. We demonstrated that patients with the GG genotype (11% of the patients in our cohort) of *TRPV1 (*rs879207) have an almost 5-fold higher risk of developing severe neuropathy when treated using platinum-based therapy. Naturally, functional validation of the exact roles of *BACH2* and *TRPV1* in platinum-induced nephro- and neurotoxicity warrants further investigation, and our findings need to be replicated. Nevertheless, our results can serve as an incentive for personalized clinical management. For example, when a genetic biomarker indicates a higher risk of developing nephrotoxicity, therapy monitoring in these patients should be carried out more intensively to detect a clinically relevant decline in kidney function earlier. Another potentially useful approach is to intensify preventive strategies in these patients, for example, by obtaining an optimal hydration state and earlier discontinuation of nephrotoxic co-medication. Based on genetic variants, both the optimal treatment and the intensity of clinical follow-up can be personalized in advance. This approach could also result in reduced healthcare costs and, even more importantly, less patient burden. Future research has yet to confirm whether applying genetic biomarkers contributes to optimizing platinum-based therapy. Since platinum agents have been used for a long time at a relatively low cost, the research interest for these agents could have unduly diminished in contrast to expensive drugs recently introduced to the market. In the short term, it will be challenging to determine how much burden of proof is necessary before it is generally acceptable to use genetic biomarkers for individualized platinum-based therapy in clinical practice. Open access publication and collaboration in a large (inter)national context (as described in Chapter 2.3) are warranted to overcome (several of) these limitations. In addition, future studies should focus on creating more diverse cohorts to unravel ethnic disparities.[13]

In addition to genetic biomarkers (Chapter 2), anthropometric and serum biomarkers (Chapter 3) could support individualized treatment. As described in Chapter 3.1, skeletal muscle area (SMA) segmentation was performed on abdominal imaging at the third lumbar vertebra level (L3) in all patients in the PGxLUNG study cohort. Patients with a pretreatment low skeletal muscle mass (SMM) had a significantly higher risk of developing

grade 3/4 haematological and dose-limiting toxicities. These findings raise the question of whether the addition of skeletal muscle measurements regarding platinum dosing could further reduce the risk of toxicity without compromising treatment response. Whether patients with low SMM could benefit from improved physical fitness and higher SMM status (prehabilitation) before chemotherapy, in line with preoperative physical exercise interventions[14,15], is currently not known. Since the predictive value of low SMM for developing anticancer drug-related toxicity is observed across many cancer types[16], further research regarding possible interventions to improve SMM status and adjusting treatment regimens based on the presence of low SMM is warranted. Moreover, the presence of less dense muscle seems to be associated with a worse survival in patients treated with immunotherapy.[17] This strengthens the necessity to investigate the association of skeletal muscle measurements with treatment-related response and toxicity even more, especially in patients treated with platinum-based therapy combined with immunotherapy.

Chapter 3.2 represents a study performed in a cohort of 593 patients with advanced (N)SCLC treated using first-line platinum-based therapy. This retrospective, single-center follow-up study investigated carcinoembryonic antigen (CEA) and lactate dehydrogenase (LDH) as biomarkers for the early assessment of treatment response in terms of radiological response and overall survival. Biomarker decrease (≥ 20%), particularly early in treatment, was significantly associated with an improved radiological response. Decreases in LDH and CEA levels (≥ 20%) at week three following treatment initiation were associated with an improved radiological response at week six (1.7- and 2.3-fold higher probability, respectively). Moreover, compared with unchanged or decreased biomarker levels, CEA and LDH increases (≥ 20%) were associated with a significant (1.7- and 1.6-fold higher) probability of reduced overall survival. In addition, a 1.4-fold higher probability of lower overall survival was found in patients with high pretreatment LDH levels (≥ 247 U/L). Implementing these biomarkers into clinical decision-making should be of great interest since our study results demonstrate that (changes in) CEA and LDH levels are strongly associated with treatment response. Moreover, determining these parameters is easy, non-invasive, and affordable. Interestingly, high pretreatment LDH levels[18] and changes in CEA levels[19] also appear to be associated with treatment response in patients receiving immunotherapy.

