PERSONALIZING CHEMOTHERAPY IN ADVANCED NON-SMALL-CELL LUNG CANCER:

new insights from pemetrexed

Sabine Visser

COLOFON

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Personalizing Chemotherapy in Advanced Non-Small-Cell Lung Cancer: New Insights from Pemetrexed

Het personaliseren van chemotherapie voor het gevorderd niet-kleincellig longcarcinoom: nieuwe inzichten vanuit pemetrexed

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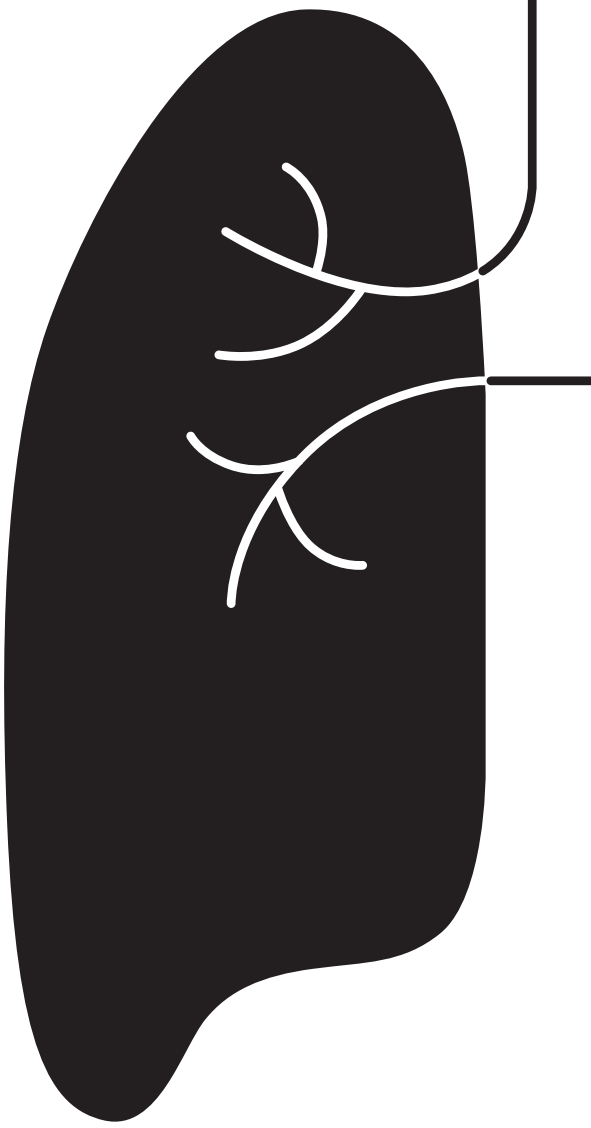
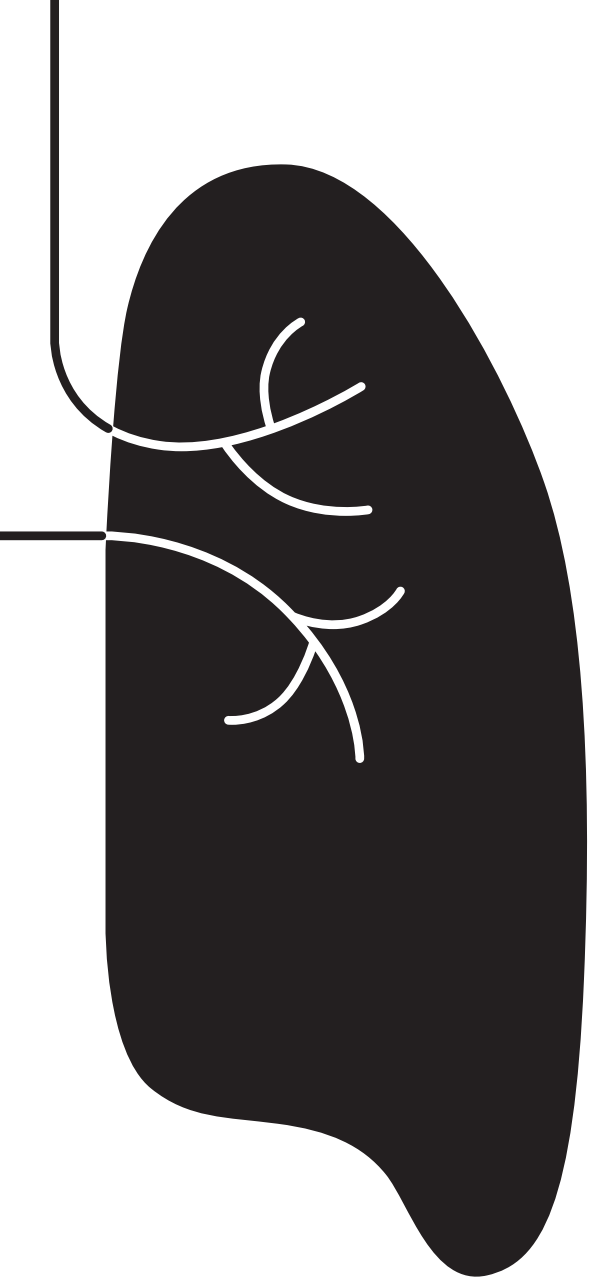




CHAPTER 1

Introduction

After the discovery of the cytotoxic effect of mustard gas during World War I, the first clinical trials with nitrogen mustard during World War II ultimately led to development of chemotherapy.[1] Ever since, chemotherapy has been a cornerstone for many anticancer treatment regimens of hematological and solid malignancies.[2] In the 1930s, a "new haemopoietic factor" was identified in yeast that was able to cure macrocytic anemia,[3] which later has been identified to be often caused by folate acid and vitamin B12 deficiency. In the process of chemically identifying this factor ultimately as folic acid, closely related compounds were synthesized with interesting abilities to interfere in the folic acid pathway. It was then reported in 1948 by Sidney Farber that one of these folate analogues, aminopterin, was effective in the treatment of childhood leukaemia by producing complete remission, due to the major role folates play in the de novo biosynthesis of purines and thymidine.[4]

Further research into the development of antifolates as antitumor agents has been actively pursued since the 1950s, and aminopterin was replaced a few years later by methotrexate because of greater efficacy and a less toxic profile.[5] It was discovered that the antifolate action of methotrexate was mainly due to the potent inhibition of dihydrofolate reductase (DHFR), an important enzyme in the folate acid cycle. It plays a role in maintaining the intracellular tetrahydrofolate (THF) cofactor pool that is needed by several THF-cofactor dependent enzymes. Ultimately, these are required for the purine and thymidine production, critical to RNA and DNA synthesis.[6] After the implementation of methotrexate as a standard therapy in different cancer treatment regimens there was an arising awareness of the effectivity of antifolates and the relative mild toxicity. In the mid-70s of the previous century, our knowledge of the working mechanism of methotrexate was further broadened. Like physiologic folates, methotrexate is metabolized into polyglutamates intracellularly. These polyglutamates are better retained intracellularly, resulting in enhanced chemotherapeutic efficacy.[7] But also, polyglutamation showed to alter the spectrum of activity of methotrexate: as a polyglutamate it also inhibited other THF cofactor-dependent enzymes to an important extent, in contrast to the monoglutamate form only inhibiting DHFR.[8,9] In the next decades, these new insights led to the increasing interest in the identification of new antifolates achieving their major activities in their polyglutamate forms. In 1992, this resulted in the discovery of the first multi-targeted antifolate structure, now known as pemetrexed.[10] Pemetrexed is more easily polyglutamated than methotrexate and its stronger affinity for target enzymes both involved in the purines and thymidine formation, leads to a beneficial multilevel inhibition of DNA synthesis.[11]

ROLE OF PEMETREXED IN TREATMENT OF NON-SMALL-CELL LUNG CANCER

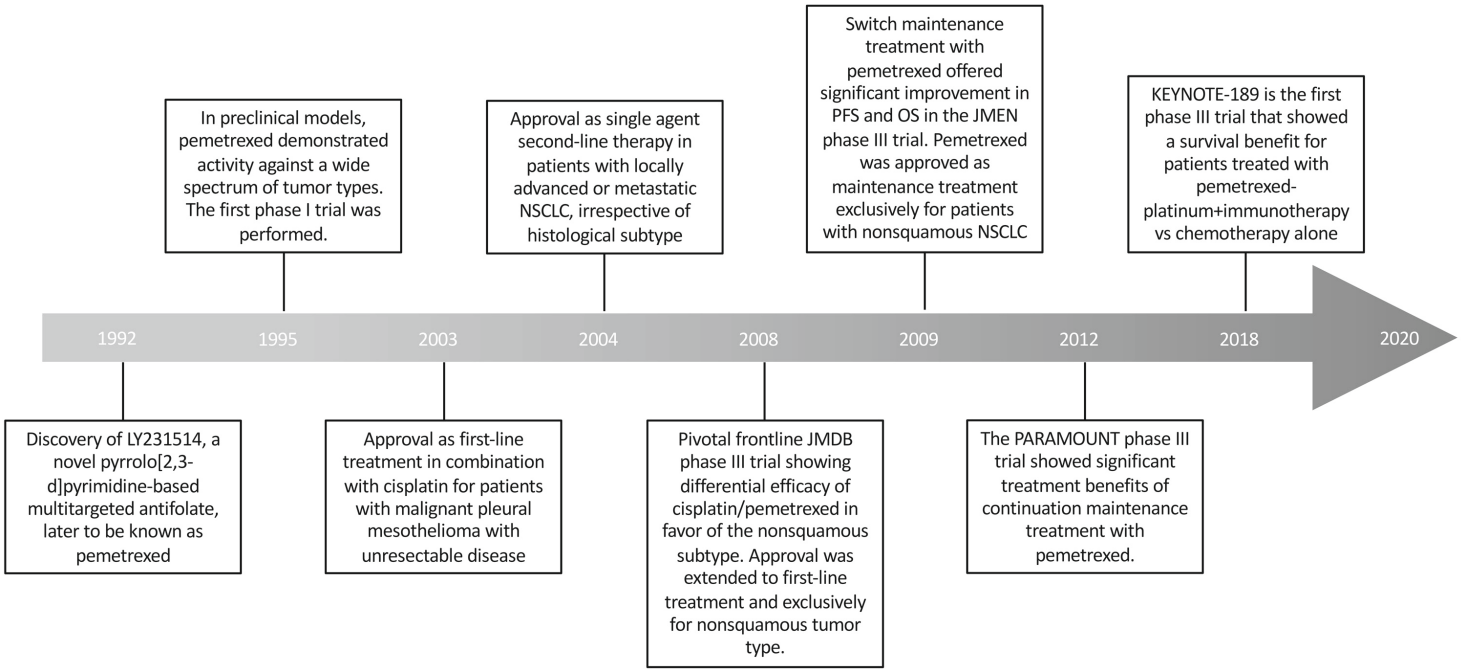
After the revolutionary findings in the 1990s about the beneficial effects of chemotherapy on survival compared to best supportive care in advanced non-small-cell lung cancer (NSCLC),[12] it became evident that combination chemotherapy increased objective response rates and overall survival (OS), but also toxicity.[13] An important research question to answer became which combination treatment could offer the superior effectiveness with the best tolerability. Except that combination treatments should include a platinum compound as those offered better response rates than combination regimens based on third-generation chemotherapy agents, no regimen was preferred over the others until the introduction of pemetrexed.[14,15] Figure 1 shows the timeline of the evolving role of pemetrexed in the treatment of NSCLC.

Frontline and second-line treatment

Pemetrexed showed good antitumor activity in a wide variety of tumor types including NSCLC, malignant pleural mesothelioma, breast, colorectal, head and neck, gastric, bladder, cervix and pancreas cancers.[16,17] The drug was first approved to use in combination with cisplatin as a treatment for patients with unresectable malignant pleural mesothelioma.[18] Soon afterwards, pemetrexed also received approval as a second-line therapy in locally advanced or metastatic NSCLC after the phase III trial of Hanna et al showed that pemetrexed had equivalent efficacy outcomes, but with a more favourable toxicity profile than docetaxel.[19] Interestingly, in a retrospective analysis of this trial, significant associations were already identified between histological subtypes of NSCLC and efficacy outcomes for pemetrexed.[20]

In 2008, the landmark phase III trial showed that the combination cisplatin-pemetrexed was non-inferior compared to cisplatin-gemcitabine with regard to overall survival (10.3 vs 10.3 months) and progression-free survival (PFS; 4.8 months vs 5.1 months) in chemotherapy-naïve patients with advanced NSCLC.[21] But, more importantly, the preplanned subgroup analysis confirmed the differential efficacy between histologies. Patients with nonsquamous NSCLC treated with cisplatin/pemetrexed vs cisplatin/gemcitabine had a longer OS (11.8 vs 10.4 months; HR 0.81; 95% CI, 0.70 - 0.94). The other way around, in patients with squamous NSCLC overall survival with cisplatin/pemetrexed vs cisplatin/gemcitabine was worse (9.4 vs 10.8 months; HR 1.23; 95% CI, 1.00 - 1.51). Patients treated with cisplatin/pemetrexed experienced less severe hematologic adverse events than patients with cisplatin/gemcitabine, but the safety profile was not affected by histology. Based on these data, pemetrexed received approval in first-line advanced NSCLC for nonsquamous histology and the second-line indication was also changed to exclusively nonsquamous type tumors.

Figure 1. Timeline with the key findings changing clinical practice of the use of pemetrexed in NSCLC after its discovery



Maintenance treatment

The U.S. National Cancer Institute's medical dictionary defines maintenance treatment as "a treatment that is given to help keep cancer from progressing after it has been successfully controlled by front-line therapy; It may include treatment with drugs, vaccines, or antibodies that kill cancer cells and it may be given for a long time".[22] Two maintenance treatment paradigms have emerged: continuation maintenance and switch maintenance.

Continuation maintenance treatment entails the ongoing administration of a component of the initial first-line treatment, with the obvious purpose to continue an effective treatment. In switch maintenance treatment, a new agent is introduced directly after finishing first-line treatment, which may decrease chemotherapy resistance according to the Goldie and Coldman hypothesis. According to this hypothesis, tumors acquire random mutations over time depending on the rate of tumor growth, leading to the emergence of chemotherapy-resistant clones.[23] Therefore, cancers may be more sensitive to a new agent at maximum tumor shrinkage than at subsequent tumor progression. Switch maintenance may also maximize antitumor efficacy by a different mechanism: The Norton-Simon hypothesis states that more rapidly growing cells are more drug-sensitive than slower growing, more resistant cells.[24] The administration of sequential non-cross-resistant therapies then seems required to achieve an optimal antitumor effect. Another important rationale for maintenance treatment is to increase the exposure to effective therapies, because many patients who achieve disease control after first-line treatment do not receive second-line treatment at the time of progression.

Switch maintenance therapy of pemetrexed offered improvement in OS (13.4 months vs 10.6 months; HR 0.79, 0.65-0.95) compared to placebo in a patient population including squamous cell subtype.[25] Again, a significant treatment-by-histology interaction was detected and the improvements in overall survival were mainly recorded in patients with nonsquamous histology. During this study, the indication for pemetrexed treatment was already shifted towards patients with nonsquamous histology exclusively and therefore only continuation maintenance would be possible. In patients with advanced nonsquamous NSCLC, continuation maintenance with pemetrexed compared to placebo resulted in improved PFS (4.1 vs 2.8 months; HR 0.62, 95%CI 0.49-0.79) and OS (13.9 vs 11.0 months; HR 0.78; 95%CI 0.64-0.96).[26,27]

Use of pemetrexed in the era of targeted therapy and immunotherapy

In parallel to the introduction of pemetrexed in the treatment of NSCLC, increasing knowledge about the disease biology has led to other important advancements in treatment. Clonal "driver" genetic alterations occurring early during cancer development were identified and changed the paradigm of treatment of oncogene-addicted cancers. In the 1990s, molecularly targeted therapies were with introduced with EGFR tyrosine kinase inhibitors (TKI) gefitinib and later erlotinib.[28,29] They were initially used in

unselected patient populations, but retrospective studies subsequently demonstrated that activating EGFR mutations were observed in the vast majority of patients who benefited from EGFR TKIs.[30] Since then, discoveries of additional driver alterations, including ALK rearrangements, ROS1 fusions and BRAF mutations led to the development of effective targeted therapies.[31] But despite these important findings, the majority of patients with advanced NSCLC does not have targetable genetic mutations or alterations up to now.

Most recently, the idea of harnessing the host immune response to treat cancer has led to a whole new direction of lung cancer care. Currently the most relevant targets for immunotherapy are inhibitory checkpoint molecules, such as those regulating the immunological synapse between T-cells and dendritic cells in lymph nodes (CTLA-4), thereby suppressing T-cell activation, and between T-cells and tumor cells in the tumor environment (programmed-death 1 (PD-1) and programmed-death ligand 1 (PD-L1)), hampering the effector phase. Several checkpoint inhibitors interfering in the programmed-death 1 (PD-1) and programmed-death ligand 1 (PD-L1) axis, received their approval as a standard of care in patients with advanced NSCLC after platinum-containing first-line treatment because they had superior efficacy compared to docetaxel.[32–35] In selected patients with high tumor expression of PD-L1 (>50%), monotherapy with pembrolizumab as frontline therapy led to substantially improved clinical outcomes, including OS and PFS but also reduced toxicity and improved quality of life compared with platinum-based chemotherapy.[36,37] Similar results with regard to improved clinical effectiveness were recently demonstrated for atezolizumab vs chemotherapy in the same category of patients.[38]

Several preclinical reports have highlighted the immunogenic potentials of chemotherapy,[39,40] and therefore it was a logical step to explore the additive or synergistic effects of cytotoxic treatment and immunotherapy combined. KEYNOTE-189 was the first phase III trial including treatment-naïve patients witch nonsquamous NSCLC, in which an evident benefit was shown for the combination of chemotherapy with pembrolizumab vs chemotherapy with regard to overall response rate (48% vs 19%), median PFS (9.0 vs 4.9 months) and median OS (22.0 vs 10.7 months).[41,42] These results and those of various other phase III trials have led to the approval of both pembrolizumab and atezolizumab in combination with platinum-based chemotherapy (and bevacizumab) in first-line treatment of advanced NSCLC without targetable driver mutations and with a PD-L1 expression <50%.[41,43–45]

Despite the introduction of molecular-targeted agents and immunotherapy, pemetrexed-based chemotherapy still has an important role in the treatment of nonsquamous non-small-cell lung cancer (NSCLC). Approximately 15-20% of the patients with nonsquamous NSCLC will have treatable oncogenic alterations by first-line molecular-targeted drugs and of the remaining patients +-30% is expected to have a high PD-L1 expression.[36,46]

Currently, for patients with nonsquamous NSCLC with negative (0%) or low positive PD-L1 expression (1-49%), the combination of platinum/pemetrexed and pembrolizumab is the preferred treatment regimen.[47,48] Thus, still the majority of patients with nonsquamous NSCLC (+-60%) will be recommended treatment with pemetrexed-platinum combined with immunotherapy as first-line treatment. The expected number eligible for chemotherapy/immunotherapy combination may be even higher; In patients with a high PD-L1 expression the three-drug combination can be considered with the goal to achieve a higher response rate, if there is a high symptom and/or disease burden or large visceral tumor load.[48]

PRECISION MEDICINE AND BIOMARKERS

For decades, lung cancer was solely categorized by its histological features: lung cancer compromised small-cell lung cancer (SCLC; approximately 15% of all lung cancers) and NSCLC (approximately 85%). Patients with stage IV NSCLC were exclusively treated by chemotherapy consisting of a platinum compound combined with a third-generation drug (gemcitabine, vinorelbine or a taxane compound), without further selection based on histology or any other marker.[14] Now, our understanding of NSCLC has evolved from a single disease entity that was treated with a one-size-fits-all approach to a disease comprising clinically, histologically and genetically diverse subtypes (Figure 2).[49,50] Hand in hand with emergence of precision medicine, the search for and use of biomarkers is expanding. A biomarker is a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or response to an exposure or intervention.

NSCLC can be further divided histologically in three subgroups: adenocarcinoma (60%), squamous cell carcinoma (30%) and NSCLC not otherwise specified or large cell carcinoma (10%).[51] The era of precision medicine in NSCLC started with the treatment allocation according to histological subtype in the early 2000s. As mentioned above, pemetrexed was approved for treatment of advanced NSCLC solely for tumors of the nonsquamous subtype based on differential treatment efficacy. Another example is bevacizumab, an anti-vascular endothelial growth factor (VEGF) monoclonal antibody. The tolerance of this drug was severely compromised by the development of proteinuria, hypertension and bleeding events, predominantly in squamous NSCLC.[52] In parallel with pemetrexed, this drug received approval for treatment of advanced NSCLC in combination with first-line platinum-based chemotherapy, but only in the nonsquamous subtype because of these safety issues.

Subsequent to the treatment based on histologic features, the identification of targetable gene alterations as molecular biomarkers has further transformed the management of lung cancer and this is still a rapidly moving field. Importantly, not only new targetable gene alterations and treatments are discovered, but the resulting treatment benefit

in terms of overall survival is also evident. Patients with oncogenic driver mutations who received targeted therapies live longer than patients who did not receive targeted therapies or patients without driver mutations (median survival, 3.5 years vs 2.4 years and 2.1 years respectively).[53] The last few years, a better (but still incomplete) understanding of the immune landscape of tumors, including immune-evasion strategies, has led to breakthrough therapeutic advanced with immune checkpoint inhibitors. The only predictive biomarker currently used in clinical practice is the immunohistochemical marker PD-L1 expression. Although far from perfect, in general a correlation between PD-L1 expression and efficacy of PD-1 or PD-L1 targeted treatment has been reported.

Despite improvements in clinical outcome for these selected patient subgroups, progress on new biomarkers is still profoundly needed for the majority of tumors. Until now, no useful biomarkers to predict treatment effectiveness or toxicity are known for (pemetrexed-based) chemotherapy in advanced NSCLC. Molecular, (pharmaco)genetic, and pharmacokinetic characteristics are types of biomarkers that will be further explored in this thesis, with the aim to improve patient selection with regard to treatment efficacy and toxicity of pemetrexed. Now, the different biomarkers which form the outline of a part of this thesis will be discussed.

Protein expression

In histopathology, measurement of protein expression by immunohistochemistry (IHC) analysis is routinely performed as a diagnostic marker to classify NSCLC, especially when tissue or cytological samples of lung cancer do not show clear morphologic features of adenocarcinoma (e.g., TTF-1 or mucin) or squamous cell carcinoma (e.g., p40 or p63). The use of IHC became more expanded, since the requirement for more exact histopathological is now mandatory with certain drugs approved for nonsquamous NSCLC (bevacizumab, pemetrexed) and the observation that targetable genetic mutations are found primarily in adenocarcinoma. In other types of tumors, IHC testing is also used as a predictive biomarker for therapeutic decision-making. In breast cancer for example, HER2 overexpression testing determines which patient is likely to respond to trastuzumab, a monoclonal antibody against HER2.[54] As earlier described, nowadays in advanced NSCLC, PD-L1 expression on tumor cells is quantified by an IHC assay in order to predict responsiveness to immunotherapy.

The main determinant of pemetrexed responsiveness is thought to be the level of expression of thymidylate synthase (TS), the primary intracellular target of pemetrexed.[11,55] However, in clinical practice the relationship between protein expression levels of TS, measured by immunohistochemistry (IHC) methods, and the clinical efficacy of pemetrexed is controversial.[56–60] Earlier research implemented a more refined molecular classification of NSCLC subtypes based on gene expression profiles independent of histology.[61] Furthermore, response to pemetrexed was predicted based on expression of genes encoding different pemetrexed target enzymes including but not limited to TS,

and expression signatures of correlated genes were identified.[61] In **Chapter 2**, we used resected tumor samples from pemetrexed-naïve NSCLC patients. We explored whether these differential gene expression profiles between responders and non-responders can be used to define a prediction model based on IHC scores of selected molecular markers. A retrospective cohort of patients with advanced NSCLC treated with first-line pemetrexed-based chemotherapy was used for external validation.

Pharmacokinetics

For many chemotherapeutic drugs, there is a narrow therapeutic window which states the boundaries in between systemic drug concentrations should balance for the optimal clinical effect: if systemic drug concentrations are (too) low, drug exposure to the tumor might not be sufficient to lead to clinical benefit. However, if drug concentrations are (too) high, there is a risk of severe treatment toxicity due to high exposure to healthy tissues. Comparable with many cytostatic agents, the administered dose of pemetrexed is based on body surface area (BSA), which is calculated from a patient's height and weight. Theoretically, this should lead to equal drug concentrations as larger patients have a higher clearance and volume of distribution.[62]

It is important to define the influence of different factors, such as the activity of drug metabolizing enzymes, drug in- and efflux transporters and organ function involved in drug metabolism and excretion, on drug exposure. One can quantify the influence of these factors on drug exposure by looking at the change in interindividual variability. There is a lack of rationale to use BSA-based dosing if the BSA is not an important predictor of the inter-patient variability of total exposure.[63] Pemetrexed is primarily eliminated by the kidneys, so there might be a rationale for renal based dosing strategy. In **Chapter 3**, population pharmacokinetic /dynamic (PopPK/PD) modelling of pemetrexed was performed to determine whether total systemic exposure of pemetrexed predicts clinical effectiveness and/or treatment toxicity. Different dosing schedules (flat-fixed, BSA-based and renal function based) were simulated to minimize the estimated variability of total systemic exposure. In **Chapter 4**, we evaluated a limited sampling schedule for the assessment of pemetrexed pharmacokinetics. Since toxicity correlates well with the total exposure to pemetrexed and large interindividual variability in exposure,[64,65] pharmacokinetically guided dosing, also known as therapeutic drug monitoring (TDM), may be a feasible strategy to optimize treatment.

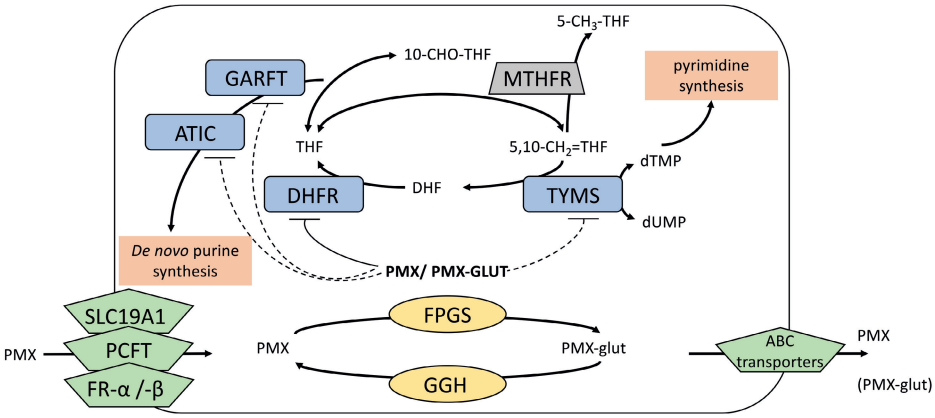
Genetics

Although the precise reasons for interindividual variability in treatment effectiveness and toxicity of pemetrexed have not yet been discovered, its mechanism of action is already well known (Figure 2, adapted from **Chapter 5)**. Uptake into the cells is regulated by different membrane transporters, i.e. proton-coupled folate transporter (PCFT), folate receptors α and β, and reduced folate carrier (RFC), while ATP-binding cassette transporters (ABC) of the multidrug resistance protein family ABCC1-5 are primarily

responsible for the cellular efflux of (anti-)folates.[66,67] Intracellularly, pemetrexed undergoes rapid polyglutamation facilitated by folylpoly-γ-glutamate synthetase (FPGS) and γ-glutamyl hydrolase (GGH) is involved in the reverse process of deglutamation.[66] The formation of polyglutamates is thought to be a major determinant of its antitumor activity as polyglutamates are no substrates for most efflux ABCC transporters, except ABCC5, and therefore are longer retained intracellularly. Moreover, polyglutamates have a stronger affinity for the target enzymes of pemetrexed.[55]

Thymidylate synthetase is the main target enzyme of pemetrexed and results in disturbed *de novo* thymidine production needed for DNA synthesis. By binding to its secondary target enzymes glycinamide ribonucleotide formyltransferase (GARFT) and 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase (ATIC) *de novo* purine synthesis is inhibited, while binding to dihydrofolate reductase (DHFR) results in a diminished active tetrahydrofolate pool needed for purine and thymidine synthesis. Another potential determinant of pemetrexed activity is 5,10-methylenetetrahydrofolate reductase (MTHFR), which is an important regulator of the folic acid pathway.[11]

Figure 2. Important proteins involved in the working mechanism of pemetrexed



Green boxes: enzymes involved in the cell transport of pemetrexed. The most important import transporter reduced folate carrier (FRC) is encoded by SLC19A1. Pemetrexed and its polyglutamates are excreted from the cell via ABC transporters, but polyglutamates to a lesser extent. Yellow boxes: FPGS is responsible for the polyglutamylation of pemetrexed and GGH for the deglutamylation. Blue boxes: TYMS, DHFR, GARFT and ATIC are the target enzymes of pemetrexed. The dashed lines respresent the increased inhibitory ability of the pemetrexed polyglutamates compared to pemetrexed. Grey box: MTHFR has a major impact on the regulation of the folic acid pathway due to conversion of 5,10-methylenetetrahydrofolate to 5-methyl-THF, which is the methyl donor for methylation of dUMP to dTMP for de novo dTMP synthesis. Abbreviations: PMX, pemetrexed; PMX-glut, pemetrexed polyglutamates; dTMP, deoxythymidylate; dUMP, deoxyruridine monophosphate.

Single nucleotide polymorphisms (SNPs) are germline genetic aberrations due to a substitution of a single nucleotide at a specific site in the genome, that is present in a sufficiently large fraction of the population. As this genetic variant is already present before the start of antitumor treatment, it is expected to play a role in the *innate* resistance to the drug. In **Chapter 5**, a SNP analysis is performed to investigate the association between polymorphisms in multiple candidate genes covering the folate pathway, cell transport, intracellular metabolism and target enzymes of pemetrexed, and clinical effectiveness and treatment toxicity**.** Additionally, the relationship between pharmacogenetic SNPs with the PK of pemetrexed was explored by implementing genotypes of SNPs encoding enzymes involved in the cell transport and polyglutamation of pemetrexed, in the previously developed PopPK model discussed in **Chapter 3**.

Increasing evidence supports the existence of intratumor heterogeneity in NSCLC, which can pose an important challenge on achieving and maintaining tumor control.[68,69] *De novo* somatic mutations during anticancer treatment lead to spatial and temporal heterogeneity in a tumor and can affect *acquired* resistance to an anticancer drug. For targeted agents, an increasing number of mechanisms of acquired resistance to sensitizing driver mutations or translocations have been unraveled now.[70] In contrast, no such essential information about tumor evolution is known for treatment with (pemetrexed) chemotherapy.

A rapidly evolving technology in the field of multiple cancer types is the use of non-invasive genotyping of tumors. The use of cell-free DNA (cfDNA) appears to be a promising approach to noninvasively monitor for the emergence of resistance mutations during treatment with ALK and EGFR inhibitors.[71,72] This so called 'liquid biopsy' not only has the potential to detect (targetable) driver mutations or translocations, e.g. KRAS, EGFR and ALK, at the time of diagnosis,[73–75] but serial plasma genotyping also has the potential to detect drug-resistance mechanisms over time.[76,77] Moreover, tissue biopsies are invasive, often not well attainable with the risk of failed biopsies and the turnaround time is slow.[78,79] Another advantage of plasma genotyping is the ability to take into account the heterogeneity and evolution of tumors, in contrast to the limitations of tissue biopsies both spatially and temporally.[80,81] In **Chapter 6**, acquired resistance to pemetrexed is examined by performance of whole exome sequencing of circulating DNA in plasma from patients with advanced NSCLC treated with pemetrexed. Specific attention has been paid to candidate genes playing a role in the working mechanism of pemetrexed.

TOXICITY

Next to the prolongation of survival of cancer patients, another important goal of palliative oncological treatment is to maintain or even improve a patient's quality of life (QoL). Treatment-related adverse events (AEs) can have a considerable impact on

health-related QoL.[82] Most common pemetrexed-induced severe AEs are hematologic toxicities, fatigue and gastro-intestinal complaints.[19,21]

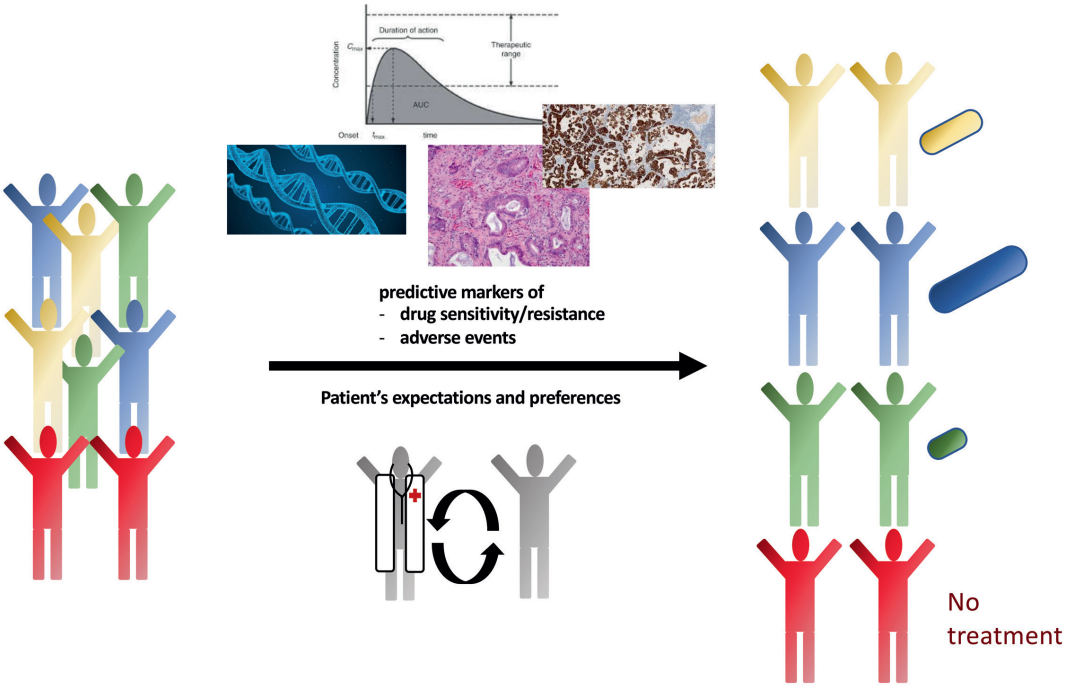
Patients with (lung) cancer are at increased risk of developing acute kidney injury,[83] which is a predictor of immediate and long-term unfavorable outcomes as well as a risk factor for the development of chronic kidney disease. Moreover, to gain optimal clinical effectiveness of chemotherapeutic agents as well as immunotherapy, patients should be able to start as well as continue multiple lines of treatment for which it is required to maintain an adequate renal function. Although the pivotal trials leading to approval of pemetrexed treatment did not describe a high incidence of nephrotoxicity, it is known that clinical trial populations are highly selected and might underestimate the risk and consequences of toxicities in clinical practice.[84] In **Chapter 7** the incidence of acute kidney injury and sustained impairment of renal function after discontinuation of pemetrexed treatment is described, both prospectively and retrospectively, in two real-world observational cohorts treated with pemetrexed per standard of care. Also, it was examined whether patients at increased risk of kidney injury during pemetrexed treatment could be identified.

The recent shift in treatment paradigm of advanced NSCLC has led to the common use of platinum and pemetrexed plus pembrolizumab induction regimen followed by pemetrexed plus pembrolizumab maintenance. One of the major concerns about combination treatment with different types of anticancer drugs is toxicity, is that it may lead to more (severe) toxicities. Indeed, with regard to this specific chemo- and immunotherapy treatment combination, more severe adverse events (AEs) and withdrawal of induction treatment were observed.[41,85] Although the overall reported frequencies are still low, renal impairment is more commonly found in patients treated with the combination of chemo- and immunotherapy than with either therapy alone.[36,41] Platinum derivates, pemetrexed and pembrolizumab can induce renal impairment, but the mechanism of renal damage is different. It is a challenge to distinguish between chemotherapy- and immunotherapy induced renal adverse events. In **Chapter 8**, the mechanisms of renal side effects caused by platinum agents, pemetrexed and pembrolizumab are described. Ultimately, an algorithm covering the diagnostic approach and treatment has been established, which may function as a supportive tool for clinicians.

PATIENT-REPORTED OUTCOMES

Precision medicine is sometimes also called personalized medicine. The benefit of the latter term may be that it also assumes the incorporation of patients' goals and preferences next to the individualization of cancer diagnostics and treatment (Figure 3).

Figure 3. Precision and personalized cancer therapy



The process of shared decision-making leads to a higher reported patient quality of care.[86] This is particularly important in cancer patients with a poor prognosis. Despite the improvement in survival and QoL provided by chemotherapy and other anticancer treatments in patients with advanced NSCLC, survival gain remains limited and therapy is often accompanied by AEs. Therefore, patients' well-being during treatment and how patients perceive the treatment and side effects is of upmost importance. Patients' well-being can be evaluated with the use of patient reported outcomes (PROs). Evaluation of PROs is increasingly incorporated as an outcome parameter in (lung) cancer.[87,88] Moreover, clinical trials investigating new therapies are now obliged to monitor the effects of treatment on patients' well-being in parallel to the measurement of the traditional clinical effectiveness and toxicity outcomes in order to facilitate approval and legislation of a drug.

The implementation of PROs has been shown to improve the assessment of, and communication about, symptoms and QoL.[89] Measurement of health-related QoL (HRQoL) has gained importance in treatment decision-making, as it embodies the influence of AEs (treatment- and cancer related) and it serves as a prognostic factor for survival.[82,90,91] HRQoL has the focus on the impact of disease or treatment on the feelings and experiences patients' have about their physical possibilities and ability to function.[92] The EORTC-QLQ-C30 is a broadly used cancer-specific HRQoL instrument, with the focus

on the impact of treatment and disease on patient's functioning.[93] However, QoL is a broader concept than only HRQoL. According to the World Health Organization (WHO), QoL is "an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns".[94] This concept is influenced by patients' physical and psychological health, but also by social relationships and their environment. In 2004, the WHO released the WHOQoL-BREF, a generic QoL questionnaire with the purpose to rapidly perform QoL assessment in epidemiological surveys and clinical studies. In patients with advanced lung cancer, satisfactory validity and reliability of this questionnaire has been established and minimal clinically important differences are available to facilitate application in daily practice.[95]

PROs and shared decision-making

It has been well established that there is a considerable variability in how patients individually value the importance of survival benefit weighed against cancer- and treatment related toxicities.[96] Physicians often make treatment decisions based on a patient's functional status, comorbidity and potential toxicities, while patients might focus more on survival benefits at the cost of QoL. Moreover, patients with cancer usually want to be involved in treatment decisions.[97] By measuring (HR)QoL though, patients' preferences, expectations and satisfaction with regard to the choice of therapy, treatment effectiveness and occurrence and management of AEs are not taken into account. More insight into these values provides opportunities for physicians to improve the process of shared decision-making and ultimately personalize the course of treatment. In **Chapter 9**, the reliability and validity of the Cancer Therapy Satisfaction Questionnaire (CTSQ) was tested in patients with advanced NSCLC receiving chemotherapy. This questionnaire was developed in 2005, to assess patients' opinions and feelings concerning their cancer therapy and associated adverse events.[98] Subsequently in **Chapter 10**, in the same study population, the association between satisfaction with treatment and patient- and treatment-related factors and patients' feelings about side effects was explored. Furthermore, the added value of the measurement of satisfaction with treatment alongside widely accepted clinical outcomes QoL and adverse events was assessed.

It is common that treatment-related toxicities are routinely assessed during clinical trials. However, in daily practice adverse events are often not scored systematically according to standardized methods and thus toxicities may well be underreported. The use of PROs can better estimate the frequency of treatment-related toxicities than observations of clinicians and may also provide more reliable information with regard to the burden of adverse events.[99] In **Chapter 11**, the association between patients' feelings about side effects and (HR)QoL was further investigated. Also, the underlying factors related to feelings about side effects were further explored.

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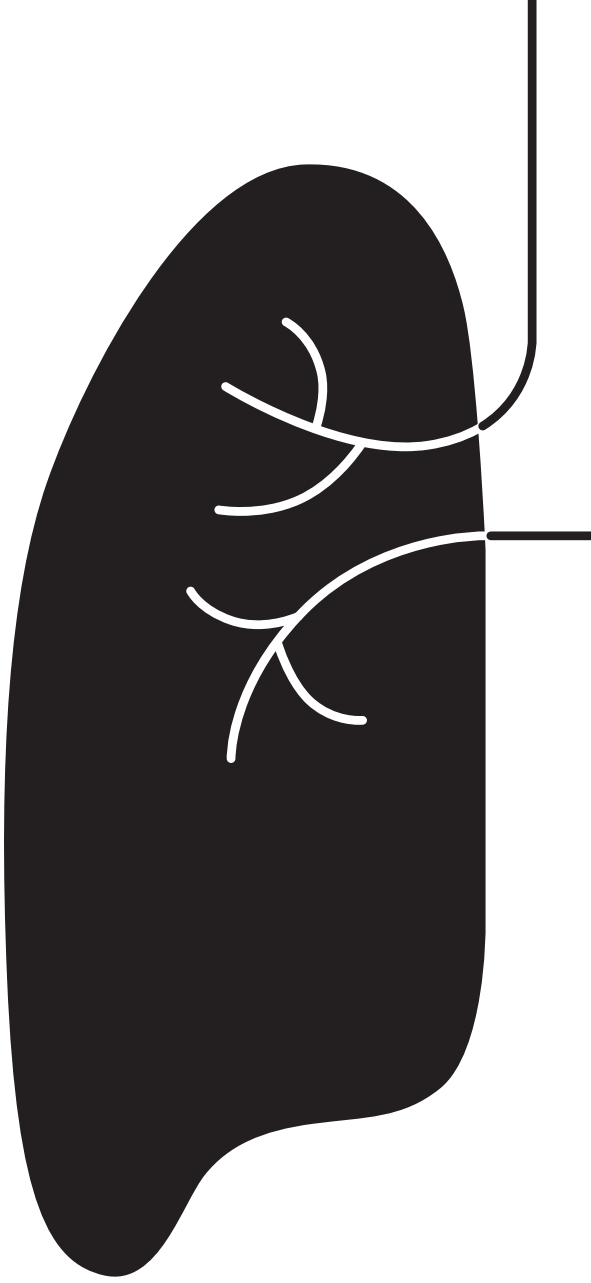

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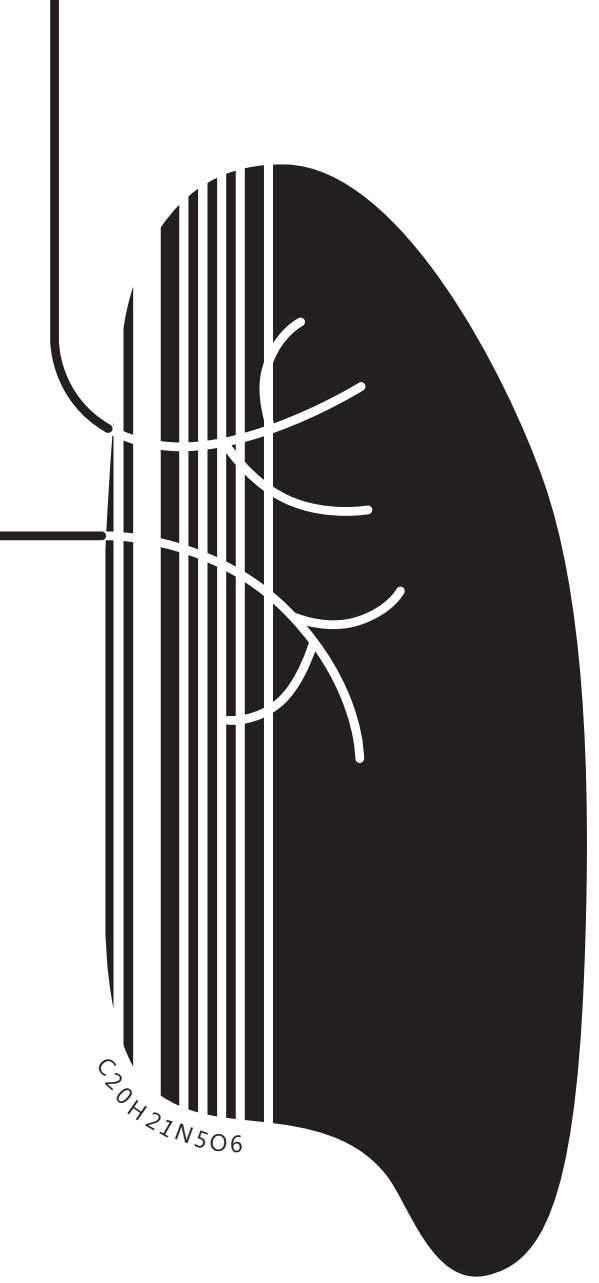

PREDICTION OF TREATMENT RESPONSE AND TOXICITY

CHAPTER 2

Prediction of response to pemetrexed in non-small-cell lung cancer with immunohistochemical phenotyping based on gene expression profiles

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ABSTRACT

Background

Palliative pemetrexed-based chemotherapy remains a standard of care treatment for the majority of patients with advanced nonsquamous non-small-cell lung cancer (NSCLC). Currently, no predictive markers for pemetrexed treatment are available.