The results of these studies raise the question of how this valuable biomarker information can be translated into daily clinical practice. Risk stratification is especially relevant in cases of a discrepancy between clinical and radiological responses. For example, in routinely response evaluation performed for a patient with NSCLC treated in a palliative

setting following two cycles of platinum-based therapy, a CT scan revealed stable disease. However, the treatment was compromised by a decline in kidney function, severe fatigue, and invalidating neuropathy. In such a case, the treating physician and the patient face the dilemma of continuing, adjusting, or stopping the treatment. Unfortunately, in current practice, adequate data is often lacking to guide such vital decisions. Therefore, a reliable tool incorporating (dynamic) biomarkers and clinical data, in terms of response and toxicity, would be helpful in guiding treatment decisions for an individual patient. A tool that is already being used for patients with curative breast or lung cancer at treatment initiation is adjuvant online.[20] Estimated survival rates are generated using a prediction algorithm based on risk factors and treatment options, however this tool does not incorporate data regarding treatment-related toxicity. Another algorithm-based instrument is currently being developed to support clinical decision-making in patients with stage IV NSCLC.[21] Based on the most recent literature, the following patient characteristics should be considered in such an algorithm: age, gender, and ECOG performance status. These factors have proven to be well-known predictors for overall survival in advanced NSCLC.[22] In addition, tumor histology and the treatment regimen are essential predictors of treatment outcome as well. Based on the results of our data, incorporating pharmacogenetic biomarkers and skeletal muscle measurements could enrich a predictive model in terms of toxicity. Combining biomarker information may be particularly relevant, since most biomarkers are only suitable to predict either treatment response or toxicity. However, in clinical practice, treatment decisions are based on the balance between both benefit and harm. A synergetic effect may be achieved by combining information from different biomarkers in a multifactorial algorithm. In addition to the patients personal treatment goals, such a multifactorial algorithm should combine static (e.g. genotypes, anthropometric measurements at treatment initiation) and dynamic information (e.g. serum levels of CEA, LDH, anthropometric biomarkers during treatment). Obviously, more accurate prediction of both treatment response and toxicity will influence clinical decision-making.

When implementing such a multifactorial algorithm in daily clinical practice, accurate and up-to-date registration by those involved in the anticancer treatment is essential for enabling continuous treatment assessment using dynamic biomarkers and up-to-date clinical information. Ongoing evaluation is particularly relevant given the recent introduction of immunotherapy in addition to platinum-based therapy in treating NSCLC, which has resulted in new treatment perspectives and strategies but also differences in treatment-related toxicities.[23] Although chemotherapy-induced toxicities most frequently

occur after the first chemotherapy course[24], there is no set time window during which patients could experience treatment-related toxicities from immunotherapy.[25] Accounting for timing (e.g., time-to-event, time-to-dose reduction) could be crucial when studying the factors that predict toxicity.[24] Performing such analyses in the PGxLUNG study cohort and using data from patients treated using a combination of platinum-based therapy and immunotherapy could be valuable and is recommended.

To summarize, the results presented in this thesis provide evidence for associations between genetic, anthropometric, and serum biomarkers for platinum-based therapy-related response and toxicity in patients with NSCLC in a daily clinical practice setting. These results encourage an individualized approach to platinum-based therapy. A synergetic effect could be achieved by combining information from different biomarkers when treating patients using platinum-based therapy. This approach should be investigated in future studies and confirmed in daily clinical practice.

Translating biomarker evidence from daily clinical practice into real-time evaluation

When a biomarker is able to stratify patients into groups that differ in platinum-based therapy-related response and toxicity, the next challenge is to implement this into daily clinical practice. This section provides future perspectives on translating our novel insights, with the ultimate goal to further individualize platinum-based therapy in patients with NSCLC.

Optimizing data collection and analysis

As described in this thesis, post-marketing evaluation of treatment response and toxicity is relevant for tailoring therapy. In daily clinical practice, anticancer treatments are carried out using a multidisciplinary approach, in which the collaboration of many healthcare professionals (i.e., oncologists, radiologists, nurse practitioners, hospital pharmacists, clinical pharmacologists, and medical dietitians) is critical. These medical care providers have access to the patient's medical record, a digital record in hospital electronic information systems that is largely text-based and often has only a partially structured form. This data could be of significant value in outlining patient profiles and determining whether an individual will respond to treatment. In the PGxLUNG study (Chapter 2.1), 350 patients were recruited from six hospitals in the Netherlands. Data collection was performed manually, which limits its feasibility, given its time-consuming

nature. Since treatment evaluation is critical in daily clinical practice, automating this process is warranted. Automation could be achieved through structured registration by clinicians of, for example, side effects according to validated tools such as the common toxicity criteria.[26] Systematic registration could pave the road for automated information extraction and real-time evaluation. An example of such a registration database is the nationwide Dutch Melanoma Treatment Register[27], in which detailed information is obtained continuously from all patients diagnosed with melanoma. Moreover, for data that has already been reported in electronic patient reports, other (artificial intelligence) techniques, such as text mining, could be used to optimize the use of existing data. Several initiatives in this area have already been examined and proven useful.[28-30] To summarize, efforts should be made to optimize data collection in daily clinical practice. Hence, rapid commissioning of systematic registration and automated information extraction tools should be promoted to support the process of treatment optimization.