Methods

Resected tumor samples from pemetrexed-naïve NSCLC patients were collected. Gene expression profiling with respect to predicted sensitivity to pemetrexed classified predicted responders (60%) and non-responders (40%) based on differentially expressed genes encoding for pemetrexed target enzymes. Genes showing a strong correlation with these target genes were selected for measurement of corresponding protein expressions by immunohistochemical (IHC) staining. A semi-quantitative IHC scoring method was applied to construct a prediction model for response to pemetrexed. A retrospective cohort of patients with advanced NSCLC treated with first-line pemetrexed-based chemotherapy was used for external validation.

Results

From ninety-one patients resected tumor samples were collected. The majority of patients had early or locally advanced NSCLC (96.3%). Gene expression profiling revealed five markers, which mRNA levels strongly correlated to pemetrexed target genes mRNA levels: TPX2, CPA3, EZH2, MCM2 and TOP2A. Of 63 (69%) patients IHC staining scores of these markers were obtained, which significantly differed between predicted non-responders and responders ($P < 0.05$). The optimized prediction model included EZH2 (OR = 0.56, 95%CI 0.35-0.90) and TPX2 (OR = 0.55, 95%CI 0.30-1.01). The model had a sensitivity of 86.8%, specificity of 63.6% and showed a good ability to distinct between responders and non-responders (C-index 0.86).

In the external study population (N = 23) the majority of patients had metastatic NSCLC (95.7%). Partial response (PR) was established in 26.1%. The sensitivity decreased drastically to 33.3%, with a specificity of 82.4% and a C-index of 0.73.

Conclusions

Using external validation this prediction model with IHC staining of target enzyme correlated markers showed a good discrimination, but lacked sensitivity. The role of IHC markers as response predictors for pemetrexed in clinical practice remains questionable.

BACKGROUND

In the management of advanced non-small cell lung cancer (NSCLC) systemic treatment options are rapidly expanding with the increasing use of molecular-targeted agents and immunotherapy.[1–4] One of the most important therapeutic advances has been the identification of predictive molecular markers to guide patient selection for frontline treatment with these agents, like sensitizing mutations within the EGFR gene to EGFR tyrosine kinase inhibitors and protein PD-L1 overexpression to anti PD-(L)1 checkpoint inhibitors.[1, 2, 5] Despite the changing treatment landscape with increasing use of molecular targeted agents and immunotherapy, pemetrexed-based chemotherapy is still widely used as standard treatment in patients with advanced nonsquamous non-small-cell lung cancer.[6, 7] Unfortunately, to date useful biomarkers predicting response to this treatment regimen are lacking.

Pemetrexed treatment shows a variable clinical efficacy, apparently dependent on the histologic subtype of lung cancer. Clinical trials demonstrated efficacy of pemetrexed in nonsquamous NSCLC, while efficacy was worse in squamous NSCLC and small-cell lung cancer.[8–10] However, tumor response to pemetrexed also differs significantly between patients with similar histology.[9, 11] In patients treated with pemetrexed monotherapy, the response rate to pemetrexed was evidently different between histological subtypes but low in both patients with squamous and nonsquamous NSCLC (2.8% vs 11.5%).[10] In this study pemetrexed was administered as second-line treatment and patients with poor ECOG performance score were included. Although the response rate to pemetrexed was significantly higher in patients with nonsquamous versus squamous NSCLC in the first-line pivotal trial,[9] still more than 20% of patients with squamous NSCLC had a response to pemetrexed while the response rate was merely ~30% in patients with nonsquamous histology.[11] These findings highlight the need for predictive molecular markers for pemetrexed-based treatment.

The main determinant of pemetrexed responsiveness is thought to be the level of expression of thymidylate synthase (TS), the primary intracellular target enzyme of pemetrexed.[12, 13] Overexpression of TS mRNA has been correlated with reduced sensitivity to pemetrexed in vitro,[14–17] and with worse clinical outcomes in patients treated with pemetrexed.[18] Moreover, the abundance of TS expression is higher in squamous cell NSCLC than in other histologic subtypes,[19, 20] which constitutes the biological hypothesis behind the superior efficacy of pemetrexed in nonsquamous NSCLC. However, in clinical practice the relationship between protein expression levels of TS, measured by immunohistochemistry (IHC) methods, and the clinical efficacy of pemetrexed remains controversial.[21–25]

Our study group earlier presented an approach to implement a more refined molecular classification of NSCLC subtypes based on gene expression profiles independent of

histology.[26] Furthermore, response to pemetrexed was predicted based on expression of genes encoding different pemetrexed target enzymes including but not limited to TS, and expression signatures of correlated genes were identified. In the current study, we explore whether these differential gene expression profiles between responders and non-responders can be used to define a prediction model based on IHC scores of selected molecular markers.

METHODS

Training cohort

Tumor samples from pemetrexed-naïve NSCLC patients who had undergone curative surgical resection at the Erasmus Medical Center (Rotterdam) between 1992 and 2004 were used. A detailed description of tissue collection, microarray preparation and data processing, the derivation of a gene-expression based predictive algorithm for tumor response and the identification of pemetrexed resistance-associated genes has been previously described.[26] In short, the predictive algorithm predicted tumor response based on the expression difference between internal reference genes and pemetrexed target genes TS, dihydrofolate reductase (DHFR) and glycinamide ribonucleotide formyltransferase (GARFT). Using percentile-rank based target gene expression levels relative to the internal reference genes, patients were stratified as predicted responders (±60%) and non-responders (±40%). Subsequently, significance analysis of microarray identified differentially expressed genes between these classified groups.[27] A minimized signature containing 25 genes performed optimally in predicting pemetrexed response (Supplemental Table S1). For the current study, we selected molecular markers from this signature if they showed a strong correlation with the gene expression of TS and if IHC stainings for these markers were commercially available. Written informed consent was obtained from all these patients. The study was conducted in accordance with the REMARK guidelines.[28]

Validation cohort

In order to externally validate the model, we obtained formalin-fixed paraffin-embedded pre-treatment biopsies of a retrospective cohort of patients newly diagnosed with advanced stage (IIIB/IV) NSCLC in a large teaching hospital (Amphia hospital, Breda, the Netherlands) between January 2007 and December 2010. Patients were eligible for enrolment if they had received ≥2 cycles of platinum-combined pemetrexed chemotherapy as first-line treatment. Medical charts and radiological imaging data were reviewed to collect information regarding sociodemographic characteristics, tumor histology, ECOG performance status, treatment and observed tumor response (RECIST 1.1). Patients with early stage (IA-IIB) or locally advanced (IIIA) disease, (neo)adjuvant chemotherapy, combination treatment with bevacizumab and without tissue samples from primary tumor or (lymph node) metastases were excluded.

Tissue microarray analysis and immunohistochemistry

The tissue microarrays (TMAs) were composed of 68 of the 91 tumor tissues, in triplicate, from the Erasmus MC patient cohort used for the expression microarray analyses. TMA blocks containing 0.6mm cores of formalin-fixed paraffin-embedded tumors were cut and antigen retrieval was performed by a 20-minute incubation at 95°C using Trisethylenediaminetetraacetic acid buffer (Klinipath, Duiven, The Netherlands). Subsequently, TMAs were stained with primary antihuman antibodies of the selected candidate markers: EZH2, TOP2A, TPX2, MCM2 and CPA3 (Supplemental Table S2). For each TMA multicontrol stainings were performed using a combination of tissues (liver, pancreas, tonsil, colon and appendix). The slides were stained and processed in the Ventana Benchmark ULTRA strainers, using DAB as substrate and Hematoxylin as counterstain. Tumor tissues from the validation group were equally handled, except that the tumor samples were cut in 0.4mm instead of 0.6mm cores for TMA blocks.

Immunohistochemical staining score

A semi-quantitative scoring method was applied to classify the intensity and quantity of IHC staining of candidate markers. The quantity score was defined as: 1: 0-30%; 2: 30-60%; 3: 60-100%. The intensity score was defined as: 0 (negative), no appreciable staining in the tumor cells; 1 (weak), barely detectable cytoplasmic/membranous or nuclear staining of tumor cells; 2 (moderate), readily appreciable staining of tumor cytoplasm/nucleus; 3 (strong), strong staining obscuring nucleus/cytoplasm of tumor cells. Multiplying quantity and intensity score yielded a total score with a range between 0 and 9. TMAs from the training group were evaluated and scored for protein expression simultaneously by K.B. and J.H. Samples were individually discussed until consensus was reached. For the validation group, TMA evaluation and protein staining quantification were performed independently by K.B. and S.V.

Statistical analyses

Sociodemographic and disease- and treatment-related variables were described for all patients who were included in this study and were compared between the training and validation group. We used the independent samples *t*-test and the χ^2-test or Fisher's exact test for continuous and categorical variables respectively. Degree of agreement on quantity and intensity scores of the different IHC stainings was evaluated using weighted linear Cohen's kappa scores (κ) in the validation group. Degree of agreement was determined according to widely used scale described by Landis and Koch.[29] As IHC staining scores from the training group were obtained by discussion, and thus not independently, no interobserver agreement could be calculated.

Pearson correlation coefficients (ρ) were calculated between gene expression of the candidate markers and TS in the training group and subsequently between the gene expression of those markers and their associated protein expressions. Using the described prediction algorithm of response to pemetrexed, patients from the training

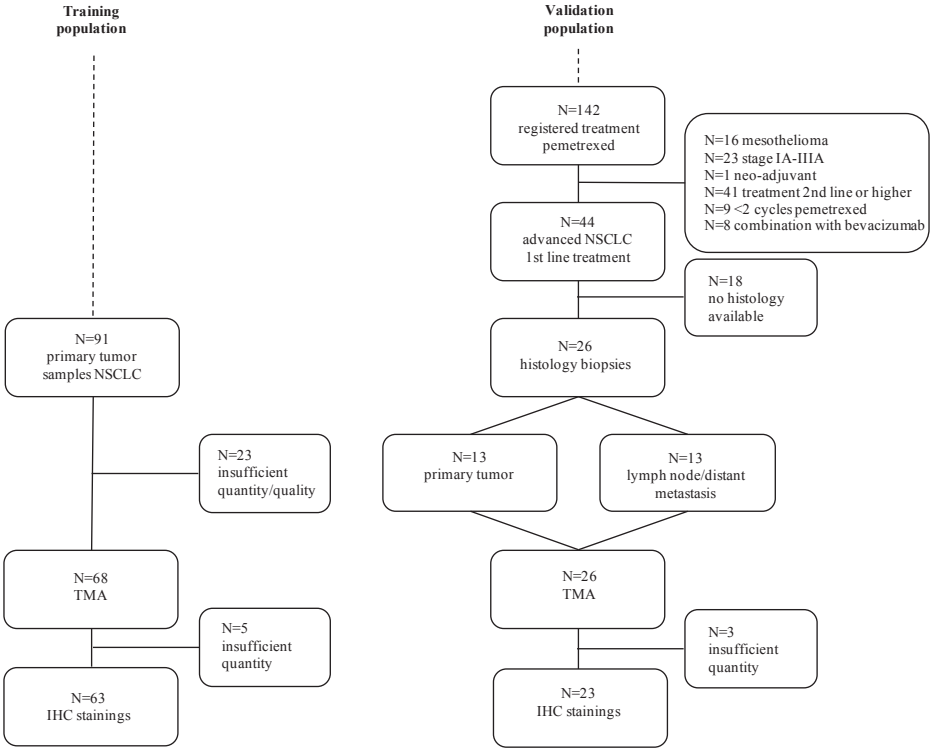
group were divided in predicted responders and non-responders. In the training group, we compared gene and protein expression from selected molecular markers between predicted responders and non-responders.

Logistic regression with dependent variable predicted tumor response by gene expression signature was applied to the training cohort to build a prediction model with the IHC staining scores of selected molecular markers as independent variables. Optimized model derivation was performed using purposeful selection by stepwise in- and exclusion of molecular markers.[30] Univariate logistic regression identified molecular markers associated with predicted tumor response. We specified a priori that molecular markers with $P < 0.2$ on univariate analysis would be candidate variables for multivariable logistic regression model. In the iterative process of variable selection, variables were removed from the model if they were non-significant and not a confounder. We used backward selection with a *P*-value < 0.05 and/or change of effect size of (an)other included variable(s) >20% to remain in the model. The fit of a reduced model versus full model was compared with the likelihood ratio test (LRT), following a chi-squared distribution. Subsequently, the model was externally validated in the validation cohort. In both cohorts, the model performance of the derived model was assessed by examining the predictive classification accuracy and discriminatory ability (C-index). A C-index of 1.0 would indicate perfect discrimination, whereas a C-index of 0.5 indicates total absence of discrimination. All statistical analyses were performed with the use of SPSS, version 24.0 (IBM Corporation, Armonk, NY).

RESULTS

The selection of patients in both training and validation cohort is depicted in Figure 1. Of the 91 surgically resected samples of the primary tumor, 68 (74.7%) samples were suitable for further processing into TMAs. Since samples of five patients could not be used due to insufficient TMA material, the training cohort ultimately consisted of 63 patients whose samples were prepared with additional IHC stainings. For validation, 44 of 142 (31%) patients who received pemetrexed had advanced stage NSCLC treated with ≥2 cycles first-line treatment with platinum-combined pemetrexed, excluding combinations with bevacizumab. Of these patients, 18 (40.9%) were excluded from further analysis because their diagnosis was cytology-based and no histologic biopsy was obtained. Histology samples were retrieved from primary tumor (N = 13) or lymph nodes or distant metastases (N = 13). Three patients had insufficient tumor material available for additional IHC staining and therefore the validation cohort consisted of 23 patients.

Figure 1. Flowchart of training and validation population



Abbreviations: NSCLC, non-small-cell lung cancer; TMA, tissue micro array; IHC, immunohistochemical

Patient characteristics

Patient and treatment characteristics are shown in Table 1. In the training cohort 46 patients (73.0%) were male compared to 10 (43.5%) in the validation cohort. Eighteen patients (28.6%) had squamous NSCLC in the training cohort while in the validation cohort only patients were included with nonsquamous histology. The majority of patients (95.2%) in the training group had early stage NSCLC opposed to all patients with advanced disease stage in the validation group. No data were available with regard to the ECOG performance score of the patients in the training cohort. In the validation cohort, 21.7% of the patients had a performance score of 2. Corresponding to the differences in disease stage between the cohorts, all patients in the training cohort underwent surgical resection in contrast to palliative pemetrexed-based chemotherapy in the validation cohort. Patients in the training cohort had worse overall survival compared to the validation cohort (4.5 months vs 28 months).

Table 1. Characteristics of patients in the training population and the validation population

	Training cohort (N = 63)	Validation cohort (N = 23)
Age, mean (SD)	61.9 (±10.7)	58.7 (±8.7)
Gender, male	46 (73.0)	10 (43.5)
Smoking status		
Never smoker	1 (1.6)	1 (4.3)
Ever smoker	31 (49.2)	21 (91.3)
Unknown	31 (49.2)	1 (4.3)
ECOG performance score		
0 or 1		17 (73.9)
2		5 (21.7)
Unknown	63 (100)	1 (4.3)
Histology		
ADC	18 (28.6)	21 (91.3)
LCC	24 (38.1)	2 (8.7)
SCC	15 (23.8)	
Other	6 (9.5)	
Tumor stage		
IA-IIB	56 (88.9)	
IIIA	4 (6.3)	
IIIB		1 (4.3)
IV	3 (4.8)	22 (95.7)
Treatment		
Surgery	63 (100)	
CISPEM		18 (78.3)
CARPEM		5 (21.7)
No. cycles chemotherapy, median (IQR)		3 (3-4)
Treatment effect		
PR		6 (26.1)
SD		7 (30.4)
PD		10 (43.5)
OS, median (IQR)	28.0 (10.0-67.6)	4.5 (3.2-7.3)

Data are expressed as numbers (%) unless stated otherwise. Abbreviations: SD, standard deviation; ADC, adenocarcinoma; LCC, large cell carcinoma; SCC, squamous cell carcinoma; CISPEM, cisplatin combined with pemetrexed; CARPEM, carboplatin combined with pemetrexed; OS, overall survival; IQR, interquartile range; PR, partial response; SD, stable disease; PD, progressive disease.

Selection molecular markers

Of the 25-genes containing optimized gene expression signature predicting response to pemetrexed in the training group, five molecular markers were selected based on their correlation with the gene expression level of target genes (TS, DHFR, GARFT) and the commercial availability of corresponding IHC stainings: Enhancer of zeste homolog 2 (EZH2), Topoisomerase II (TOP2A), Microtubule Nucleation Factor (TPX2), Carboxypeptidase A3 (CPA3) and Minichromosome Maintenance Complex Component 2 (MCM2). All markers showed a positive correlation to the mRNA level of TS (EZH2, $\rho = 0.732$; MCM2, $\rho = 0.804$; TOP2A, $\rho = 0.814$; TPX2, $\rho = 0.825$), except for CPA3 which was negatively correlated ($\rho = -0.467$) (Supplemental Figure S1). The correlation of gene mRNA level with their corresponding IHC staining score had a range between 0.303 (CPA3) and 0.578 (EZH2) (Supplemental Figure S2).

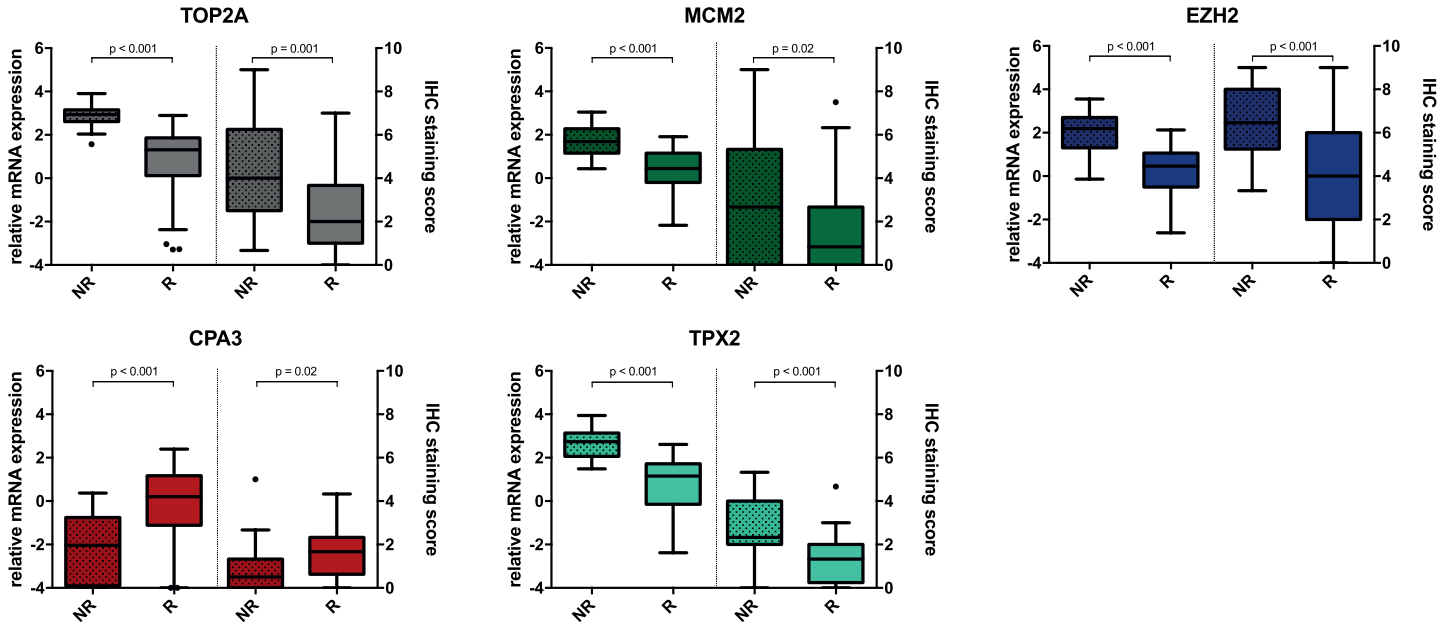
The IHC stainings of the same markers were applied to the TMAs of the samples of patients in the validation cohort. The strength of agreement between the observers with regard to the intensity score ranged between $\kappa = 0.515$ (CPA3) and $\kappa = 1$ (MCM2), and with regard to the quantity score between $\kappa = 0.547$ (TPX2) and $\kappa = 0.851$ (CPA3). Weighted kappa values of IHC staining scores are outlined in Supplemental Table S3.

Of all selected markers, both mRNA levels and IHC staining scores were significantly higher in predicted non-responders than responders in the training group, except for CPA which mRNA level and IHC staining score were significantly lower in non-responders compared to responders. These results are depicted in Figure 2.

Model derivation

The model coefficients and odds ratios (OR) with corresponding 95% confidence intervals (CI) of the prediction model with dependent variable tumor response to pemetrexed using univariable and multivariable logistic regression analyses are presented in Table 2. Univariable analyses of the relationship between the IHC staining scores of the selected markers and the gene expression based predicted tumor response to pemetrexed were performed using the training cohort. Higher IHC staining scores of all markers were significantly associated with worse predicted tumor response, except for CPA3 which repeatedly showed the reverse association compared to the other markers. Using multivariable analysis, only a higher IHC staining score of EZH2 was significantly related with a worse predicted tumor response to pemetrexed (OR 0.56, 95% CI 0.35-0.90; $P = 0.015$). The staining scores of all other markers failed to demonstrate a significant association on multivariable analysis. Although IHC staining score of TPX2 was not significantly associated with tumor response in the multivariable model (OR 0.55, 95% CI 0.30-1.01; $P = 0.056$), this variable was still included in the final optimized prediction model. Removal of this variable led to a significantly reduced model fit ($P < 0.001$).