In addition, attention should be focused on further automating diagnostic tools. For example, manually segmenting skeletal muscle mass in clinically acquired CT scans (described in Chapter 3.1) requires multiple, time-consuming steps, limiting its use in clinical practice. Fortunately, implementing routine skeletal muscle measurements in clinical practice is emerging with the development of automated methods.[31] Automated body-composition analysis could be fully integrated into patients' electronic health records and used quickly and optimally without additional costs or patient burden. Moreover, using deep-learning techniques to analyze CT scans, accurate and reproducible body-composition segmentation could provide additional information and serve as imaging biomarkers. For example, Pieters *et al*. recently described how deep-learning-based extraction of body-composition parameters in abdominal CT scans could be used to estimate creatinine production reliably.[32] The presented algorithm could improve the estimation of renal function in patients who have recently undergone a CT scan. The proposed methods provide an improved estimation of renal function that is fully automatic and can be readily implemented in routine clinical practice. This information could be particularly relevant when calculating the carboplatin dosage for patients with a different body composition.

Patient-reported outcome measurements: real-time registration

Communication between clinicians and patients is increasingly executed digitally, offering the potential to incorporate the patient's treatment experiences directly into a medical record. An example is the use of digital applications for registering chemotherapy-

related side effects. Direct registration by patients or supported by their relatives or caregivers provides valuable information regarding treatment response and toxicity.[33] Hence, registration should not be limited to pre-defined moments during clinical follow-up, usually only planned immediately before a new chemotherapy course begins. Up-to-date information regarding the patient's condition could support earlier recognition of complications and lead to earlier intervention if side effects occur, preventing worsening symptoms. For example, involuntary weight loss and/or loss of appetite between two hospital visits could be detected earlier if a registration application is used, enabling action (dietician counseling, starting supplementary feeding) to be taken more quickly. Such interventions could also increase the likelihood of completing the predetermined number of chemotherapy cycles without adjustments, potentially increasing treatment effectiveness and improving the quality of care. Such patient registration tools are already available or being developed.[34]

In addition, participants in the PGxLUNG study were asked to complete questionnaires on health-related quality of life during treatment. These results are highly relevant and could contribute to a better understanding of the effect of platinum-based therapy on quality of life in daily clinical practice. Moreover, future studies could also benefit from these results by using them to compare their own interventions. Furthermore, it would be of significant interest to investigate the impact of decision support tools on treatment-related regret since the risk of regret exists in almost every medical decision a patient makes.[35] Treatment-related regret has already been described by many patients with advanced NSCLC receiving systemic treatment.[36] Therefore, active surveillance of decision regret, once a treatment option is completed and when the treatment is ongoing, would be valuable. Eventually, this approach could help identify patients at risk of regret, enabling clinicians to anticipate and support a patient's decision-making, considering personal needs and (changing) treatment goals.[36-38]

In summary, structured data collection by clinicians, automated and sophisticated data evaluation, and real-time registration of patient-reported outcomes, provide opportunities to support further individualization of platinum-based therapy.

Conclusions

The studies described in this thesis support identifying patients at a higher risk of developing platinum-based therapy-related toxicity based on specific genetic (i.e., *BACH2*, *TRPV1* variants) and anthropometric (i.e., low skeletal muscle mass and density) biomarkers. In addition, CEA and LDH levels, at the initiation and during treatment, may serve as valuable biomarkers to determine treatment response in patients receiving platinum-based therapy. Since no single treatment fits every patient, clinicians should consider all available patient characteristics to achieve individual treatment goals. Future studies should focus on validating our findings, automating information extraction, and combining real-time (patient-reported) data with validated algorithm-based care. Ultimately, this recommendation should lead to an improved benefit-harm ratio that provides and encourages an individualized approach to platinum-based therapy in patients with NSCLC.

Declarations

Authors' contributions

C. de Jong wrote the General Discussion of this thesis. Dr. V.H.M. Deneer, dr. G.J.M. Herder and prof. dr. A.C.G. Egberts reviewed the manuscript critically for important intellectual content and approved the final version.

Competing interests

The authors declare that they have no competing interests.

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Chapter 5

Summary
Nederlandse samenvatting

Summary

Biomarkers for individualizing platinum-based therapy of patients with non-small cell lung cancer

This thesis presents the results of several studies focusing on biomarkers for individualizing platinum-based therapy in patients with non-small cell lung cancer (NSCLC).