Figure 2. Boxplots of gene and protein expression levels of selected markers in predicted responders and non-responders to pemetrexed



Boxplots (Tukey) representing medians and interquartile ranges of relative mRNA expression and IHC staining scores respectively. Whiskers represent minimum and maximum 1.5 interquartile range and dots are outliers. Abbreviations: IHC, immunohistochemical; EZH2, Enhancer of zeste homolog; TOP2A, Topoisomerase II; TPX2, Microtubule Nucleation Factor; CPA3, Carboxypeptidase A3; MCM2, Minichromosome Maintenance Complex Component 2. Abbreviations: IHC, immunohistochemical; EZH2, Enhancer of zeste homolog; TOP2A, Topoisomerase II; TPX2, Microtubule Nucleation Factor; CPA3, Carboxypeptidase A3; MCM2, Minichromosome Maintenance Complex Component 2

Table 2. Prediction model derivation to predict tumor response using IHC staining scores of selected molecular markers in training group (N = 63)

	Univariable analysis		Optimized model	
	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P*-value
IHC score CPA3	1.82 (1.08-3.07)	0.025		
IHC score EHZ2	0.47 (0.30-0.71)	<0.001	0.56 (0.35-0.90)	0.015
IHC score TPX2	0.43 (0.26-0.70)	0.001	0.55 (0.30-1.01)	0.054*
IHC score MCM2	0.75 (0.0.59-0.96)	0.022		
IHC score TOP2a	0.67 (0.51-0.88)	0.003		

*Model fit was significantly worse (based on difference -2 Log Likelihood) if TPX2 was excluded. Abbreviations: CI, confidence interval; EZH2, Enhancer of zeste homolog; TOP2A, Topoisomerase II; TPX2, Microtubule Nucleation Factor; CPA3, Carboxypeptidase A3; MCM2, Minichromosome Maintenance Complex Component 2

Model performance and validation

In Table 3 the different test characteristics describing the performance of the model in the training and validation cohort are shown. In the training cohort, 38 patients were predicted responders (63.3%) by gene expression profiling and 86.8% (33 of 38) were correctly classified responders by the prediction model (sensitivity 86.8%, 95% CI 71.9-95.6). Fourteen patients were predicted non-responders (36.7%) by gene expression profiling while 63.6% (14 of 22) were correctly classified as non-responders by the model (specificity 63.6%, 95% CI 40.7-82.8).

In the validation cohort, the same classification by the prediction model was applied, however an actual tumor response was obtained as these patients were treated with pemetrexed. The response rate was 26.1% and therefore the prevalence of response was substantially lower than in the training cohort. Of the six patients who experienced a partial response, two patients were correctly classified by the prediction model resulting in a sensitivity of 33.3% (95% CI 4.3-77.7). Herewith, the sensitivity in this cohort is significantly worse than the sensitivity in the training cohort (Fisher's exact test, P = 0.011). The positive predictive value (PPV) also decreased substantially. If we classified both patients with a partial response and stable disease as responders (56.5%), performance characteristics of the model declined dramatically (Table 3).

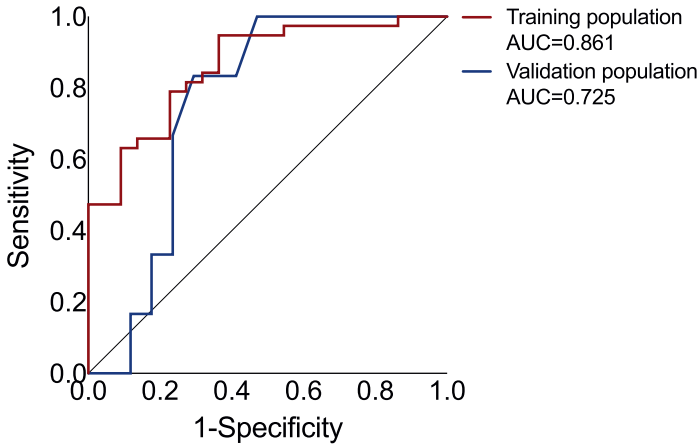
The ROC curve showed a C-index of 0.86 (95% CI 0.77-0.96) in the training cohort, representing a good discriminatory performance. The C-index decreased to 0.73 (95% CI 0.52-0.93) if the prediction model was applied to the validation cohort (Figure 3).

Table 3. Conditional and post-test probability performance of the IHC based prediction model in the training and validation cohort

	Training cohort	Validation cohort Responder: PR	Validation cohort Responder: PR + SD
Prevalence (responder)	63.3 (49.9-75.4)	26.1 (10.2-48.4)	56.5 (34.5-76.8)
Sensitivity	86.8 (71.9-95.6)	33.3 (4.3-77.7)	15.38 (1.9-45.5)
Specificity	63.6 (40.7-82.8)	82.4 (56.6-96-2)	70.0 (34.6-93.3)
LR+ (weighted by prevalence)	2.39 (1.36-4.21)	1.89 (0.41-8.71)	0.51 (0.10-2.51)
LR- (weighted by prevalence)	0.21 (0.09-0.50)	0.81 (0.44-1.49)	1.21 (0.76-1.93)
PPV	80.5 (70.1-87.9)	40.0 (12.6-75.5)	40.0 (12.0-76.5)
NPV	73.7 (53.8-87.1)	77.8 (65.6-86.5)	38.9 (28.5-50.4)

Data are expressed as percentages, except LR+ and LR- (odds), with 95% confidence intervals. Abbreviations: LR+, positive likelihood ratio; LR-, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value

Figure 3. Receiver operating characteristic curve showing model performance of two-protein prediction model in training and validation population.



Diagonal line reflects total absence of discrimination (AUC = 0.5). Abbreviations: AUC, area under the curve

DISCUSSION

Currently, the profit of and need for molecular markers to select therapy for individual patients is increasingly recognized. The last several years, treatment of advanced NSCLC obviously has become more complex and therefore tools to choose therapies that are most likely to benefit patients are required. Indeed the registration of new therapeutic agents as frontline therapy is accompanied by selective markers. For patients with EGFR mutation-positive, ALK rearrangement-positive or ROS1 rearrangement-positive tumors

first-line molecular targeted tyrosine kinase inhibitors are recommended, while patients with high PD-L1 expression in the tumor are suitable for pembrolizumab first-line treatment.[6] Despite the wide implementation of molecular markers, the administration of chemotherapy in NSCLC patients is still solely based on histology even though its capacity to predict response has been proved to be suboptimal.

In an earlier study, we demonstrated an approach to predict response to pemetrexed based on the use of gene expression profiles.[26] Samples of different histological subtypes including squamous NSCLC were used. Prediction of response to pemetrexed based on gene expression profiling of its target enzymes failed to show the expected disadvantage for the squamous cell histological subtype and these results therefore challenge the restricted use of pemetrexed in nonsquamous NSCLC. In the present study, we developed a prediction model using immunohistochemistry scores of selected candidate genes (EZH2 and TPX2) from the gene expression signature predicting pemetrexed response. In the training group, the use of the model resulted in good performance characteristics of the model in the sense of a high sensitivity and PPV. The model also showed the ability to discriminate well between responders and non-responders. Unfortunately, the results of the obtained model could not be validated when applied to an external cohort of patients treated with pemetrexed.

Although it is hypothesized that superior efficacy of pemetrexed in nonsquamous over squamous NSCLC is related to the level of TS expression, multiple clinical studies failed to demonstrate the association between TS protein expression and clinical outcomes.[22, 25] This can be ascribed firstly to the fact that current semi-quantitative IHC methods might lack sensitivity required to measure protein expression of TS opposed to quantitative analysis of mRNA expression. And secondly, the biological significance of TS for pemetrexed responsiveness might be of less importance than other molecular processes, e.g. gene expression or amplification of other (target) genes. To overcome these limitations in the present study, we carefully selected markers related to mRNA gene expression of TS but also to other target enzymes DHFR and GARFT.

In the literature, both EZH2 and TPX2 have been previously linked to survival in NSCLC. TPX2 is involved in key steps during mitotic events and increased expression has been associated with poor overall survival in NSCLC.[31, 32] EZH2 epigenetically silences multiple genes involved in cell differentiation, growth and invasion. It is often overexpressed in NSCLC promoting cancer progression and a more aggressive tumor behaviour.[33, 34] Downregulation of EZH2 has been associated with higher expression of oestrogen receptor and increased sensitivity to tamoxifen in advanced breast cancer patients.[35] Similarly, one can speculate that EZH2 might change the expression of genes related to responsiveness of pemetrexed through its ability to silence other genes. Although the lack of a control arm precludes discrimination between a prognostic or predictive factor, we purposefully focused on radiological response rather than survival.

It is crucial to predict treatment effects for individual patients in order to avoid unnecessary toxicities and to offer alternative treatment options, with few false positives.[36] The clinical value of the derived model is probably limited as the sensitivity was poor in the validation group. Moreover, the low PPV makes the classifier not useful for clinical decision-making, as many patients who are predicted responders will then actually undergo potentially harmful treatment with low chance of tumor response. The failure of the prediction model to adequately perform in the external patient population might be ascribed to an insufficient sensitivity of used IHC assays to measure significant differences in protein expression or a discrepancy between protein expression and protein activity. Additionally, spatiotemporal heterogeneity might have led to different intrinsic tumor properties in the validation group as these patients had advanced disease and in half of the cases tumor samples were obtained from lymph node or distant metastases. Finally, other factors might influence pemetrexed activity such as cell transport and intracellular formation of polyglutamate metabolites.[37]

Our study was limited in the number of patients included, especially in the external validation cohort. We recognize that the differences in histology between the training and validation cohort is a major shortcoming. It was impossible to include patients with nonsquamous NSCLC to the validation cohort, as selection was treatment-based and pemetrexed is only recommended in patients with nonsquamous NSCLC. For ALK and ROS1-rearrangement positive adenocarcinoma patients might experience more benefit to pemetrexed-based chemotherapy,[38, 39] molecular characteristics would have been desirable. Unfortunately, those data were not available in our cohort, but the high number of smokers profoundly reduces the chance of rearrangements. Although response rates were in accordance with the literature, patients in the validation cohort experienced a very poor median overall survival of only 4.5 months. This can be partially explained by the presence of a substantial group of patients (>20%) with a poor ECOG performance score and suboptimal treatment with carboplatin instead of cisplatin combination. Whether these patients appropriately represent the population with advanced NSCLC is therefore highly questionable and we cannot exclude their genetic profile to be different. However, given the results we do not expect that expanding the number of samples will lead to a clinically useful biomarker.

CONCLUSION

There remains an unmet need to identify biomarkers to select patients for standard pemetrexed-based treatment. Prediction of pemetrexed responsiveness with IHC stainings of markers correlated to TS and other target enzymes could not be validated using external validation. Future research focusing on metabolomics, pharmacokinetics and pharmacogenetics might offer new insights into tailoring therapy. Until a well-validated biomarker is identified, histology should remain the standard to select advanced NSCLC patients eligible for treatment with pemetrexed.

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SUPPLEMENTAL TABLES AND FIGURES

Table S1. Minimized signature for prediction of pemetrexed response

ProbesetID	Gene Symbol	NR : R Ratio	NR Mean	R Mean
1554696_s_at	TYMS	3.79	1.11	-0.77
202589_at	TYMS	3.70	1.15	-0.83
202954_at	UBE2C	3.19	1.04	-0.79
201292_at	TOP2A	3.05	1.04	-0.88
223381_at	NUF2	3.04	0.93	-0.74
204162_at	NDC80	2.84	0.87	-0.73
207828_s_at	CENPF	2.79	0.97	-0.80
210052_s_at	TPX2	2.77	1.04	-0.79
204146_at	RAD51AP1	2.74	0.86	-0.64
222958_s_at	DEPDC1	2.73	0.80	-0.67
218755_at	KIF20A	2.70	0.79	-0.68
219918_s_at	ASPM	2.67	0.93	-0.75
203358_s_at	EZH2	2.65	0.87	-0.70
201291_s_at	TOP2A	2.63	1.05	-0.85
204822_at	TTK	2.55	0.88	-0.72
204962_s_at	CENPA	2.54	0.88	-0.68
219306_at	KIF15	2.53	0.73	-0.55
202107_s_at	MCM2	2.52	0.90	-0.65
205053_at	PRIM1	2.50	0.74	-0.55
222680_s_at	DTL	2.48	0.83	-0.67
218039_at	NUSAP1	2.39	0.79	-0.68
204444_at	KIF11	2.35	0.69	-0.57
204023_at	RFC4	2.27	0.85	-0.65
39248_at	AQP3	0.40	-1.34	0.78
205624_at	CPA3	0.35	-1.18	0.75

NR: predicted non-responder to Pemetrexed
R: predicted responder to Pemetrexed

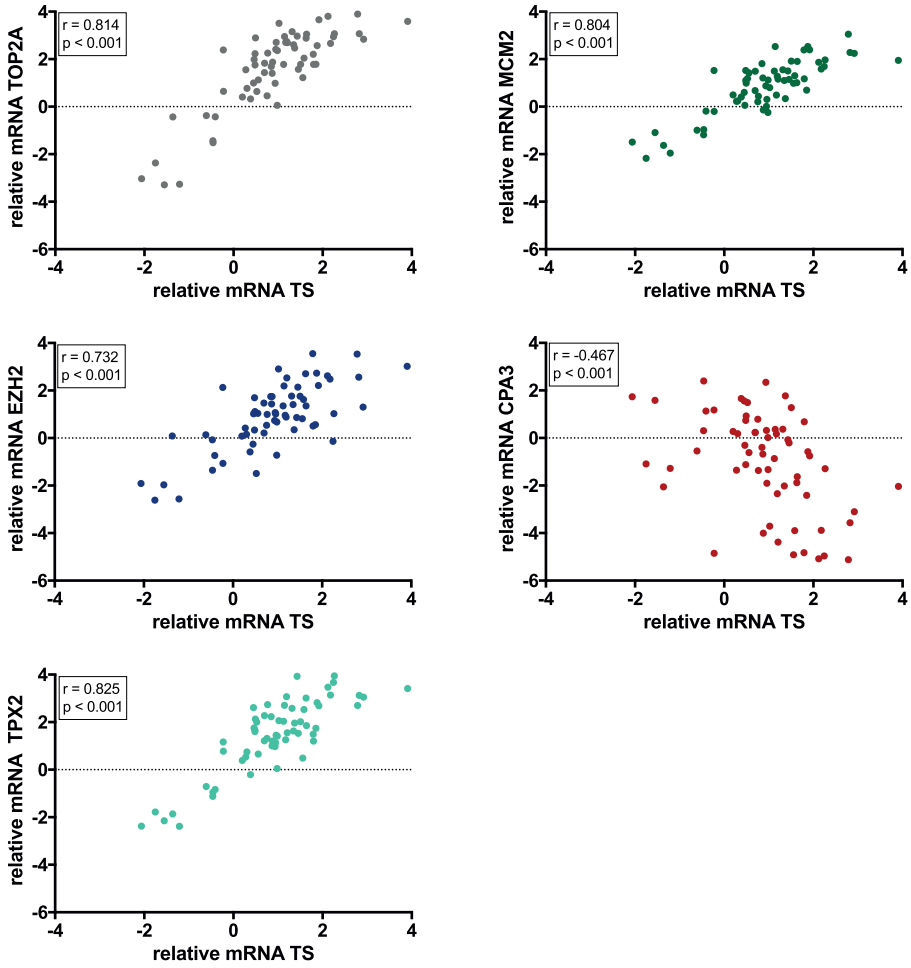
Table S2. Antibodies used for immunohistochemical analyses

Name	Company	Titer	Antibody type
EZH2	Leica	1:200	Anti-human
TOP2A	Leica	1:40	Anti-human
TPX2	Sigma	1:100	Anti-human
MCM2	Bio Connect	1:100	Anti-human
CPA3	Sigma	1:1000	Anti-human

Table S3. Interobserver agreement of the IHC staining score with regard to tumor quantity and intensity of staining

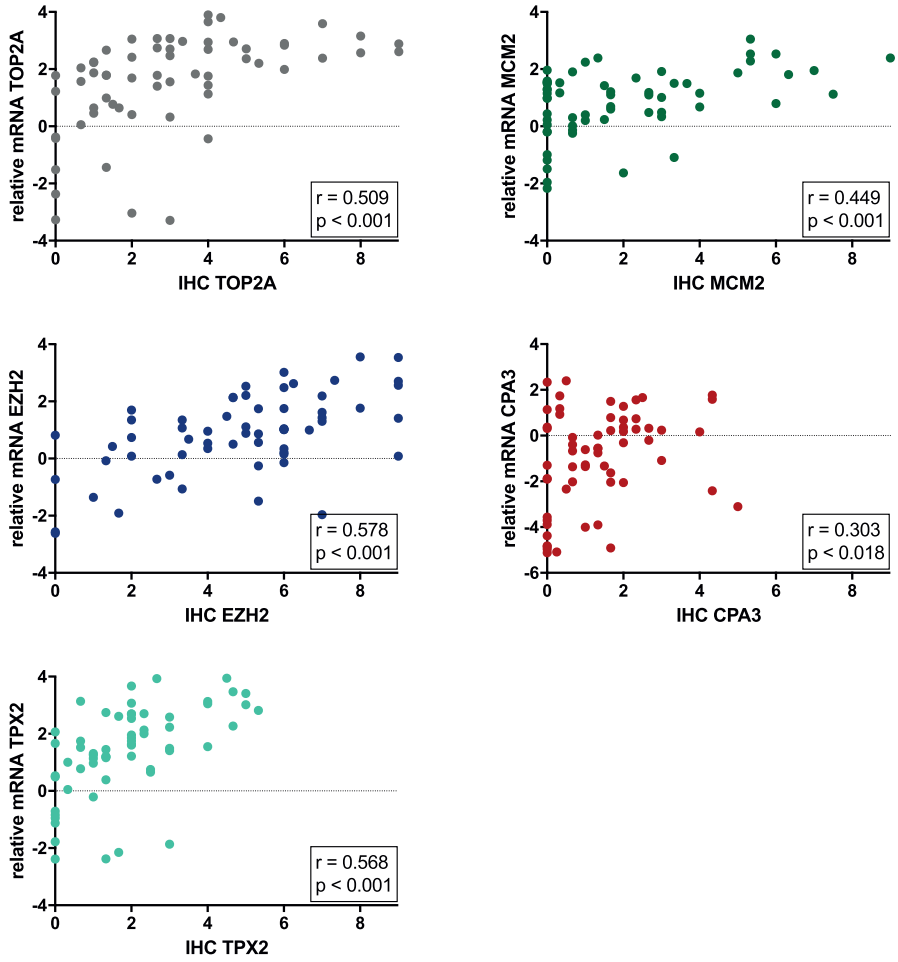
	Tumor quantity N = 23		Tumor Intensity N = 23	
	weighted kappa (κ)	95% CI	weighted kappa (κ)	95% CI
TPX2	0.547	0.220-0.873	0.577	0.025-1.130
CPA3	0.851	0.684-1.019	0.515	0.203-0.827
EZH2	0.723	0.441-1.006	0.733	0.514-0.951
MCM2	0.741	0.531-0.951	1	
TOP2A	0.785	0.563-1.006	0.708	0.336-1.080

Figure S1. Scatter plots of gene expression levels of selected molecular markers and TS gene expression.



Dot plots showing correlations between relative mRNA expression of TS and mRNA expression of TOP2A, MCM2, EZH2, CPA3, TPX2. Abbreviations: IHC, immunohistochemical; EZH2, Enhancer of zeste homolog; TOP2A, Topoisomerase II; TPX2, Microtubule Nucleation Factor; CPA3, Carboxypeptidase A3; MCM2, Minichromosome Maintenance Complex Component 2.

Figure S2. Scatter plots of gene expression levels of selected molecular markers with their protein expression level and the associated correlations.



Dot plots showing correlations between relative mRNA expression and IHC staining score of TOP2A, MCM2, EZH2, CPA3, TPX2. Abbreviations: IHC, immunohistochemical; EZH2, Enhancer of zeste homolog; TOP2A, Topoisomerase II; TPX2, Microtubule Nucleation Factor; CPA3, Carboxypeptidase A3; MCM2, Minichromosome Maintenance Complex Component 2.

CHAPTER 3

A genetic polymorphism in *ATIC* is associated with effectiveness and toxicity of pemetrexed in non-small-cell lung cancer

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ABSTRACT

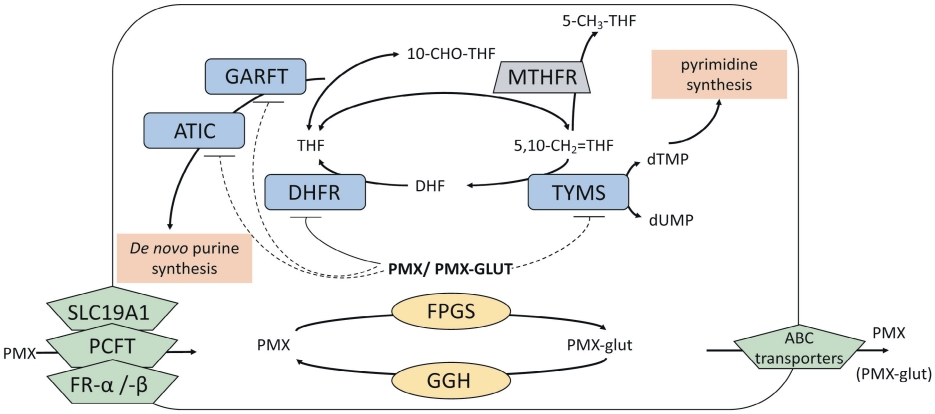
Patients with advanced non-small-cell lung cancer (NSCLC) who are treated with pemetrexed display a wide variation in clinical response and toxicity. In this prospective, multi-center cohort study, we investigated the association with treatment effectiveness and toxicity of 10 polymorphisms in 9 candidate genes, covering the folate pathway (*MTHFR*), cell transport (*SLC19A1/ABCC2/ABCC4*), intracellular metabolism (*FPGS/GGH*), and target enzymes (*TYMS/DHFR/ATIC*) of pemetrexed. Adjusted for sex, ECOG performance score and disease stage, the association between *ATIC* (rs12995526) and overall survival (HR 1.59, 95% CI 1.06-2.39) was significant. Regarding toxicity, this *ATIC* polymorphism was significantly associated with severe laboratory (P = 0.014) and clinical (P = 0.016) chemotherapy-related adverse events, severe neutropenia (P = 0.007) and all-grade diarrhea (P = 0.034) in multivariable analyses.