Lung cancer remains the leading cause of cancer-related death worldwide. In the Netherlands, more than 14,000 patients are diagnosed with lung cancer each year. Despite the rapid introduction of therapeutic innovations, platinum-based therapy remains a cornerstone of NSCLC treatment. However, platinum-based therapy is often accompanied by dose-limiting and severe toxicities, such as haematological toxicities, nephro- and neurotoxicity. Frequently, severe toxicities necessitate dose reduction, resulting in treatment delay, treatment changes, or even early treatment termination, which can affect treatment response and, thus, disease prognosis. Some patients seem to be more susceptible to developing disabling side effects. However, identifying those patients with the highest risk of developing toxicity is hardly possible. Risk stratification is especially relevant in cases of a discrepancy between clinical and radiological responses. For example, in a routine response evaluation of a patient with NSCLC treated in a palliative setting following two cycles of platinum-based therapy, a CT scan revealed stable disease. However, the treatment was compromised by a decline in kidney function, severe fatigue, and invalidating neuropathy. In such a case, the treating physician and the patient face the dilemma of continuing, adjusting, or stopping the treatment. Unfortunately, in current practice, adequate data is often lacking to guide such vital decisions. Hence, improved prediction of treatment outcomes for an individual patient, in terms of response and toxicity, followed by informed decision-making, is warranted.

Therefore, the studies described in this thesis were designed to provide novel insights into the association between genetic, anthropometric, and serum biomarkers for platinum-based therapy-related response and toxicity in patients with NSCLC in a daily clinical practice setting.

Genetic biomarkers for platinum-based therapy-related toxicity

Chapter 2 of this thesis focuses on the genetic predisposition for developing platinum-based therapy-related toxicity, described in four studies. Chapter 2.1 presents the design of the PGxLUNG study, a multicenter prospective cohort study. The primary study

objective was to investigate the association between genetic variants and therapy-related toxicity in patients with NSCLC undergoing first-line platinum-based therapy. Secondary objectives included exploring the association between anthropometric and serum biomarkers for platinum-based therapy-related response and toxicity. Between February 2016 and August 2019, 350 patients were recruited from an academic hospital (University Medical Center Utrecht), two teaching hospitals (St. Antonius Hospital Nieuwegein/ Utrecht and Meander Medical Center Amersfoort), and three general hospitals (Diakonessenhuis Utrecht, Groene Hart Ziekenhuis Gouda, and Ziekenhuis Rivierenland Tiel) in the Netherlands. A significant advantage of this study's design is that the data was collected prospectively in a daily clinical practice setting. Consequently, the results reflect daily clinical practice, strengthening the findings' translation to current practice.

Chapter 2.2 describes a young woman diagnosed with squamous cell cervix carcinoma with severe and irreversible nephropathy following three low weekly doses of cisplatin. Besides several known risk factors for cisplatin-induced nephrotoxicity (such as hypomagnesemia and hypoalbuminemia), the patient proved to be homozygous for two polymorphisms within the *COMT* gene (c.615+310C>T [rs4646316] and c.616–367C>T [rs9332377]). Since polymorphisms within the *COMT* gene have been associated with cisplatin-induced ototoxicity, a link between these polymorphisms and the observed cisplatin-induced nephrotoxicity was suggested, and recommendations were made for further investigation.

Chapter 2.3 describes a study evaluating genetic risk factors for cisplatin-induced nephrotoxicity. A genome-wide study (GWAS) was conducted on genetically estimated Europeans in a discovery cohort of cisplatin-treated adults from Toronto, Canada, complemented by a validation study in the PGxLUNG study cohort. In addition, previously reported genetic associations were further examined in the discovery and validation cohorts. Nephrotoxicity was assessed in two ways: (I) decreased estimated glomerular filtration rate (eGFR), calculated using the Chronic Kidney Disease Epidemiology Collaboration formula (CKD-EPI), and (II) increased serum creatinine, according to the Common Terminology Criteria for Adverse Events (CTCAE v4.03), for acute kidney injury. In the discovery cohort (n = 608), five single-nucleotide polymorphisms (SNPs) achieved genome-wide significance. Carrying the minor allele A (minor allele frequency = 0.23) in *BACH2* (rs4388268), a common intronic variant, increased the risk of cisplatin-induced nephrotoxicity nearly 4- and 1.7-fold in the discovery and validation cohorts, respectively. The genetic predisposition of *BACH2* (rs4388268) could be significant in the development of cisplatin-induced nephrotoxicity, indicating opportunities for mechanistic understanding, tailored therapy,

and preventive strategies. Previously reported genetic associations from candidate gene studies could not be confirmed after correction for multiple testing.