BACKGROUND

Pemetrexed is widely used in the treatment of advanced non-small-cell lung cancer (NSCLC) as first-line treatment in combination with a platinum agent, and recently also immunotherapy, second-line therapy and maintenance treatment.[1] Pemetrexed shows a substantial variation in clinical effectiveness and toxicity, which cannot be predicted for individual patients. Importantly, toxicity is related to the pharmacokinetic (PK) parameters of pemetrexed, which have a wide interpatient variability.[2]

Here, we aimed to investigate whether polymorphisms of genes associated with the pharmacodynamics (Figure 1) which cover the folate pathway (*MTHFR*), cell transport (*SLC19A1/ABCC2/ABCC4*), intracellular metabolism (*FPGS/GGH*) and target molecules (*TYMS/DHFR/ATIC*) of pemetrexed, are associated with clinical effectiveness and toxicity of pemetrexed in a large cohort of patients exposed to this drug. Additionally, we explored the relationship of these pharmacogenetic single nucleotide polymorphisms (SNPs) with the PK of pemetrexed.

Figure 1. Important proteins involved in the working mechanism of pemetrexed.



Green boxes: enzymes involved in the cell transport of pemetrexed. The most important import transporter reduced folate carrier (FRC) is encoded by SLC19A1. Pemetrexed and its polyglutamates are excreted from the cell via ABC transporters, but polyglutamates to a lesser extent. Yellow boxes: FPGS is responsible for the polyglutamylation of pemetrexed and GGH for the deglutamylation. Blue boxes: TYMS, DHFR, GARFT and ATIC are the target enzymes of pemetrexed. The dashed lines represent the increased inhibitory ability of the pemetrexed polyglutamates compared to pemetrexed. Grey box: MTHFR has a major impact on the regulation of the folic acid pathway due to conversion of 5,10-methylenetetrahydrofolate to 5-methyl-THF, which is the methyl donor for methylation of dUMP to dTMP for de novo dTMP synthesis. Abbreviations: PMX, pemetrexed; PMX-glut, pemetrexed polyglutamates; dTMP, deoxythymidylate; dUMP, deoxyruridine monophosphate.

MATERIALS AND METHODS

Pharmacogenetic data were available from the 'PEmetrexed and biomaRkerS: an observatiONAL study' (PERSONAL), a prospective multi-center cohort study in the Netherlands. Adult patients with locally advanced or metastatic (stage IIIB/IV) nonsquamous NSCLC receiving per standard of care (Supplemental material) platinum-combined pemetrexed therapy as first-line treatment, followed by maintenance pemetrexed if indicated, or pemetrexed monotherapy as second-line treatment, were recruited from October 2012 until November 2014. The Institutional Review Board of the Erasmus University Medical Center approved this study and all patients provided written informed consent.

Adverse events (AEs) were registered weekly during the entire treatment period and graded according to the NCI-CTCAE version 4.03 (Grade≥3 was marked as severe toxicity).[3] Clinical effectiveness endpoints were overall survival (OS), progression-free survival (PFS) and best tumor response according to RECIST 1.1.

DNA isolation and genotyping are described in the Supplemental material. Within the *TYMS* gene, two polymorphisms were selected and combined as one genotype, resulting in a high-expression (3RG/3RG), intermediate expression (3RG/3RC, 2R/3RG) and low expression genotype (2R/2R, 2R/3RC, 3RC/3RC).[4]

For details on SNP selection and statistical analyses, see Supplemental material. We used our recently developed population-PK model as a base model for the current pharmacokinetic/pharmacodynamic analyses,[5] in which SNPs were included as covariables on pemetrexed clearance. Cox regression analysis was applied in treatment-naïve patients to test the association between polymorphisms and OS/PFS. Adjustment for sex, ECOG performance score and disease stage was performed. Polymorphisms were tested against toxicity endpoints using cause-specific Cox regression analyses. If the patient died before completion of four cycles of chemotherapy, censoring for death was performed to take this into account as a competing risk.[6] In univariable analyses, correction for multiple testing was applied using the false discovery rate (FDR) Benjamini-Hochberg procedure (significance level $p < 0.1$). A two-sided $P < 0.05$ was regarded as significant in the multivariable analyses.

RESULTS

Hundred sixty-three patients were recruited, of whom 161 (99%) patients provided blood samples for pharmacogenetic analysis (Supplemental Table S1). Half of the patients were male with a mean age of 63.3 ± 9. Most patients had metastatic NSCLC (87%), and received first-line platinum-combined chemotherapy (91%). Treatment-naïve patients (n = 147) had a median OS of 7.7 months and PFS of 4.7 months. Forty-four patients (30%) continued with pemetrexed maintenance after induction treatment. The results of the pharmacogenetic analyses are demonstrated in Table 1.

Table 1. Investigated SNPs in the total cohort (N =161)

Gene	rsID	Variant	Assay-ID		WT (%)	HET (%)	HVAR (%)	MAF	HWE
SLC19A1	rs1051298	c.*746C>T	C__26854602_10		58 (36.0)	81 (50.3)	22 (13.7)	39%	0.45
GGH	rs3780126	c.109+1307C>T	C__26361922_20		65 (40.4)	74 (46.0)	22 (13.7)	37%	0.90
FPGS	rs1544105	g.2572C>T	C___8342611_10		53 (32.9)	79 (49.1)	29 (18.0)	43%	0.95
ABCC2	rs717620	c.-24C>T	C___2814642_10		100 (62.1)	56 (34.8)	5 (3.1)	20%	0.39
ABCC4	rs7317112	c.75-23516T>C	C__29165801_20		73 (45.3)	80 (49.7)	8 (5.0)	30%	0.02[†]
ATIC	rs12995526	c.815-102T>C	Assay-by-Design		39 (24.2)	79 (49.1)	43 (26.7)	49%	0.82
DHFR	rs1650697	c.-473T>C	C__27863089_10		90 (55.9)	57 (35.4)	14 (8.7)	26%	0.26
MTHFR	rs1801133	c.665C>T	C___1202883_20		72 (44.7)	77 (47.8)	12 (7.5)	31%	0.16
*TYMS**	rs45445694 c.-86G>C rs183205964	5'UTR TSER*2/ TSER*3	*See Reference [4]*	Genotypes 2R/2R 2R/3RC 2R/3RG 3RC/3RC 3RC/3RG 3RG/3RG Undetermined	 37 (23.0) 42 (26.1) 28 (17.4) 13 (8.1) 24 (14.9) 13 (8.1) 4 (2.5)			25%	0.34

* *TYMS* was categorized into a high-expression genotype (3G/3G), an intermediate expression genotype (3G/3C, 2R/3G) and a low expression genotype (2R/2R, 2R/3C, 3C/3C) depending on the 5'-UTR VNTR polymorphism and the C/G polymorphism within the third VNTR. [†] Since *ABCC4* 2168T>C was not in Hardy-Weinberg equilibrium, this SNP was excluded from all further analyses.
Abbreviations: rsID, reference SNP ID number; WT, wildtype; HET, heterozygous; HVAR, homozygous variant; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; TSER, thymidylate synthase enhancer region

Table 2. Association between polymorphisms and PFS and OS in treatment-naïve patients and toxicity in all patients

Endpoint	SNP	rsID	Model	Genotype	Univariable			Multivariable*	
					HR (95% CI)	*P*-value	Adjusted *P*-value	HR (95% CI)	*P*-value
Clinical effectiveness (n=147)									
OS	*ATIC*	rs12995526	Recessive	CC vs CT + TT	1.65 (1.11-2.45)	0.010	0.080	1.59 (1.06-2.39)	0.025
	TYMS	rs45445694 rs183205964	High vs intermediate + low	3G/3G vs 3G/3C, 2R/3G + 2R/2R, 2R/3C, 3C/3C	1.79 (0.97-3.28)	0.062	0.244		
PFS	*FPGS*	rs1544105	Dominant	TT + CT vs CC	0.65 (0.46-0.93)	0.012	0.096	0.72 (0.50-1.05)	0.084
Toxicity (n=161)									
Clinical									
Any event, grade 3/4	*ATIC*	rs12995526	Recessive	CC vs CT + TT	1.80 (1.10-2.96)	0.012	0.096	1.86 (1.12-3.07)	0.016
Diarrhea, all grade	*ATIC*	rs12995526	Recessive	CC vs CT + TT	2.01 (1.07-3.78)	0.012	0.096	1.99 (1.05-3.77)	0.034
Fatigue, severe	*ATIC*	rs12995526	Recessive	CC vs CT + TT	3.33 (1.47-7.56)	0.004	0.032*		
Anorexia, severe	*SLC19A1*	rs1051298	Dominant	TT + CT vs CC	0.15 (0.03-0.72)	0.008	0.064*		
Laboratory									
Any event, grade 3/4	*ATIC*	rs12995526	Recessive	CC vs CT + TT	1.89 (1.15-3.10)	0.011	0.088	1.87 (1.14-3.09)	0.014
Neutropenia, grade 3/4	*ATIC*	rs12995526	Recessive	CC vs CT + TT	2.24 (1.25-4.03)	0.007	0.056	2.25 (1.25-4.06)	0.007
Anemia, severe	*ATIC*	rs12995526	Recessive	CC vs CT + TT	4.38 (1.67-11.52)	0.003	0.024*		

*For clinical effectiveness endpoints adjusted for sex, disease stage and ECOG performance score. For toxicity endpoints adjusted for sex and age. † significant p<0.1 after false discovery rate correction. * Only tested univariably due to the number of events. Abbreviations: rsID, reference SNP ID number; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval

None of the polymorphisms were associated with tumor response (Supplemental Table S2). In the univariable analyses, only the *ATIC* polymorphism (rs12995526) was significantly correlated with OS after FDR correction (Table 2). Adjusted for sex, disease stage and ECOG performance score, the association between *ATIC* and OS remained (HR 1.59, 95% CI 1.06-2.39). Patients with a homozygous variant genotype (CC) had a significantly shorter OS compared to patients with CT/TT genotypes (6.2 months, 95%CI 3.4-9.0 vs 9.0 months, 95%CI 5.6-12.3, *P*=0.012), but this association was not found for PFS (Supplemental Figure S1).

Detailed information about frequencies of treatment-related AEs and univariable analyses between SNP polymorphisms and toxicity are provided in Supplemental Table S3 and S4. In multivariable analyses (Table 2), the homozygous variant genotype of *ATIC* was significantly associated with a 1.9-fold higher risk of severe laboratory and clinical AEs, a 2.0-fold higher risk of developing diarrhea and a 2.3-fold higher risk of severe neutropenia. Univariably, the CC genotype of *ATIC* was also associated with experiencing severe fatigue and severe anemia, and having at least one mutant *SLC19A1* allele was associated with an almost 7-fold lower risk of experiencing severe anorexia. These associations could not be tested multivariably due to a too low number of events.

Treatment-naïve patients with the CC genotype of *ATIC* had more dose reductions (OR 4.16 95%CI 1.59-10.93, *P* = 0.004), which was not significantly associated with OS. They continued less often with maintenance treatment than patients with the CC/CT genotypes (20% vs 33%, *P* = 0.09). Receiving maintenance therapy was associated with improved OS (HR 0.59, 95%CI 0.40-0.87, *P* = 0.01). Patients who experienced severe clinical toxicities during induction treatment received less often maintenance treatment than patients without these toxicities (19% vs 39%, *P* = 0.01).

No significant associations were observed between the selected SNPs and pemetrexed clearance in the pharmacokinetic/pharmacodynamic analyses (Supplemental Table S5).

DISCUSSION

We have found new associations between a genetic polymorphism in a gene encoding for pemetrexed target enzyme ATIC and overall survival, as well as pemetrexed-induced (severe) toxicity. None of the investigated polymorphisms could explain a part of the interpatient variability in pemetrexed pharmacokinetics.

Patients with homozygous variant *ATIC* alleles had a 1.6-fold higher risk of death and they experienced ~2 times more treatment-related toxicities than patients with CT/TT genotypes. Patients with this genotype also had a ~4 times higher risk of receiving dose reductions due to toxicity and they received less maintenance treatments. According to our data, a lower OS in patients with homozygous variant alleles of *ATIC* may be

explained by more severe treatment-related toxicity leading to dose reductions and less maintenance treatment. But, a decreased OS is not mediated by increased tumor growth due to increased purine synthesis or decreased activations of AMP-activated protein kinase, as *ATIC* genotype was not associated with tumor response and/or PFS. The effect of the intronic polymorphism rs12995526 on ATIC functionality has not been clarified yet. Recently, Zhang et al. did find an association between the CC genotype of the same *ATIC* polymorphism and worse tumor response, but no survival analyses were performed and therefore we cannot easily compare these findings with our results.[7] However, the specific ethnic Han Chinese population together with the high number of never smokers (66%), in contrast to our population (never smokers 3%), probably results in genetically different tumors, which might alter tumor behavior and response to treatment.

Although this could not be confirmed multivariably, the CT+TT genotype of *SLC19A1* was univariably associated with a 7-fold lower risk of severe anorexia. Adjei et al. showed an association between CT+TT genotype and a shorter PFS/OS.[8] Although the influence of the SNP, located in the 3'-UTR region, on gene functionality and expression is unknown, one could speculate that the CT+TT genotype may lead to a decreased influx of pemetrexed into the cell and thereby lower toxicity and less effectiveness.

To our knowledge, this is the largest NSCLC patient population treated with first-line pemetrexed in which pharmacogenetic analyses have been performed. However, a limitation of our study is still the relatively small sample size, which may have led to missed (weaker) associations between SNP genotypes and treatment outcomes due to a lack of statistical power.

A recent shift in the treatment paradigm of advanced NSCLC has led to the common use of platinum and pemetrexed plus pembrolizumab induction regimen followed by pemetrexed plus pembrolizumab maintenance. Although the survival has improved with the combination treatment of chemo/immunotherapy,[9] the combination also leads to more severe toxicity and withdrawal of induction treatment.[9,10] To date, germline genetic aberrations in genes involved in the PD-1 pathway have no clinical utility in predicting PD-1 inhibitor associated toxicities.[11] But now, polymorphism analysis of *ATIC* (rs12995526) could provide valuable information on which patients are more vulnerable to severe pemetrexed-related toxicities. Our suggestion that decreased survival in patients with the CC genotype of *ATIC* may be a result of increased toxicity is alarming, but warrants further validation.

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SUPPLEMENTAL MATERIAL

RATIONALE FOR CANDIDATE GENE AND SNP SELECTION

Although the precise reasons for this interindividual variability have not yet been discovered, several pharmacokinetic processes of pemetrexed and its mechanism of action are already well known (Figure 1). Pemetrexed is primarily eliminated via the kidneys, and hence pemetrexed clearance and total exposure are associated with renal (dys)function.[1,2] Uptake into the cells is regulated by different membrane transporters, i.e. proton-coupled folate transporter (PCFT), folate receptors α and β, and reduced folate carrier (RFC), while ATP-binding cassette transporters (ABC) of the multidrug resistance protein family ABCC1-5 are primarily responsible for the cellular efflux of (anti-)folates.[3,4] Intracellularly, pemetrexed undergoes rapid polyglutamation facilitated by folylpoly-γ-glutamate synthetase (FPGS) and γ-glutamyl hydrolase (GGH) is involved in the reverse process of deglutamation.[3] The formation of polyglutamates is thought to be a major determinant of its antitumor activity as polyglutamates are no substrates for most efflux ABCC transporters, except ABCC5, and therefore are longer retained intracellularly. Moreover, polyglutamates have a stronger affinity for the target enzymes of pemetrexed [5]. Thymidylate synthetase (TYMS) is the main target enzyme of pemetrexed and results in disturbed *de novo* thymidine production needed for DNA synthesis. By binding to its secondary target enzymes glycinamide ribunecleotide formyltransferase (GARFT) and 5-aminoimadizaole-4-carboxamide ribonucleotide formyltransferase (ATIC) *de novo* purine synthesis is also inhibited, while binding to dihydrofolate reductase (DHFR) results in a diminished active tetrahydrofolate pool needed for purine and thymidine synthesis. ATIC may also play a role in cell growth and proliferation by inhibition of the mammalian target of rapamycin (mTOR) pathway.[6] Knockdown of ATIC by pemetrexed leads to an endogenous increase in 5-aminoimidazole-4-carboxamide-1-*β*-D-ribonucleotide (AICAR), which activates AMP-activated protein kinase (AMPK) and inhibits its downstream pathway mTOR, and thereby ultimately leads to a decrease in cell proliferation and an increase in cell apoptosis.[7,8] Another potential determinant of pemetrexed activity is 5,10-methylenetetrahydrofolate reductase (MTHFR), which is an important regulator of the folic acid pathway.[9] It is both involved in DNA synthesis and methylation. Different levels of activity of all these different proteins, for example due to genetic variations, may lead to altered exposure and sensitivity to pemetrexed. In our study, we aimed to investigate whether polymorphisms of genes (Figure 1), which encode for or regulate these enzymes, are associated with clinical effectiveness and toxicity of pemetrexed in a large cohort of patients exposed to this drug.

Based on its role in the working mechanism of pemetrexed, earlier findings with regard to the relation of polymorphisms and clinical outcomes and a minor allele frequency of >10% in the European subpopulation of the 1000 Genome project using LDpop,[10] we selected SNPs of the above mentioned genes.

The polymorphism *746C>T of *SLC19A1*, encoding the major entrance transporter RFC, has been associated with progression-free and overall survival (PFS/OS) in a small group of NSCLC patients treated with the combination pemetrexed-bevacizumab and in a mixed NSCLC/mesothelioma cohort.[11,12] This polymorphism is located in the 3'-UTR region of *SLC19A1*. The SNP *ABCC2* -24C>T, has been reported to lower the expression of the protein,[13] which theoretically leads to intracellular accumulation of pemetrexed (polyglutamates) and might explain the better objective tumor response in patients with the -24CC polymorphism and the increased gastrointestinal toxicity observed with the TT polymorphism in patients treated with pemetrexed.[14,15] In patients with acute lymphocytic leukemia receiving treatment with methotrexate, closely resembling the mechanism of cell transport of pemetrexed, the wildtype variant of SNP *ABCC4* 75-23516T>C was associated with a higher risk of mucositis.[16] The polymorphism is located in intron 1 of the *ABCC4* gene, its role has not been clarified yet. With regard to the metabolizing enzymes, there is evidence that alterations in FPGS and GGH function may alter the cellular retentions of (anti)folates.[17,18] *GGH* intronic polymorphism 109+1307C>T was associated with worse median overall survival and less hematological toxicity.[11] The wildtype variant of *FPGS* 2572C>T correlated with a higher protein expression of FPGS and higher response rate.[19] *TYMS* mRNA expression is regulated by different polymorphisms, among others various number of 28-base-pair tandem repeats (VNRT) in 5' UTR enhancer region of the *TYMS* gene, and a SNP -86G>C inside this second tandem repeat.[20] Patients with a low expression genotype had a more favorable clinical response to pemetrexed, while they experienced more hematological toxicities.[21–25] The T missense variant of the *MTHFR* 677C>T has been associated with reduced enzyme activity, and thus the carrying TT genotype would be expected to lead to a favorable clinical response. Reports on *MTHFR* 677TT genotype showed contradictory results with regard to OS/PFS.[12,26,27] The *DHFR* variant c.-473T>C is located in the 5'-promotor region of the gene and wildtype T allele forms part of a promoter region haplotype that is reported to upregulate *DHFR* expression. Carrying the TT genotype was associated with increased risk of adverse events.[12] With regard to the *ATIC* polymorphism c.815-102T>C, its relation with treatment effectiveness outcomes are contradictory. Woo et al. found that patients with the CC genotype had a better tumor response and overall survival, while Zhang et al. observed a worse tumor response in patients with the CC genotype without having performed survival analysis.[14,28] The effect of the intronic *ATIC* SNP on protein expression or functionality is not clear.

MATERIALS AND METHODS

Standard of care platinum-combined pemetrexed chemotherapy

Patients received platinum-combined pemetrexed chemotherapy or pemetrexed monotherapy treatment as first-line or second-line treatment per standard of care for a maximum of 4 cycles. Pemetrexed was dosed at 500 mg/m^2 and cisplatin at 75 mg/m^2. Carboplatin dosage was calculated using the Calvert formula with a target AUC of 5

or 6. Dose adjustments (i.e. reductions) at the start of subsequent courses of therapy were based on nadir counts (neutrophils, platelets) or maximal non-hematologic toxicity from the preceding cycle of therapy. Patients were recommended to continue with pemetrexed maintenance therapy if they had no progressive disease, no intolerable toxicities and underwent no sequential radiotherapy or surgery.

DNA isolation and genotyping

Four hundred microliters of whole-blood specimens collected in EDTA tubes were extracted on the MAGNAPure Compact (Roche Diagnostics GmbH, Germany) using the Total Nucleic Acid Isolation Kit I (Roche Diagnostics GmbH, Germany) and a final elution volume of 200 ml.

Taqman genotyping

The genotyping of *SLC19A1* 746C>T (rs1015298), *GGH* 6699G>A (rs3780126), *FPGS* 2572C>T (rs1544105), *ABCC2* -24C>T (rs717620), *ABCC4* 2168T>C (rs7317112), *ATIC* 815-102T>C (rs12995526), *DHFR* -473T>C (rs1650697), *MTHFR* 677C>T (rs1801133), *TYMS* VNTR polymorphism (rs45445694) and c.-86G>C (rs183205964) was performed using TaqMan 5'-nuclease analyses (ThermoFisher, Carlsbad, CA, USA). The assay IDs are listed in Table 1. Each assay consisted of two allele-specific minor groove binding (MGB) probes, labeled with the fluorescent dyes VIC and FAM. Polymerase chain reactions (PCRs) were performed in a reaction volume of 10 ml, containing assay-specific primers, allele-specific Taqman MGB probes (Applied Biosystems), Abgene Absolute QPCR ROx Mix (Thermo Scientific, Life Technologies Europe BV, Bleiswijk, The Netherlands) and genomic DNA (20 ng).

Statistical analyses

The distribution of genotypes was tested for Hardy-Weinberg equilibrium (HWE) using the chi-squared test. Since *ABCC4* 75-23516T>C was not in HWE in our cohort (Table 1), this SNP was excluded from all further analyses.