Chapter 2.4 describes a candidate gene approach focusing on the association between genetic variants and chemotherapy-induced peripheral neuropathy. The evaluated SNPs were selected based on a review of the existing evidence. The relationship between 34 SNPs in 26 genes and chemotherapy-induced peripheral neuropathy (CIPN) was investigated. Neuropathy was clinically evaluated at baseline, before each chemotherapy cycle and, at three and six months after treatment initiation, using the CTCAE v4.03 for peripheral sensory neuropathy (any grade and severe CIPN defined as grades ≥ 1 and ≥ 2, respectively). In total, 320 patients in the PGxLUNG study cohort were included, of which 26.3% (*n* = 84) and 8.1% (*n* = 26) experienced any grade and severe CIPN, respectively. The GG genotype (rs879207, A>G) of *TRPV1* (minor allele frequency G-allele = 0.32), a gene expressed in peripheral sensory neurons, was associated with an almost 5-fold higher risk for the development of severe neuropathy following treatment using platinum-based therapy. Future studies should be conducted in an independent cohort to validate these findings. In addition, the implementation of these results in daily clinical practice should be investigated in clinical intervention studies focused on further individualization of platinum-based therapy to prevent the occurrence of neuropathy.

Anthropometric and serum biomarkers for platinum-based therapy-related response and toxicity

Chapter 3 of this thesis describes anthropometric measurements and serum biomarkers as predictors for treatment response and toxicity, outlined in two studies.

Chapter 3.1 describes the use of pretreatment diagnostic CT scans to predict the value of skeletal muscle mass (SMM) for toxicity. Patients in the PGxLUNG study cohort were included if pretreatment imaging was available. Skeletal muscle area (SMA) segmentation was performed on abdominal imaging at the third lumbar vertebra level (L3). The SMA at L3 was corrected for squared height (m^2) to yield the lumbar skeletal muscle mass index (LSMI). Skeletal muscle density (SMD) was calculated as the mean Hounsfield Unit (HU) of the segmented SMA. The SMM and SMD were categorized as low, intermediate, and high, based on the LSMI and mean HU tertiles, respectively. Chemotherapy-induced toxicity was scored using CTCAE v4.03 and categorized into haematological (anemia, leukocytopenia, neutropenia, and thrombocytopenia), non-haematological (nephrotoxicity, neurotoxicity, and esophagitis), and dose-limiting toxicity (DLT) (treatment switch, delay, de-escalation,

discontinuation, or hospitalization). Among 297 patients, 36.6% (*n* =108) experienced haematological toxicity grade 3/4, 24.6% (*n* = 73) experienced non-haematological toxicity grade ≥ 2, and 55.6% (*n* = 165) experienced DLT. Patients with low SMM pretreatment had a significantly higher risk of haematological toxicities grade 3/4 and DLT. Patients with high SMD had a significantly lower risk of DLT. Future studies should investigate whether platinum dosing based on skeletal muscle measurements and/or improvement in SMM/SMD pretreatment could reduce the risk of toxicity without compromising efficacy.

Chapter 3.2 describes a study in which pretreatment serum levels of carcinoembryonic antigen (CEA) and lactate dehydrogenase (LDH), and changes from pretreatment levels, were studied as biomarkers for early assessment of radiological response and overall survival. A retrospective follow-up study of 593 consecutive patients with advanced (N) SCLC, treated using first-line platinum-based therapy in a large teaching hospital in the Netherlands from 2012 to 2017, was conducted. Decreases in CEA and LDH (≥ 20%), particularly in early treatment, were significantly associated with improved radiological response (partial response [PR] or complete response [CR], according to RECIST 1.1). Increases in these biomarkers (≥ 20%) and high pretreatment LDH levels (≥ 247 U/L) were significantly associated with lower overall survival. These results support the earlier use of CEA and LDH levels to assess response to platinum-based therapy in patients with advanced (N)SCLC. Biomarker assessment could be particularly relevant alongside radiological evaluation to evaluate patients with a mixed radiological response or in the case of a discrepancy between clinical and radiological responses.

In conclusion, this thesis provides novel insights into biomarkers for platinum-based therapy-related response and toxicity. Chapter 4, General Discussion, places the results of the individual studies presented in this thesis in a broader perspective. In addition, the main findings and potential clinical implications are discussed. These results encourage an individualized approach and contribute to optimizing platinum-based therapy in patients with NSCLC.

Samenvatting

Biomarkers voor individualisatie van platinumhoudende therapie bij patiënten met niet-kleincellige longkanker

Dit proefschrift beschrijft de resultaten van verschillende studies naar het gebruik van biomarkers voor de individualisatie van de behandeling met platinumhoudende therapie van patiënten met niet-kleincellige longkanker.