With regard to toxicity endpoints, AEs were selected if they occurred in >10% of the patients. If an adverse event was already present in an equal or higher degree before start of treatment, it was not considered as an event. Adverse events were considered treatment-related if defined as possibly, probably or definitely related by the investigator. For both clinical effectiveness and toxicity end points, multivariable analysis was only performed in case of approximately 10 or more events per assessed variable in order to avoid bias of the regression coefficients. The selected polymorphisms were fitted and the most appropriate model was selected from four models: dominant, recessive, additive model and a multiplicative model.[29]

With a sample size of patients treated with first-line pemetrexed N = 147 and event rate (death) of 92% observed in our study, we were able to detect a hazard ratio of ≥2.0 (or

≤0.5) at a two-sided significance level of 0.025 (α = 0.05) between two genotype groups with a power of 0.8 or higher, if the proportion of the dominant or recessive genotype group was ≥ 0.14. This is the case for all SNPs, except for the recessive genotypes of *MTHFR* (MAF 31%, N = 12 (8.2%)), *ABCC2* (MAF 20%, N = 5 (3.4%)), *DHFR* (MAF 26%, N = 13 (8.8%)) and the high-expression genotype vs other of *TYMS* (MAF 25%, high-expression genotype N = 12 (8.2%)). For these genotypes the power of detection of HR ≥2.0 (or ≤0.5) was 0.35 (*ABCC2*), 0.6 (*MTHFR* and *TYMS*) and 0.64 (*DHFR*). Statistical analyses were performed with the use of SPSS, version 24.0 (IBM Corporation, Armonk, NY).

Population pharmacokinetic model

The PK data were described by a two-compartment model (population estimate (% standard error of the estimate) in terms of pemetrexed clearance CL (4.58 L/h (3.1%)), central volume of distribution V_c (15.9 L (3.3%)), peripheral volume of distribution V_p (21.6 L (5.0%)) and intercompartmental clearance (Q; 0.05 L/h (4.7%)).[30] Despite a reduction of approximately 20% in between-patient variability of pemetrexed clearance after inclusion of covariable estimated glomerular filtration rate (eGFR), still 16.7% (coefficient of variation) of the between-patient variability remained unexplained.

Genotypes encoding enzymes involved in the cell transport and polyglutamation of pemetrexed (SLC19A1, GGH, FPGS, ABCC2) were added to the previously developed population PK model and were included as dichotomous or ordinal covariables on pemetrexed clearance using the following equation:

$$CL = \theta_x * \frac{eGFR}{median}^{\theta_y} * (\theta_z)^{pg},$$

Where pg was scored '1' for patients of whom the genotype of interest was present and '0' for patients of whom the genotype was absent if the genotype was considered as a dichotomous variable (recessive or dominant genotype). If the genotype was included ordinally (additive genotype), pg was scored '0' for patients with the homozygous major allele genotype (wild-type), '1' for heterozygous patients and '2' for patients with the homozygous minor allele genotype (variant). q_x is the typical parameter value for the homozygous major allele population, q_y is the covariable effect size estimate of eGFR and q_z is the covariable effect size estimate of the SNP. First, the potential association of all SNPs was univariably tested. The threshold of this step was set at $P < 0.01$ (likelihood ratio test, Δ objective function value (OFV) >6.64, degrees of freedom = 1 or ΔOFV >9.21, degrees of freedom = 2). In the next step, all potentially related covariables were included in the full model. During a backward elimination procedure, covariables were removed one at a time from the full model again if the fit of the model did not decrease significantly ($P < 0.005$) tested using the likelihood ratio test (ΔOFV >7.88, df = 1 or (ΔOFV >10.6, df = 2).

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SUPPLEMENTAL TABLES AND FIGURES

Table S1. Patient characteristics at baseline (N = 161)

Characteristic	All patients (n = 161)
Age (yr), mean (SD)	63.3 (9.2)
Gender, male	82 (50.9)
Ethnicity, Caucasian	151 (93.8)
ECOG performance score	
0 or 1	140 (87.0)
≥ 2	19 (11.8)
Missing	2 (1.2)
Packyears	36.9 (33.9)
Never smokers	4 (2.5)
Type of tumor	
Adenocarcinoma	156 (96.9)
Large cell carcinoma	5 (3.1)
Cancer stage	
Locally advanced (IIIB)	21 (13.0)
Metastatic (IV)	140 (87.0)
Line of therapy	
First-line	147 (91.3)
Second-line	14 (8.7)
Combination therapy	
Cisplatin	99 (61.5)
Carboplatin	59 (36.6)
Monotherapy	3 (1.9)
Comorbidity	
Cardiovascular disease	68 (42.2)
COPD	23 (14.3)
Diabetes	22 (13.7)

Data are expressed as numbers (%) unless stated otherwise. Abbreviations: SD, standard deviation.

Table S2. Associations of polymorphisms with objective tumor response (N = 122)

Endpoint	SNP	Model*	Genotype	Significance Test	Univariable OR (95% CI)	*P*-value
ORR	*ATIC*	Recessive	CC vs CT + TT	Fisher's exact	0.90 (0.38 – 2.14)	0.810
	TYMS	High + intermediate vs low	3G/3G +3G/3C, 2R/3G vs 2R/2R, 2R/3C, 3C/3C	Chi-squared	0.68 (0.31 – 1.52)	0.351
	SLC19A1	Recessive	TT vs CT + CC	Fisher's Exact	3.31 (1.06 – 10.33)	0.063
	GGH	Dominant	GA + AA vs GG	Chi-squared	1.86 (0.84 – 4.11)	0.12
	FPGS	Dominant	TT + CT vs CC	Chi-squared	0.62 (0.29 – 1.36)	0.235
	ABCC2	Dominant	TT + CT vs CC	Fisher's exact	1.23 (0.56 – 2.70)	0.610
	DHFR	Additive	TT à CT à CC	Logistic regression	1.12 (0.61 – 2.06)	0.720
	MTHFR	Recessive	TT vs CT + CC	Fisher's exact	0.91 (0.22 – 3.70)	0.892

*Best fitting model. Abbreviations: OR, odds ratio; ORR, objective response rate

Table S3. Adverse events in patients with first- and second-line treatment (N = 161)

	Frequency (%)	
Adverse event	**All grades**	**Grade ≥ 3**
Treatment-related[a]		
Any event	158 (98)	99 (62)
Clinical		
Any event	157 (98)	68 (42)
Fatigue	140 (87)	23 (14)
Nausea and vomiting	106 (66)	4 (2)
Anorexia	101 (63)	10 (6)
Oral mucositis/stomatitis	75 (47)	5 (3)
Constipation	66 (41)	2 (1)
Taste alteration	62 (39)	0
Dry skin	53 (33)	0
Dizziness	49 (30)	0
Neuropathy sensory	46 (29)	0
Dry eyes/watering eyes	44 (27)	0
Diarrhea	41 (25)	4 (2)
Infection with normal neutrophil count	38 (24)	19 (12)
Dysphagia	37 (23)	2 (1)
Rash	30 (19)	0
Weight loss	29 (18)	0
Alopecia	24 (15)	0
Abdominal distension	20 (12)	1 (1)
Pruritus	18 (11)	0
Laboratory		
Any event	154 (96)	68 (42)
Anemia	139 (86)	17 (11)
Decreased white cell count	106 (66)	27 (17)
Decreased neutrophil count	97 (60)	47 (29)
Alanine aminotransferase elevation	80 (50)	2 (1)
Decreased thrombocyte count	78 (48)	17 (11)
Alkaline phosphatase elevation	63 (39)	0
Aspartate aminotransferase elevation	68 (38)	2 (1)
Blood creatinine level elevation	54 (34)	2 (1)

Listed are adverse events that are reported in at least 10% of the patients. [a]Adverse events were scored as treatment-related if investigator defined relatedness as possibly, probably or definitely.

Table S4. Association between SNPs and chemotherapy-related toxicity with $P < 0.1$ in the univariable analysis

Endpoint	SNP	Model	Univariable		
			OR (95% CI)	*P*-value	Significance Test
Severe toxicity, all	*ATIC*	Recessive	2.21 (1.02-4.81)	0.042	Chi-squared
Severe toxicity, laboratory	*ATIC*	Recessive	2.42 (1.19-4.94)	0.012†	Chi-squared
	MTHFR	Recessive	4.58 (1.19-17.6)	0.017	Chi-squared
Severe toxicity, clinical	*ATIC*	Recessive	2.42 (1.19-4.94)	0.012†	Chi-squared
Clinical					
Anorexia, all grade	*TYMS*	High vs intermediate + low	0.33 (0.10-1.07)	0.073	Fisher's exact
Anorexia, severe	*SLC19A1*	Dominant	0.14 (0.03-0.72)	0.011†	Fisher's exact
Diarrhea, all grade	*ATIC*	Recessive	2.56 (1.20-5.47)	0.011†	Chi-squared
	MTHFR	Dominant	0.47 (0.23-0.97)	0.039	Chi-squared
AKD during induction	*MTHFR*	Recessive	4.58 (1.19-17.6)	0.017	Chi-squared
	ATIC	Recessive	2.12 (1.05-4.31)	0.035	Chi-squared
Fatigue, severe	*ATIC*	Recessive	3.77 (1.52-9.36)	0.003†	Chi-squared
	DHFR	Recessive	3.98 (1.20-13.2)	0.032	Fisher's exact
	ABCC2	Dominant	2.44 (1.00-5.97)	0.047	Chi-squared
	SLC19A1	Recessive	2.69 (0.93-7.82)	0.094	Fisher's exact
Nausea and vomiting, all grade	*MTHFR*	Recessive	6.44 (0.81-51.2)	0.058	Fisher's exact
Mucositis, all grade	*SLC19A1*	Dominant	0.81 (0.42-1.54)	0.057	Fisher's exact
	ATIC	Recessive	0.52 (0.25-1.07)	0.072	Chi-squared
Infection normal ANC, all grade	*SLC19A1*	Recessive	0.69 (0.22-2.17)	0.078	Fisher's exact
	ABCC2	Recessive	5.19 (0.83-32.3)	0.086	Fisher's exact
Taste alteration, all grade	*ATIC*	Recessive	0.45 (0.21-0.98)	0.042	Chi-squared
Constipation, all grade	*SLC19A1*	Dominant	0.50 (0.26-0.97)	0.038	Chi-squared

Table S4. Association between SNPs and chemotherapy-related toxicity with $P < 0.1$ in the univariable analysis (continued)

Endpoint	SNP	Model	Univariable		
			OR (95% CI)	*P*-value	Significance Test
	FPGS	Recessive	2.02 (0.90-4.55)	0.086	Chi-squared
Dry skin, all grade	*SLC19A1*	Dominant	0.56 (0.28-1.09)	0.086	Chi-squared
Dry and watery eyes, all grade	*ABCC2*	Dominant	0.45 (0.21-0.97)	0.039	Chi-squared
Dysphagia, all grade	*ATIC*	Recessive	0.35 (0.13-0.98)	0.039	Chi-squared
	ABCC2	Recessive	5.38 (0.86-33.5)	0.080	Fisher's exact
	FPGS	Dominant	2.07 (0.87-4.90)	0.096	Chi-squared
Alopecia, all grade	*TYMS*	High + intermediate vs low	0.25 (0.08-0.78)	0.018	Chi-squared
	ATIC	Recessive	2.78 (1.13-6.80)	0.022	Chi-squared
	MTHFR	Recessive	3.23 (0.89-11.7)	0.083	Fisher's exact
Pruritus, all grade	*DHFR*	Dominant	0.41 (0.14-1.20)	0.096	Chi-squared
Laboratory					
Leukopenia, severe	*ATIC*	Recessive	2.19 (0.92-5.20)	0.071	Chi-squared
	DHFR	Dominant	3.06 (1.28-7.31)	0.019	Chi-squared
Anemia, severe	*ATIC*	Recessive	4.81 (1.70-13.6)	0.003†	Fisher's exact
	DHFR	Recessive	4.12 (1.13-15.0)	0.044	Fisher's exact
Neutropenia, severe	*ATIC*	Recessive	2.55 (1.22-5.31)	0.012†	Chi-squared
	GGH	Dominant	1.91 (0.92-3.94)	0.079	Chi-squared
Trombopenia, severe	*ATIC*	Recessive	2.77 (0.99-7.72)	0.077	Fisher's exact

† Significant after false discovery rate correction. Abbreviations: AKD, acute kidney disease; OR, odds ratio

Table S5. SNP covariable analysis on pemetrexed clearance in the full model using stepwise forward inclusion

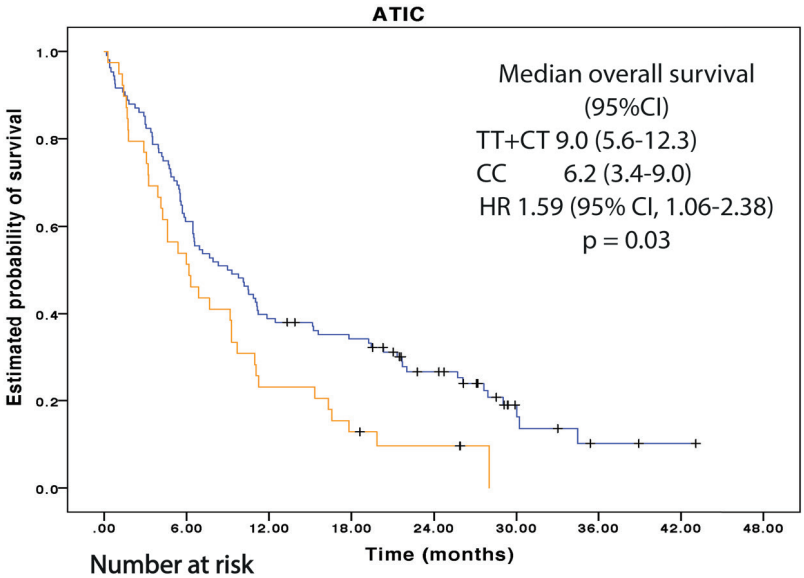
Factor	Compared genotypes	OFV	⊿OFV*
Ordinal	**Structural base model + eGFR on CL and BSA on Vc**	**-743.8**	
SLC19A1	Mut/Mut vs Mut/WT vs WT/WT	-745.3	-1.6
GGH	Mut/Mut vs Mut/WT vs WT/WT	-745.1	-1.3
FPGS	Mut/Mut vs Mut/WT vs WT/WT	-744.8	-1.0
ABCC2	Mut/Mut vs Mut/WT vs WT/WT	-744.3	-0.6
Dichotomous		**-743.8**	
SLC19A1	WT/WT vs other	-744.3	-0.5
	Mut/Mut vs other	-744.4	-0.6
GGH	WT/WT vs other	-744.7	-0.9
	Mut/Mut vs other	-743.8	-0.0
FPGS	WT/WT vs other	-744.5	-0.8
	Mut/Mut vs other	-744.3	-0.5
ABCC2	WT/WT vs other	-743.8	-0.0
	Mut/Mut vs other	-744.2	-0.5

* To determine model fit, ⊿OFV was used according to the likelihood ratio test following a chi-squared distribution. In the stepwise forward inclusion, the threshold for significant improvement of the model was set at $p < 0.01$ (dichotomous: ΔOFV > 6.64, df =1 or ordinal: ΔOFV > 9.21, df =2). In the backward elimination significant worsening of the model was set at $p < 0.005$ (Δ OFV > 7.88, df =1).

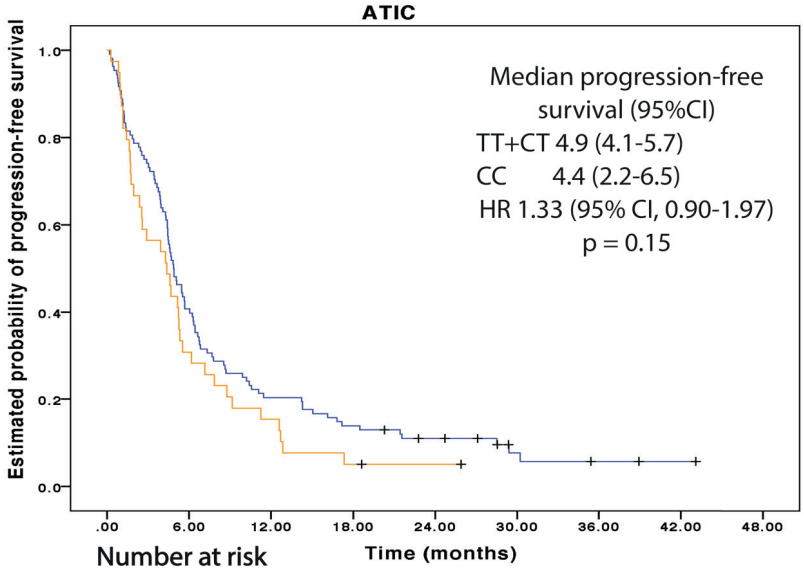
†Structural model: Two-compartment model in terms of pemetrexed clearance (CL), central distribution volume (Vc), intercompartimental clearance (Q) and peripheral volume of distribution (Vp), including between-patient variability on CL and proportional error model describing between-patient variability

Abbreviations: OFV, objective function value; CL, pemetrexed clearance; Vc, central volume of distribution; Vp, peripheral volume of distribution; BSA, body surface area; eGFR, estimated glomerular filtration rate; Mut, mutant; WT, wildtype

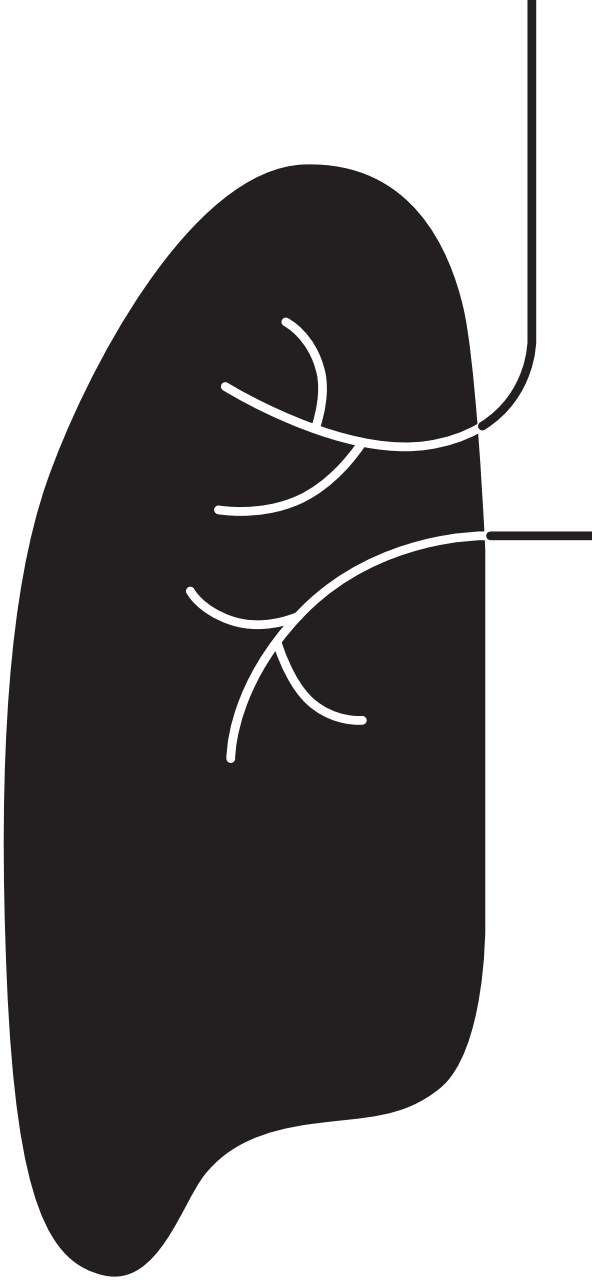
Figure S1. The overall survival and progression-free survival of pemetrexed-treated patients according to genetic polymorphisms of *ATIC*



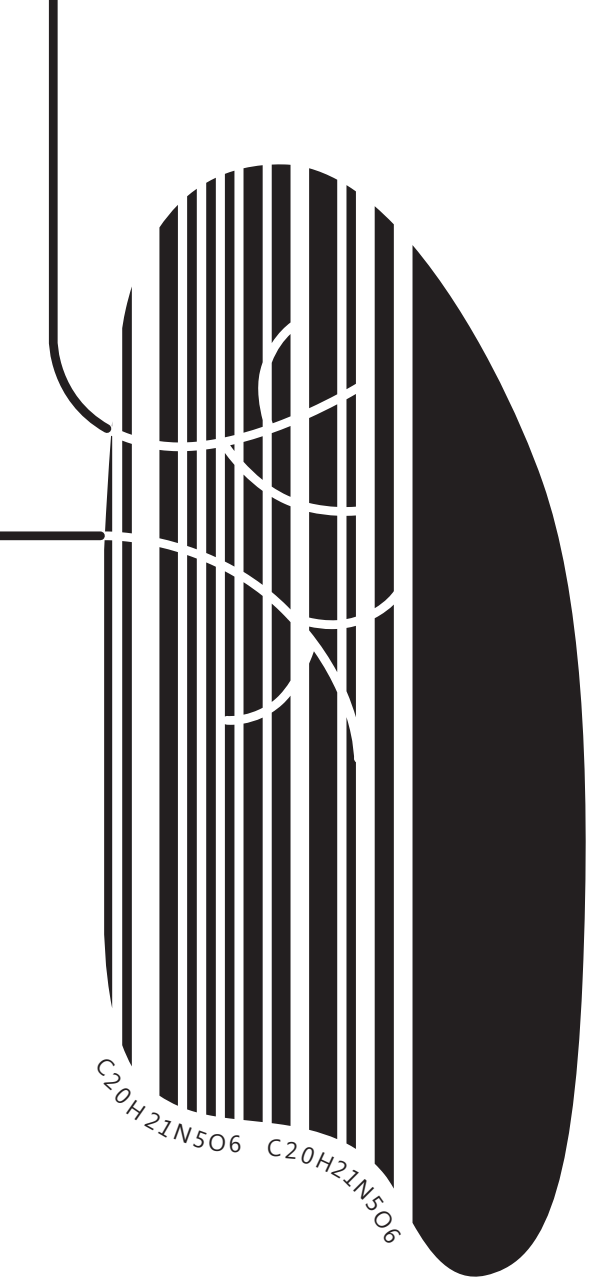
Number at risk								
TT+CT	108	66	42	35	22	7	2	1
CC	39	20	9	5	3	0	0	0



Number at risk								
TT+CT	108	44	22	15	10	4	2	1
CC	39	12	6	2	1	0	0	0



PART 2



PEMETREXED

AND RENAL TOXICITY

CHAPTER 5

Renal impairment during pemetrexed maintenance in patients with advanced non-small-cell lung cancer: a cohort study

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ABSTRACT

Background

Optimal survival benefit from different lines of anticancer treatment in advanced non-small-cell lung cancer (NSCLC) requires conservation of renal function. We evaluated the development of renal impairment during pemetrexed maintenance.