Wereldwijd is longkanker de hoofdoorzaak van alle aan kanker gerelateerde sterfte. In Nederland wordt ieder jaar bij meer dan 14.000 mensen longkanker vastgesteld. Er zijn verschillende vormen van longkanker: kleincellige longkanker (SCLC) en niet-kleincellige longkanker (NSCLC). Bij 85% van de patiënten met longkanker betreft het NSCLC. De afgelopen jaren zijn er meerdere nieuwe behandelopties beschikbaar gekomen, zoals immunotherapie. Helaas zijn deze nieuwe behandelopties voor slechts een deel van de patiënten geschikt. Daarom blijft chemotherapie, al dan niet in combinatie met immunotherapie, vaak de behandeling van eerste keus. Deze chemotherapie bestaat uit een combinatie van verschillende stoffen, waaronder platinaverbindingen zoals cisplatine en carboplatine. Behandeling met platinumhoudende therapie gaat echter vaak gepaard met ernstige toxiciteit, zoals hematologische toxiciteit, nier- en zenuwschade. Frequent resulteert deze toxiciteit in een verlaging van de dosering, uitstel van behandeling of zelfs het vroegtijdig stoppen van chemotherapie. Aanpassingen van de behandeling kunnen mogelijk nadelig zijn voor de effectiviteit van de behandeling en leiden tot een slechtere prognose. Bij sommige patiënten lijkt het risico op het ontwikkelen van (ernstige) toxiciteit verhoogd te zijn. Dit risico is echter slecht te voorspellen en daarom is er behoefte aan voorspellers (biomarkers) voor toxiciteit. Een risico inschatting kan in het bijzonder relevant zijn wanneer de beeldvorming en de klinische toestand van een patiënt niet met elkaar overeenkomen. Ter illustratie, bij een patiënt met NSCLC die in een palliatieve setting wordt behandeld is na twee kuren platinumhoudende therapie routinematig een CT-scan gemaakt. De scan laat zien dat de tumor niet groter is geworden, hetgeen aangeeft dat de chemotherapie effectief is. Echter, als gevolg van de platinumhoudende therapie heeft de patiënt nierschade, ernstige vermoeidheid en zenuwschade (neuropathie) ontwikkeld. In dit geval staan de behandelend arts en de patiënt voor het dilemma om de behandeling te continueren, aan te passen of vroegtijdig te stoppen. In de huidige praktijk ontbreekt het echter aan adequate data om een dergelijke cruciale beslissing te kunnen nemen. Biomarkers zouden kunnen helpen bij het identificeren van patiënten met een verhoogd risico op (ernstige) toxiciteit door de behandeling met platinumhoudende therapie.

Het doel van de beschreven studies in dit proefschrift is om te bepalen of er een verband bestaat tussen genetische biomarkers, de lichaamssamenstelling (antropometrische biomarkers) en biomarkers in het bloed met de behandeleffectiviteit en toxiciteit van platinumhoudende therapie. De studies zijn uitgevoerd bij patiënten met NSCLC die werden behandeld in de dagelijkse praktijk.

Genetische biomarkers voor toxiciteit van platinumhoudende therapie

Hoofdstuk 2 richt zich op het onderzoek naar genetische aanleg voor het ontwikkelen van door platinumhoudende therapie geïnduceerde toxiciteit, hetgeen wordt beschreven in vier studies.

Hoofdstuk 2.1 beschrijft de studieopzet van de PGxLUNG studie, een prospectieve cohort studie. Het primaire doel van de PGxLUNG studie was het onderzoeken van het verband tussen genetische aanleg en het optreden van toxiciteit, zoals nier- en zenuwschade, geïnduceerd door platinumhoudende therapie. Als secundair doel werd onder andere de mogelijke relatie tussen antropometrische biomarkers (waaronder skeletspiermassa) en toxiciteit van platinumhoudende therapie onderzocht. Aan de PGxLUNG studie hebben in totaal 350 patiënten deelgenomen. Deze patiënten werden in de periode tussen februari 2016 en augustus 2019 in zes verschillende ziekenhuizen in Nederland behandeld (St. Antonius Ziekenhuis Nieuwegein/Utrecht, Diakonessenhuis Utrecht, Groene Hart Ziekenhuis Gouda, Meander Medisch Centrum Amersfoort, Rivierenland Ziekenhuis Tiel, Universitair Medisch Centrum Utrecht). Een belangrijk voordeel van de studieopzet is dat de gegevens prospectief werden verzameld in verschillende ziekenhuizen. Als gevolg hiervan weerspiegelen de resultaten de dagelijkse klinische praktijk, wat extrapolatie van de resultaten naar de huidige behandeling van patiënten met longkanker mogelijk maakt.

Hoofdstuk 2.2 beschrijft een jonge vrouw met baarmoederhalskanker die na drie wekelijkse toedieningen van cisplatine ernstige en blijvende nierschade ontwikkelde. Om onderliggende oorzaken van deze nierschade uit te zoeken werd een farmacogenetische analyse uitgevoerd. Naast meerdere bekende risicofactoren voor het ontwikkelen van nierschade als gevolg van behandeling met cisplatine (zoals een laag magnesium en laag albumine gehalte in het bloed), bleek de patiënte ook drager te zijn van twee genetische variaties in het *COMT* gen (c.615+310C>T [rs4646316] en c.616-367C>T [rs9332377]). Aangezien genetische variaties in het *COMT* gen eerder in verband zijn gebracht met cisplatine-geïnduceerde gehoorschade, werd verondersteld dat de gevonden genetische aanleg bij deze patiënte een rol zou kunnen hebben gespeeld bij het ontstaan van de nierschade.

Hoofdstuk 2.3 beschrijft een genoombrede associatiestudie (GWAS), uitgevoerd in een cohort (*n* = 608) met volwassenen van Europese afkomst uit Toronto (Canada) die zijn behandeld met cisplatine. Vervolgens werd een kandidaat-gen studie uitgevoerd binnen de met cisplatine behandelde patiënten van Europese afkomst (*n* = 149) van het PGxLUNG studie cohort. Het doel van deze studie was het onderzoeken van het verband tussen nieuwe en eerder beschreven genetische varianten en het optreden van cisplatine-geïnduceerde nierschade. Uit de resultaten van deze studie werd geconcludeerd dat genetische aanleg door variaties in het *BACH2* gen (rs4388268) belangrijk zou kunnen zijn bij het optreden van door cisplatine-geïnduceerde nierschade. Toekomstige studies zouden zich verder moeten verdiepen in het onderliggende mechanisme en de bruikbaarheid van het *BACH2* gen (rs4388268) als genetische biomarker. Deze resultaten kunnen mogelijk leiden tot betere, persoonsgerichte behandeling of preventieve maatregelen ter voorkoming van door cisplatine-geïnduceerde nierschade.

Hoofdstuk 2.4 beschrijft een kandidaat-gen studie die zich richt op het verband tussen genetische aanleg en door platinumhoudende chemotherapie geïnduceerde perifere neuropathie (CIPN). Patiënten die neuropathie ontwikkelen ervaren vaak veranderingen in het gevoel (bijvoorbeeld prikkelende of tintelende handen en voeten), zenuwpijn en vermindering van spierkracht. De onderzochte genetische variaties (single nucleotide polymorfismen [SNPs]), werden geselecteerd op basis van reeds bestaande gegevens in de literatuur. De relatie tussen 34 SNPs in 26 genen en CIPN werd onderzocht. In deze studie werd de ernst van de neuropathie beoordeeld voorafgaand aan de behandeling met platinumhoudende therapie, vóór elke chemotherapiecyclus en drie en zes maanden na de start van de behandeling. De ernst van de neuropathie werd uitgedrukt op basis van de CTCAE v4.03 graderingslijst. CIPN en ernstige CIPN, waarbij beperkingen optreden in de algemene dagelijkse activiteiten, werden gedefinieerd als respectievelijk graad ≥ 1 en ≥ 2. In deze studie werden de gegevens van totaal 320 patiënten van het PGxLUNG cohort onderzocht. Van deze patiënten ondervond 26,3% (*n* = 84) enige mate van CIPN en 8,1% (*n* = 26) ernstige CIPN. Een variatie in *TRPV1* (rs879207), een gen dat tot uiting komt in perifere zenuwen, werd in verband gebracht met een bijna vijfmaal hoger risico op het ontstaan van ernstige CIPN. Toekomstige studies in een onafhankelijk cohort zouden deze bevindingen moeten valideren. Vervolgens zouden deze resultaten moeten worden onderzocht in de dagelijkse klinische praktijk, met als doel verdere individualisatie van therapie en mogelijke interventies ter preventie van CIPN.

Antropometrische biomarkers en biomarkers in het bloed voor behandeleffectiviteit en toxiciteit van platinumhoudende therapie

Hoofdstuk 3 van dit proefschrift beschrijft in twee studies antropometrische biomarkers en biomarkers in het bloed als voorspellers van behandeleffectiviteit en toxiciteit.