Patients and methods

In a prospective multi-center cohort study, we evaluated the incidence of acute/chronic kidney disease (AKD/CKD), its related treatment discontinuation frequency and associated clinical variables with AKD in patients with stage IIIB/IV NSCLC treated with pemetrexed maintenance. We validated findings in an independent cohort.

Results

In total 190 patients received pemetrexed. In the primary cohort 149 patients started induction of whom 44 (30%) continued maintenance. In the independent cohort 41 patients received maintenance. During maintenance, 13 patients (30%) developed AKD, leading to CKD and treatment discontinuation in 8 (62%) in the primary cohort. Higher eGFR (unit 5 mL/min/1.73 m^2) before maintenance and induction (OR 0.70, 95% CI: 0.54-0.90 and OR 0.78, 95% CI: 0.62-0.98, respectively) and relative decline (per 10%) in eGFR during induction (OR 2.54, 95% CI: 1.36-4.74) were associated with AKD during maintenance. In the independent cohort 20 patients (49%) developed AKD, leading to CKD in 11 (55%) and treatment discontinuation in 6 (30%).

Conclusion

Patients are at risk for renal impairment during pemetrexed maintenance, which may jeopardize further lines of anticancer treatment.

INTRODUCTION

In nonsquamous non-small-cell lung cancer (NSCLC) without actionable driver mutations or high PD-L1 expression, pemetrexed is widely used as first- and second-line treatment.[1] More recently, first-line platinum-based treatment with pemetrexed combined with pembrolizumab prolonged overall survival compared to chemotherapy regardless of PD-L1 expression.[2] In patients without disease progression after platinum-based induction therapy, pemetrexed is recommended as maintenance treatment.[3–6] Currently, both immunotherapy and the combination of docetaxel with antiangiogenic agents have demonstrated their superior efficacy compared to conventional chemotherapy and were approved for second line treatment.[7,8] However, to gain optimal survival benefits from all these agents, patients should be able to start as well as continue multiple lines of treatment for which it is required to maintain an adequate renal function.[9,10]

Patients with (lung) cancer are at increased risk of developing acute kidney injury (AKI).[11] Besides the exposure to nephrotoxic chemotherapeutic agents, decline in renal function in these patients is due to cancer- or chemotherapy-induced true or effective volume depletion, patient's advanced age and nephrotoxic concomitant medication.[12] The mechanism of renal injury by pemetrexed is postulated to be mainly tubulointerstitial, as pemetrexed enters the proximal tubular cells at the basolateral membrane by the reduced folate carrier and it is transported through the folate receptor at its apical site. Once inside the tubular cells, pemetrexed undergoes polyglutamylation which results in intracellular retention and increase in affinity towards enzymes involved in folate metabolism leading to tubular injury due to impaired DNA synthesis.[12,13] Although pemetrexed administration is not recommended in patients with a creatinine clearance <45 mL/min per 1.73 m^2.[14] studies have shown that even milder pre-existing renal impairment is a risk factor for drug-induced nephrotoxicity.[15,16]

Irrespective of its nature, acute kidney injury is a predictor of immediate and long-term unfavourable outcomes.[17–19] Moreover, AKI is an important risk factor for the development of chronic kidney disease (CKD),[20] and may jeopardize further cancer treatment.[21] Sustained impairment of the kidney function after discontinuation of pemetrexed maintenance therapy has been described in several case reports.[22,23] The PARAMOUNT study reported renal impairment in <10% of the patients treated with pemetrexed maintenance and < 5% of the patients discontinued treatment due to renal toxicity.[24] However, this trial population was highly selected and might underestimate the risk and consequences of renal toxicity in daily clinical practice.

Therefore, our objective was to describe the development of acute and chronic renal impairment during maintenance treatment with pemetrexed and its impact on treatment decisions in a real-world setting.

MATERIALS AND METHODS

Prospective multi-center cohort (Primary cohort)

PEmetrexed and biomaRkerS: an observatiONAL study (PERSONAL) is a prospective multi-center cohort study of adult patients with locally advanced or metastatic (stage IIIB/IV) nonsquamous NSCLC and unresectable mesothelioma receiving platinum-combined pemetrexed as first-line and pemetrexed monotherapy as second-line treatment. Patients were recruited between October 2012 and November 2014 from a university hospital (Erasmus University Medical Centre), two large teaching hospitals specialized in lung cancer care (Amphia hospital; Franciscus Gasthuis) and a regional hospital (Bravis hospital) in the Netherlands. Patients who received pemetrexed as second-line treatment and patients with unresectable mesothelioma were excluded from analyses in the present study. The PERSONAL cohort will be denoted as 'primary cohort' in the following parts of this paper. All patients provided written informed consent. The study was approved by the Institutional Review Board of the Erasmus University Medical Centre in Rotterdam, the Netherlands.

Per standard of care, platinum-combined pemetrexed chemotherapy was administered as an intravenous infusion every three weeks for a maximum of 4 cycles. The administered dosages of pemetrexed and cisplatin were calculated according to the body surface area, 500 mg/m^2 and 75 mg/m^2 respectively.[14] Carboplatin dosage was calculated based on estimated glomerular filtration rate (eGFR) and the target area under the curve of five or six following the Calvert formula.[25] If the chemotherapy schedule involved cisplatin, pre- and post-hydration treatment was given per protocol. Patients were recommended to continue with pemetrexed maintenance therapy if they had no progressive disease, no intolerable toxicities and underwent no sequential radiotherapy or surgery.

Prior to the initial chemotherapy cycle baseline serum creatinine (μmol/L) was obtained. Subsequently, prior to and weekly after each chemotherapy administration during the induction therapy, serum creatinine was measured. During maintenance treatment blood samples were only extracted prior to pemetrexed administration and at day 14 of each cycle. Estimations of renal function were made by calculation of the eGFR (mL/min/1.73 m^2) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[26] Renal adverse events were registered according to the Common Terminology Criteria of Adverse Events (CTCAE) version 3.0, for comparison to the registration trial of pemetrexed maintenance,[24] and the updated version 4.03:

	All grades
CTCAE 3.0	creatinine: creatinine > upper limit of normal eGFR: eGFR <75% lower limit of normal
CTCAE 4.03	Acute kidney injury: creatinine level increase of >26.5 μmol/L (0.3 mg/dL); creatinine >1.5 × above baseline

Independent cohort

To validate findings in the primary cohort, we selected all patients with advanced NSCLC who started treatment with pemetrexed maintenance between November 2014 and December 2016 in one hospital (Amphia Hospital). We used the pharmacy database of this centre to construct a second independent cohort of patients who received pemetrexed maintenance treatment after the patient enrolment in above mentioned PERSONAL study had finished. Prior to maintenance treatment, these patients received first-line platinum-combined induction treatment with pemetrexed and hydration per standard of care (See *Prospective multi-centre cohort: Primary cohort)* creatinine level before start of induction and maintenance and during maintenance prior to each pemetrexed administration. As data in this cohort were collected retrospectively, no approval by a medical research and ethics committee was necessary according to Dutch guidelines.

Definitions of acute and chronic kidney disease

In both cohorts, patients with acute kidney disease (AKD) and chronic kidney disease (CKD) during induction and maintenance therapy were identified in accordance with the Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guidelines.[27,28]

AKD	eGFR <60mL/min per 1.73 m^2 for <3 months*, OR
	Decrease in eGFR by >35%, OR
	Increase in serum creatinine >50% for <3 months
CKD	eGFR <60 mL/min per 1.73 m^2 for >3 months

* In patients with a baseline eGFR <60 mL/min per 1.73 m^2 only change in eGFR and serum creatinine during next three months were used as criteria for AKD

Besides the development of CKD, we registered clinical consequences related to decreased renal function in terms of discontinuation of therapy, hospitalization and dose adjustments and postponements.

Statistical analysis

Sociodemographic and clinical variables were described for all patients who were included in both cohorts. Patients from the primary cohort who underwent maintenance treatment were categorized into two groups (eGFR <90 mL/min vs eGFR ≥90 mL/min) according to their renal function at baseline (start of induction treatment) and at the start of maintenance therapy. For these groups, we reported the percentages of patients with AKD, CKD and clinical consequences with 95% confidence intervals (95% CI) calculated using the Wilson score method. The difference in incidence of AKD between these groups was examined using the χ^2-test or Fisher's exact test. With the use of logistic regression, we determined the univariable association of renal function before induction, change of renal function during induction (both per unit eGFR of 5 mL/min per 1.73 m^2) and other patient- and treatment-related factors with the incidence of AKD during maintenance.

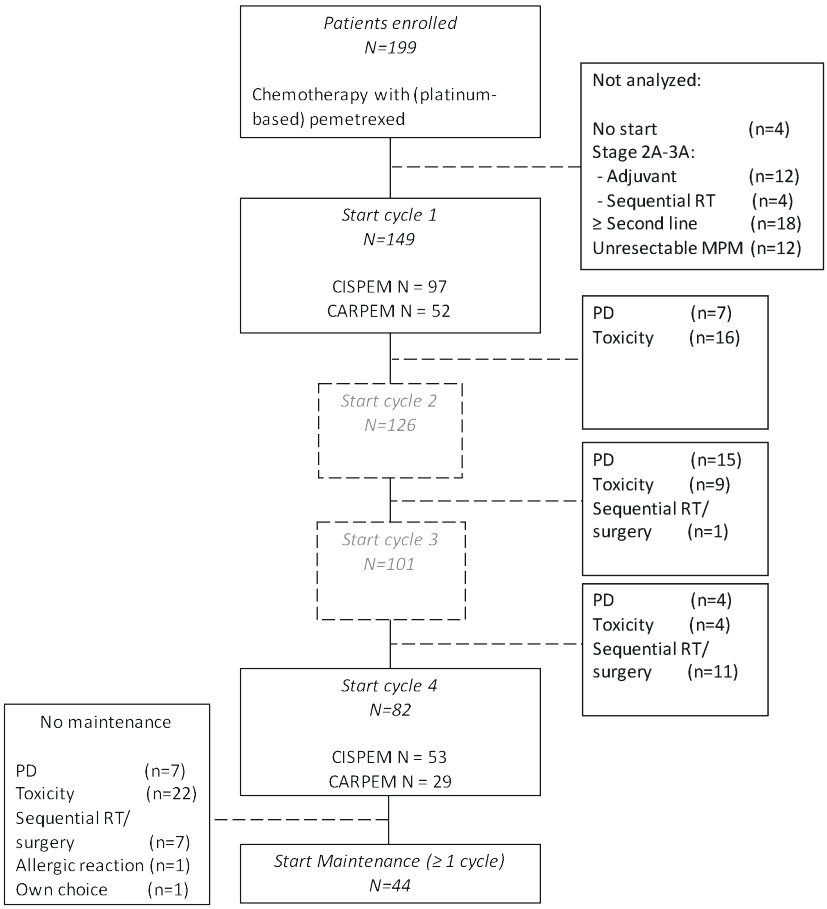
To verify findings from our prospective cohort study, we repeated these analyses in the second independent cohort.

All statistical analyses were performed with the use of SPSS, version 22.0 (IBM Corporation, Armonk, NY). A value of $P < 0.05$ was considered as statistically significant.

RESULTS

In total, 190 patients who received treatment with pemetrexed were included in the current study. In the primary cohort, 149 patients with advanced NSCLC who started first-line induction treatment with pemetrexed were enrolled. Of these patients, 44 (29.5%) ultimately received one or more cycles of pemetrexed maintenance treatment (Figure 1).

Figure 1. Flowchart of patients in the primary cohort.



Abbreviations: NSCLC, non-small cell lung cancer; MPM, malignant pleural mesothelioma; PD, progressive disease; RT, radiotherapy.

The second independent cohort consisted of 41 patients with advanced NSCLC who had received ≥1 cycle of pemetrexed maintenance therapy after first-line induction treatment.

All patient- and treatment characteristics of patients in both cohorts are outlined in Table 1. In the primary cohort, a higher percentage of patients with maintenance pemetrexed had metastatic disease (*P* = 0.003) and they had a higher serum albumin (*P* = 0.001) than patients who only received induction treatment (N = 105). Between patients who underwent maintenance in both cohorts, there were no significant differences and platinum-combination treatments were similar. Slightly more female patients underwent pemetrexed maintenance in the independent cohort than in the primary cohort (65.9% vs 50.0%, *P* = 0.188). Median follow-up time was 3.2 months (Interquartile range [IQR]: 1.9-6.1) in the second cohort and 3.5 months (IQR: 1.4-8.3) in the primary cohort.

Renal impairment in the primary cohort

Induction treatment

Calculated eGFR values at baseline were significantly different between the patients treated with CISPEM and with CARPEM (98.1 ± 16.0 vs 88.7 ± 15.9, *P* = 0.001). Over the total induction treatment of 4 cycles, the mean eGFR decreased in patients treated with CISPEM (*N* = 53) in contrast to the mean eGFR in patients treated with CARPEM (N = 29) (-9.1 ± 9.5 vs. -2.0 ± 11.0, *P* = 0.003). During the total induction period, 48 patients (49.5%) treated with CISPEM developed AKD at any time during the induction period compared to 15 patients (29%) treated with CARPEM. The proportion of patients with AKD during CARPEM treatment remained constant around 15% per cycle. In contrast, the occurrence of AKD accumulated with the number of cycles of treatment with CISPEM (20% during cycle 1, 50% during cycle 4) (Supplemental Figure S1).

Maintenance

The median number of maintenance pemetrexed cycles was five (IQR: 2-12) and the median eGFR before administration of the first maintenance cycle was 86.3 (IQR: 71.6-97.2). During maintenance treatment with pemetrexed 13 of the 44 patients (29.5%) developed AKD according to KDIGO definitions. From these 13 patients, 10 patients (77%) had all grades renal adverse events according to CTCAE 4.03 compared to only 7 patients (54%) using CTCAE 3.0. Hence, using CTCAE 3.0 we found only 16% of patients experienced renal adverse events.

Individual courses of patients' renal function are shown in Figure 2. Compared to patients with an eGFR ≥90mL/min at the start of maintenance, patients with a mildly decreased renal function (eGFR <90 mL/min) more frequently developed AKD (11/23 vs 2/21, *P* = 0.005) and their renal function more often decreased below the recommendation threshold of pemetrexed administration (eGFR <45mL/min, 6/21 vs 1/21, *P* = 0.017). Two patients with an eGFR <45 mL/min already before maintenance were excluded from this analysis.

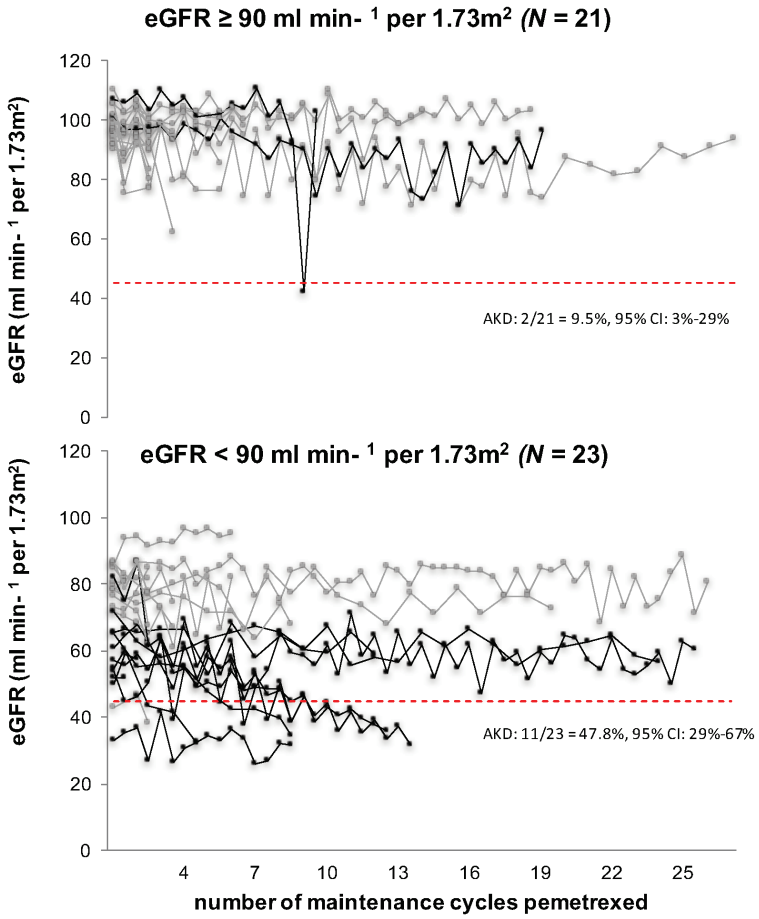
Table 1. Characteristics of all patients with advanced NSCLC who received treatment with pemetrexed (N = 190)

	COHORT 1		COHORT 2
	No maintenance pemetrexed N = 105	Maintenance pemetrexed N = 44	Maintenance pemetrexed N = 41
Age, mean (SD)	63.7 (9.4)	62.9 (7.5)	62.8 (6.7)
Sex, male	52 (49.5)	22 (50.0)	14 (34.1)
Ethnicity			
Caucasian	100 (95.2)	42 (95.5)	38 (92.7)
Negroid	1 (1.0)	0	0
Asian	2 (1.9)	0	0
Other	2 (1.9)	2 (4.5)	3 (7.3)
BMI, mean (SD)	24.3 (3.9)	25.3 (3.7)	24.8 (5.3)
Packyears (SD)	38.3 (36.4)	34.7 (23.3)	34.0 (21.0)
Type of tumor			
Adenocarcinoma	102 (97.1)	44 (100)	41 (100)
Large cell carcinoma	3 (2.9)	0	0
Cancer stage[†]			
Locally advanced (IIIB)	20 (19.0)	0	2 (4.8)
Metastatic (IV)	85 (81.0)	44 (100)	39 (95.1)
Line of induction treatment			
First-line	105 (100)	44 (100)	41 (100)
Platinum combination			
Cisplatin	65 (61.9)	32 (72.7)	31 (75.6)
Carboplatin	40 (38.1)	12 (27.3)	13 (24.4)
Laboratory values			
Creatinine (mL/min), median (IQR)	61.0 (49.0-72.5)	57.5 (52.0-70.0)	64.0 (51.5-79.0)
eGFR (mL/min per 1.73m^2), median (IQR)	96.9 (85.4-104.7)	97.6 (88.6-106.1)	95.4 (79.4-101.0)
eGFR < 60 mL/min per 1.73m^2	6 (5.7)	2 (4.5)	1 (2.4)
Albumine (g/L), mean (SD)	38.6 (5.3)	41.3 (3.8)	unknown
Comorbidity			
Cardiovascular disease	47 (44.8)	16 (36.4)	18 (43.9)
Diabetes mellitus	17 (16.2)	4 (9.1)	5 (12.2)

Data are expressed as numbers (%) unless stated otherwise. eGFR was calculated using the CKDEPI formula. [†]Patient received only palliative chemotherapy (lymfangitis carcinomatosa). Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; SD, standard deviation; IQR, interquartile range.

Per unit 5 mL/min/1.73 m^2, higher eGFR both before start of maintenance and induction treatment were associated with a lower risk of AKD as is shown in table 2 (OR 0.70, 95% CI: 0.54-0.90 and OR 0.78, 95% CI: 0.62-0.98 respectively). In contrast, 10% decline in eGFR during induction relative to baseline was associated with an increased probability of AKD (OR 2.54, 95% CI: 1.36-4.74). In patients with AKD the mean decrease of eGFR during induction was -12.2 ± 8.9 mL/min compared to -2.1 ± 8.4mL/min in patients without AKD.

Figure 2. Renal function and development of AKD during pemetrexed maintenance therapy in the primary cohort (N = 44).



Dots: individual measurements of renal function. Solid lines: course of renal function during maintenance therapy of individuals who develop AKD (black) and do not develop AKD (grey). Dashed line (red): eGFR = 45 mL/min, value below which pemetrexed administration is not recommended. Abbreviations: AKD, acute kidney disease; eGFR, estimated glomerular filtration rate; CI, confidence interval.

Table 2. Univariable analysis of clinical and treatment-related factors associated with acute kidney disease during pemetrexed maintenance

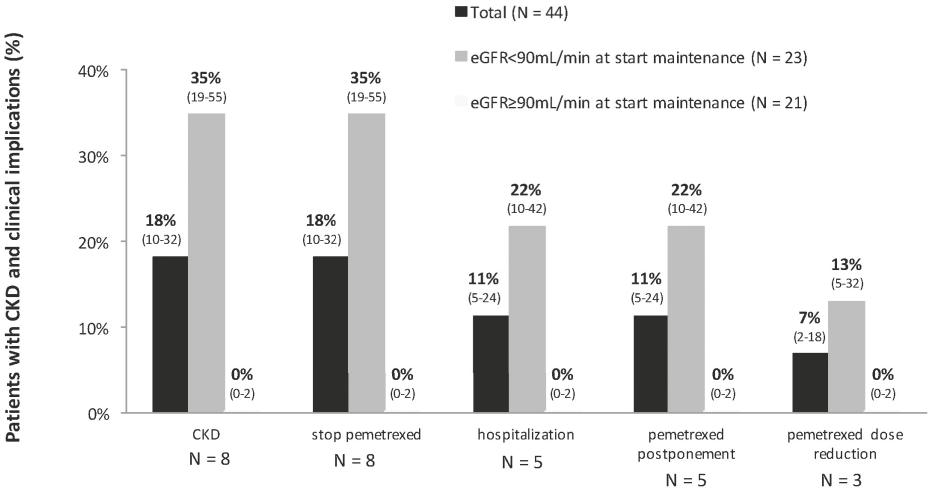
	Primary cohort (N = 44)		Independent cohort (N = 41)	
	Odds ratio (95% CI)	***P*-value**	**Odds ratio (95% CI)**	***P*-value**
Age	1.07 (0.98 - 1.18)	0.14	0.98 (0.89 - 1.07)	0.62
Sex				
male vs. female	0.52 (0.14 - 1.93)	0.34	0.28 (0.068 - 1.11)	0.069
History of cardiovascular disease				
yes vs. no	0.70 (0.18 - 2.80)	0.62	0.73 (0.21 - 2.53)	0.62
Combination CISPEM during induction				
yes vs. no	2.62 (0.49 - 14.11)	0.26	0.94 (0.23 - 3.90)	0.93
No. of cycles pemetrexed maintenance	1.08 (1.0 - 1.17)	0.059	1.09 (0.96 - 1.23)	0.2
eGFR decrease during induction*	2.54 (1.36 - 4.74)	0.004	1.56 (1.03 - 2.36)	0.038
eGFR before induction†	0.78 (0.62 - 0.98)	0.032	0.78 (0.62 - 0.98)	0.035
eGFR before maintenance†	0.70 (0.54 - 0.90)	0.005	0.64 (0.48 - 0.84)	0.001

*eGFR change relative to baseline per 10%. †eGFR per unit 5ml min^{-1} per 1.73m^2. Abbreviations: CISPEM, cisplatin and pemetrexed; eGFR, estimated glomerular filtration rate; CI, confidence interval

Clinical implications

The development of CKD and clinical consequences of renal impairment during maintenance therapy are outlined in Figure 3. Of the 13 patients (30%) who obtained AKD during maintenance therapy, 8 patients ultimately developed CKD (62%). Eight of the 13 patients with AKD (62%) were forced to discontinue maintenance treatment due to renal impairment. Importantly, all of these patients who developed CKD and stopped treatment already had a mildly impaired renal function (< 90 mL/min) before start of maintenance. Moreover, in patients whose renal function was already mildly impaired before induction (< 90mL/min) the proportion of patients who had to discontinue treatment was higher than in patients with a normal eGFR (6/11 vs 2/33, $P = 0.001$). Accordingly, patients more often developed AKD (6/11 vs 7/33, $P = 0.057$) and CKD (5/11 vs 3/33, $P = 0.016$) if renal function was mildly impaired before induction.