Hoofdstuk 3.1 beschrijft het gebruik van skeletspiermassa (SMM) metingen middels diagnostische CT-scans voor het voorspellen van toxiciteit van platinumhoudende therapie. Dit werd onderzocht bij de patiënten van het PGxLUNG cohort bij wie een CT-scan beschikbaar was (*n* = 297). Op de CT-scan, die voorafgaand aan de behandeling was gemaakt, werd het skeletspiergebied (SMA) bepaald ter hoogte van de derde lendenwervel (L3) van de onderrug. Vervolgens werd deze waarde gecorrigeerd voor lichaamslengte in het kwadraat (m^2), om de totale skeletspiermassa in verhouding tot de lengte te kunnen schatten (lumbale skeletspiermassa-index, LSMI). De gemiddelde skeletspierdichtheid (SMD) werd berekend als het gemiddelde van de CT-waarde of Hounsfield Unit (HU) van het gesegmenteerde SMA. Alle SMM en SMD metingen werden gecategoriseerd in drie gelijke groepen: laag, gemiddeld en hoog, op basis van respectievelijk LSMI en HU. De door platinumhoudende therapie geïnduceerde toxiciteit werd gescoord naar ernst en gecategoriseerd in hematologische toxiciteit (bloedarmoede, tekort aan witte bloedcellen en bloedplaatjes), niet-hematologische toxiciteit (nier-, zenuw- en slokdarmschade) en dosisbeperkende toxiciteit (DLT) (wisselen, uitstellen of stoppen van behandeling, dosisverlaging of ziekenhuisopname). Van de 297 patiënten had 36,6% (*n* = 108) last van ernstige hematologische toxiciteit, 24,6% (*n* = 73) ondervond niet-hematologische toxiciteit en 55,6% (*n* = 165) ondervond DLT. Patiënten met een lage SMM voorafgaand aan de behandeling hadden een significant hoger risico op het ontstaan van ernstige hematologische toxiciteit en DLT. Patiënten met een hoge SMD hadden een significant lager risico op DLT. Toekomstige studies moeten onderzoeken of dosering gebaseerd op skeletspiermassametingen, alsook het verbeteren van de spierkwaliteit voorafgaand aan behandeling, kan leiden tot een lager risico op toxiciteit zonder afbreuk te doen aan de behandeleffectiviteit.

Hoofdstuk 3.2 beschrijft een studie waarin de concentraties van carcinoembryonaal antigeen (CEA) en lactaatdehydrogenase (LDH) in het bloed werden onderzocht. Er werd een retrospectieve follow-up studie uitgevoerd waarin 593 patiënten met gevorderde longkanker werden geïncludeerd. Deze patiënten werden allen behandeld in het St. Antonius Ziekenhuis te Nieuwegein/Utrecht in de periode januari 2012 tot en met

december 2017. De bloedwaarden van CEA en LDH werden bestudeerd als biomarkers voor de vroege beoordeling van behandeleffectiviteit, in de vorm van radiologische respons en overleving. Hiervoor werden de veranderingen in de concentraties van CEA en LDH gedurende de behandeling met platinumhoudende therapie en de gemeten concentraties vóór behandeling met elkaar vergeleken. De resultaten toonden dat een daling (≥ 20%) van de CEA en LDH concentratie, vooral aan het begin van de behandeling, verband hield met een betere radiologische respons. Stijging van CEA en LDH concentraties (≥ 20%) en hoge LDH concentraties (≥ 247 U/L) voorafgaand aan de behandeling waren significant geassocieerd met een kortere overleving. Deze resultaten ondersteunen het meten van de CEA en LDH concentraties om de behandeleffectiviteit van platinumhoudende therapie te beoordelen.

Conclusie

De studies beschreven in dit proefschrift worden bediscussieerd in de algemene discussie (**Hoofdstuk 4**), waarbij de bevindingen in een breder perspectief worden geplaatst.

Samenvattend verschaft dit proefschrift nieuwe inzichten in genetische biomarkers, antropometrische biomarkers en biomarkers in het bloed in relatie tot de behandeleffectiviteit en toxiciteit van platinumhoudende therapie. De resultaten van de beschreven onderzoeken kunnen leiden tot een betere, persoonsgerichte behandeling met platinumhoudende therapie van patiënten met longkanker.

Chapter 6

Appendices

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Curriculum vitae

Corine de Jong was born on December 10th 1987, in Utrecht, the Netherlands. After finishing Gymnasium at the Oosterlicht College in Nieuwegein in 2006, she studied Pharmacy at the University of Utrecht. As part of her Master's of Science programme, she performed a research internship at the department of Cardiology at the St. Antonius Hospital in Nieuwegein/Utrecht and worked in a Clinical Pharmacy in British Columbia, Canada. After graduation as a PharmD in 2012, she worked as a pharmacist at the St. Antonius Hospital in Nieuwegein/Utrecht. In 2014 she started a residency in hospital pharmacy at the St. Antonius Hospital and Catharina Hospital in Eindhoven (supervisors drs. M.M. Tjoeng, prof. dr. C.A.J. Knibbe, dr. E.M.W. van de Garde, dr. R. ten Broeke). During her residency she started with the PGxLUNG study, resulting in this thesis, supervised by prof. dr. A.C.G. Egberts, dr. V.H.M. Deneer and dr. G.J.M. Herder. After registration as a hospital pharmacist in 2018, she worked at the UMC Utrecht which was combined with her PhD research. From 2021 she works as a hospital pharmacist at the Diakonessenhuis Utrecht and started her training at the UMC Utrecht to become a clinical pharmacologist. From April 2023 she will hold a position as a hospital pharmacist at Tergooi MC in Hilversum. Corine lives in Utrecht, together with her husband Paul Rootjes and their two sons, Matthijs and Bram.

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