Figure 3. Chronic kidney disease and clinical implications due to renal impairment during pemetrexed maintenance therapy in the primary cohort (N = 44).



Data are expressed as percentages with 95% confidence interval. Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate

Renal impairment in the independent cohort

In the independent cohort, the median number of maintenance pemetrexed cycles was four (IQR: 3-8) and the median eGFR before administration of the first maintenance cycle was 80.6 (IQR: 63.4-93.3). Twenty patients (49%) obtained AKD, of whom 11 patients eventually developed CKD (55%) and six discontinued pemetrexed maintenance (30%). Similarly to the primary cohort, all patients who developed CKD and stopped maintenance treatment had an eGFR <90 mL/min before start of maintenance.

We tested the same patient- and treatment related variables for their relation with development of AKD during maintenance as in the primary cohort (Table 2). Again, per unit 5 mL/min/1.73 m^2 higher eGFR before maintenance and before induction were univariably associated with a lower probability of AKD during maintenance (OR 0.64, 95% CI 0.48 - 0.84 and OR 0.78, 95% CI 0.62 - 0.98 respectively). Also, a 10% decline in eGFR during induction compared to baseline was related with an increased risk of AKD (OR 1.56, 95% CI 1.03 - 2.36).

DISCUSSION

In an era of accelerated development and adaptation of new agents with survival benefits for patients with advanced NSCLC, it becomes increasingly important to ascertain patients are able to start and continue multiple lines of treatment. Our study shows serious concerns with regard to the preservation of an adequate renal function during pemetrexed maintenance therapy, which might expose patients to a suboptimal oncological treatment. In a real-world setting, one-third of patients with metastatic NSCLC developed acute kidney disease during pemetrexed maintenance therapy and half of these patients were forced to discontinue maintenance treatment. Moreover, in the majority of patients with AKD renal function did not -or only partially- recover and these patients developed CKD. Importantly, these results were verified in an independent cohort of patients with advanced NSCLC treated with pemetrexed maintenance.

A ~20% lower risk of occurrence of AKD during pemetrexed maintenance therapy was observed in patients per 5 mL/min higher eGFR before the start of induction therapy. The proportions of patients who developed AKD, CKD and who discontinued maintenance treatment were significantly higher in patients with an impaired renal function (eGFR <90 mL/min) at the start of maintenance and before induction. It has already been recognized that decreased renal function, even mildly, can predispose to chemotherapy-induced nephrotoxicity.[15,29] Sassier et al. also reported a linkage between renal impairment before maintenance treatment and the higher probability of discontinuing double maintenance therapy with pemetrexed and bevacizumab.[30] In contrast to our study, they did not find an association between renal function before induction and treatment discontinuation. Besides almost 20% missing data of renal function before induction and a lack of patients with an eGFR <60 mL/min at baseline in that study, the different pathophysiology leading to renal damage due to bevacizumab might explain this difference.

During pemetrexed maintenance, patients were at ~2-fold higher risk of developing AKD per 10% decline of eGFR during induction therapy relative to baseline. Patients treated with CISPEM showed a decline in eGFR of approximately 10 mL/min, which is comparable to recent findings in patients who received cisplatin for treatment across multiple tumor types.[13] As treatment with CISPEM during induction therapy was not associated with AKD throughout the maintenance period, it is unlikely that nephrotoxicity during maintenance is solely a delayed cisplatin effect. This is supported by findings of follow-up studies in patients with various cancer types including lung cancer, which demonstrated that declines in eGFR did not deteriorate after discontinuation of cisplatin.[13,31] Although not statistically significant (P = 0.06), AKD occurred more often in patients who received a higher number of pemetrexed maintenance cycles. In these patients a cumulative systemic dose of pemetrexed might play a role in the development of nephrotoxicity, also recently suggested by Langer et al.[32]

The nephrotoxic potential of pemetrexed has been previously described in clinical studies. In the pivotal PARAMOUNT trial,[3] Pujol et al. reported all grades renal toxicities according to CTCAE 3.0 in 7.8% of patients and treatment discontinuation in 4.5% of patients due to renal impairment during pemetrexed maintenance.[24] Acknowledging small patient numbers, our study notes probable underestimation of renal toxicity by using the CTCAE 3.0 compared to AKD (KDIGO). By taking into account absolute increases of creatinine and its relative increase from baseline, the results of the updated version CTCAE 4.03 corresponded better with the AKD results. Additionally, the patient population in the PARAMOUNT trial was highly selected with regard to ECOG performance score, renal function at baseline and concomitant medication as opposed to our real-life population. Therefore, that trial probably underestimated the risk of renal insufficiency in daily practice. Although pemetrexed maintenance was combined with bevacizumab and therefore results cannot solely be attributed to pemetrexed, Sassier et al. reported renal adverse events resulting in treatment discontinuation in 17% of the patients.[30]

Due to a low number of event-rate per subgroup in the primary and independent cohort, we could not perform a multivariable analysis to identify patient- and treatment-related variables associated with the development of AKD during maintenance therapy. As both cohorts differed with regard to frequency and timing of data collection of renal function by design, we did not consider it suitable to perform a combined analysis of these cohorts. We cannot exclude effect modification by the platinum compound, as all patients received CISPEM or CARPEM during induction treatment without a pemetrexed monotherapy comparator arm.

In conclusion, the results of this study in a real-life setting demonstrate that patients with advanced NSCLC are at risk to develop renal impairment during pemetrexed maintenance therapy. This has important clinical consequences, as the majority of these patients develop CKD, ~15-20% are forced to stop maintenance treatment and further anticancer treatment may be jeopardized. Increased awareness and further exploration of renal protective strategies for patients at increased risk might be beneficial, such as continuation of hydration during pemetrexed maintenance.

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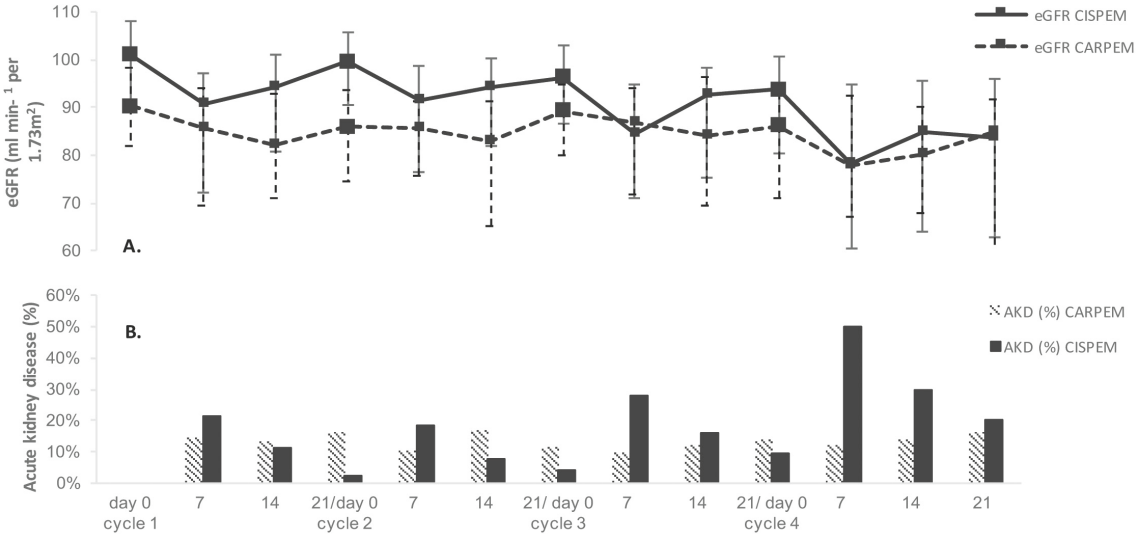
SUPPLEMENTAL FIGURE

Figure S1. Renal function and development of AKD during induction treatment (N = 149) in the primary cohort.



Number at risk													
CISPEM	97			83			67			53			
CARPEM	52			43			34			29			
Treatment discontinuation													
PD				7			15			4			
toxicity				16			9			4			
sequential RT/surgery							1			11			

(A) Squares: median eGFR with interquartile range at weekly measurements in patients at risk. Lines: course of renal function during induction pemetrexed combined with cisplatin (solid) and carboplatin (dashed). (B) Columns: weekly percentage of patients at risk who develop acute kidney disease during induction pemetrexed combined with cisplatin (filled) and carboplatin (pattern). Abbreviations: AKD, acute kidney disease; eGFR, estimated glomerular filtration rate; CISPEM, cisplatin combined with pemetrexed; CARPEM, carboplatin combined with pemetrexed; PD, progressive disease; RT, radiotherapy.

CHAPTER 6

Pemetrexed exposure predicts for toxicity in advanced non-small-cell lung cancer: a prospective cohort study

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ABSTRACT

Background

We explored whether total exposure to pemetrexed predicts effectiveness and toxicity in advanced non-small-cell lung cancer (NSCLC). Furthermore, we investigated alternative dosing schedules.

Methods

In this prospective cohort study, patients with advanced NSCLC receiving first- or second-line pemetrexed(/platinum) were enrolled. Plasma sampling was performed weekly (cyclePK) and within 24 hours (24hPK) after pemetrexed administration. With population pharmacokinetic/pharmacodynamic modelling, total exposure to pemetrexed during cycle 1 (area under the curve (AUC_1)) was estimated and related to progression-free/overall survival (PFS/OS). We compared mean AUC_1 (mg·h/L) in patients with and without severe chemotherapy-related adverse events (AEs) during total treatment. Secondly, different dosing schedules were simulated in order to minimize the estimated variability (coefficient of variation (CV)) of AUC.

Results

For 106 of the 165 patients, concentrations of pemetrexed were quantified (24hPK, N = 15; cyclePK, N = 106). Adjusted for prognostic factors sex, disease stage and WHO performance score, AUC_1 did not predict for PFS/OS in treatment-naive patients (N = 95) (OS, HR = 1.05, 95%CI: 1.00-1.11; PFS, HR = 1.03, 95%CI: 0.98-1.08). Patients with severe chemotherapy-related AEs (N = 55) had significantly higher AUC_1 values than patients without them (N = 51) (226 ± 53 vs 190 ± 31, $P < 0.001$).

Compared to BSA-based dosing (CV 22.5%), simulation of eGFR-based dosing (CV 18.5%) and fixed dose of 900 mg with 25% dose reduction if eGFR < 60 mL/min (CV 19.1%) resulted in less interindividual variability of AUC.

Conclusions

Higher exposure to pemetrexed does not increase PFS/OS, but is significantly associated with increased occurrence of severe toxicity. Our findings suggest that fixed dosing reduces interpatient pharmacokinetic variability and thereby might prevent toxicity, while preserving effectiveness.

INTRODUCTION

Despite the introduction of molecular-targeted agents and immunotherapy, pemetrexed-based chemotherapy still has an important role in the treatment of nonsquamous non-small-cell lung cancer (NSCLC).[1] Recently, the combination of immunotherapy with platinum-based pemetrexed chemotherapy showed a superior survival benefit compared to chemotherapy alone in the first-line treatment of advanced NSCLC, regardless of programmed death-ligand 1 (PD-L1) expression. This combination treatment has now become standard of care and is well tolerated in general.[2,3] However, in the combination arm of the registration study (KEYNOTE-189) adverse events led to discontinuation of a treatment component (pembrolizumab or pemetrexed) twice as often compared to platinum-based pemetrexed therapy.[2] To derive optimal benefit from the combination treatment, toxicity should be minimized.

Comparable to most chemotherapeutic agents, the dosage of pemetrexed is adjusted to a patient's body surface area (BSA), which should theoretically lead to equal drug concentrations as larger patients have a larger distribution volume and a higher clearance than smaller patients.[4] Pemetrexed is eliminated primarily via the kidneys, with 70-90% of the administered drug excreted unchanged into urine within 24 hours [5,6] and the occurrence of toxicities is associated with total systemic exposure.[5,7] Therefore, there might be a better rationale for adaptive dosing strategies other than those based on BSA.[8]

Using population pharmacokinetic/dynamic (popPK/PD) modelling, we explored whether total systemic exposure to pemetrexed predicts for progression-free and overall survival (PFS/OS) and occurrence of severe chemotherapy-related adverse events (AEs) in patients with NSCLC. Additionally, different strategies for pemetrexed dosing were simulated and compared.

METHODS

Pharmacokinetic data were available from 'PEmetrexed and biomaRkerS: an observatiONAL study' (PERSONAL). This was a prospective multi-center cohort study of adult patients with locally advanced or metastatic (stage IIIB/IV) nonsquamous NSCLC or unresectable mesothelioma receiving platinum-combined pemetrexed therapy as first-line treatment or pemetrexed monotherapy as second-line treatment. From October 2012 until November 2014, patients were recruited from a university hospital (Erasmus University Medical Center), two large teaching hospitals specialized in lung cancer care (Amphia hospital; Franciscus Gasthuis en Vlietland) and a regional hospital (Bravis hospital) located in the southwestern part of the Netherlands. For the present study, patients were eligible if blood sampling and measurement of pemetrexed concentrations was performed. Patients with unresectable mesothelioma were excluded

from all analyses. For the primary outcome exploring the relation between pemetrexed pharmacokinetics and PFS/OS only treatment-naïve patients were included in the analyses. All patients provided written informed consent. This study was approved by the appropriate institutional review boards and ethics committees at each institution.

Data collection

Per standard of care (Supplemental material), platinum-combined pemetrexed chemotherapy or pemetrexed monotherapy was administered as first-line and second-line treatment to patients as an intravenous infusion every three weeks for a maximum of 4 cycles. No patient received pembrolizumab.

We collected sociodemographic characteristics (age, sex, ethnicity), body size measures (weight, BSA), renal function and information about cancer stage and treatment. Prior to the initial chemotherapy, cycle baseline serum creatinine (μmol/L) was obtained. Subsequently, prior to and weekly after each chemotherapy administration during the induction therapy, serum creatinine was measured. Estimations of renal function were made by calculation of the eGFR (mL/min per 1.73 m^2) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[9]

Pharmacokinetic assessments

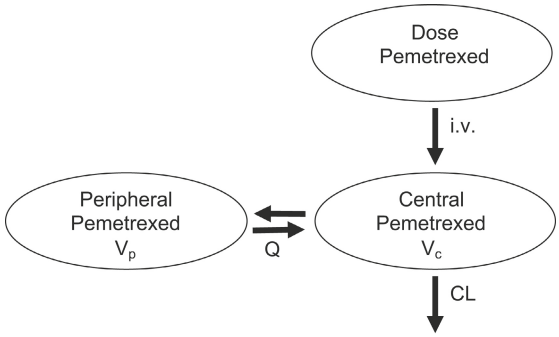
Prior to and weekly after each pemetrexed administration, sparse plasma sampling was performed (cyclePK). In a subgroup, blood samples were intensively collected on the first day of the first chemotherapy cycle at pre-infusion and 10, 30 minutes and 1, 2 ,4, 8, 24 hours after start of pemetrexed infusion (24hPK) additional to cyclePK sampling.

We validated an assay to quantitate the plasma pemetrexed concentrations, using a liquid chromatographic method coupled to tandem mass spectrometry (UP-MS/MS). A detailed description of the validation of this assay and method can be found in Supplemental material.

Pharmacokinetic model development

Plasma concentration-time data were analyzed using nonlinear mixed effect modeling. A two-compartment model for pemetrexed was structured based as schematically shown in Figure 1. Once the base model was defined, clinical variables were tested as covariables on parameters clearance (CL), central volume of distribution (V_c) and peripheral volume of distribution (V_p) using stepwise forward inclusion and backward elimination.[10] Detailed description of the model development and covariable analyses can be found in Supplemental methods.

Figure 1. Schematic representation of the population pharmacokinetic model of pemetrexed.



Abbreviations: CL, clearance; Q, intercompartimental clearance; V_c central volume of distribution; V_p, peripheral volume of distribution.

The final popPK model was internally validated using goodness of fit plots, visual predictive check plots (Supplemental Figure S1 and S2) and a bootstrap procedure. Subsequently, the final popPK model was used to estimate area under the plasma concentrations versus time curves (AUCs) for all cycles of each patient. Simulations of different dosing strategies of pemetrexed were performed with the developed final popPK model: BSA-based, renal function based and fixed dose with a dose reduction of 25% if eGFR <60 mL/min.

Endpoints

Clinical effectiveness endpoints were OS, PFS and best tumor response. Tumor response measurements were obtained according to RECIST 1.1 after the 2nd and 4th cycle of chemotherapy. Adverse events (AEs) were weekly registered during the entire treatment period and graded (severe: grade ≥3) according to the National Cancer Institute Common Terminology Criteria of Adverse Events (NCI-CTCAE) version 4.03.

Statistical analysis

Using Cox proportional hazards regression analyses, the relation between AUC during cycle 1 (AUC_1) and OS/PFS in treatment-naive patients was studied, adjusted for known prognostic factors sex, disease stage and ECOG performance status. The association of AUC_1 with best treatment response over a total treatment of 4 cycles was tested with one-way ANOVA. Differences in mean AUC_1 between patients with and without grade ≥3 chemotherapy-related AEs during the entire course of four cycles induction treatment were compared using the independent-samples *t*-test. With regard to toxicities related to pemetrexed, we distinguished clinical and laboratory AEs.

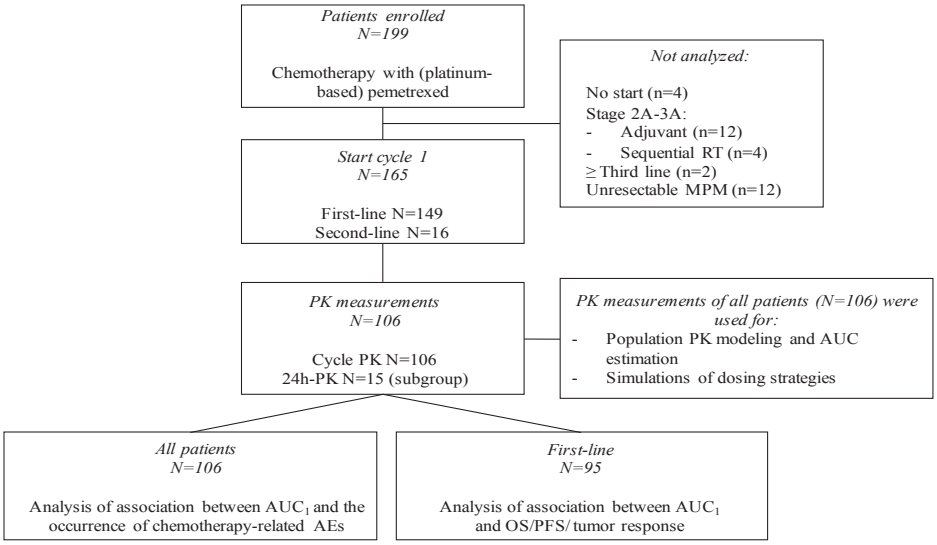
Using the final popPK model, simulations of mentioned different dosing regimens of pemetrexed were performed and explored in order to minimize the estimated variability in AUC and maintain similar population median AUC values compared to the current

dosing schedule. We compared the interindividual variation of AUCs of the distinct dosing regimens using coefficients of variation (CV) and graphically visualized systemic patterns in predicted exposures plotted against corresponding BSA and renal functions of these patients. Statistical analyses were performed with the use of SPSS, version 22.0 (IBM Corporation, Armonk, NY).

RESULTS

In total, 199 patients were included in the PERSONAL study. Of these patients, 165 (83%) started pemetrexed-based chemotherapy as first- or second-line treatment. The first 106 of these 165 patients (64%) were consecutively selected for the current study, as in these patients weekly pemetrexed cyclePK measurements were performed (Figure 2). In a subgroup of these patients (N = 15, 14%), we also collected repeated samples during the day of chemotherapy infusion (24hPK). Reasons for withdrawal of chemotherapeutic treatment are displayed in Supplemental Figure S3.

Figure 2. Flowchart of patients in the study population.



Abbreviations: NSCLC, non-small-cell lung cancer; MPM, malignant pleural mesothelioma; RT, radiotherapy; PK, pharmacokinetic(s); AUC_1 Area under the curve during cycle 1; AE, adverse event.

Patient characteristics

Patient characteristics are outlined in Table 1. The mean age in this population was 63.3±9.3 years and slightly more than half of the patients were male (55%). The majority of patients had metastatic NSCLC (85%) and received pemetrexed as first-line treatment (90%), mostly combined with cisplatin (67%). The mean body surface area of these patients was 1.8 ± 0.2 m^2 and they had a renal function with median eGFR 98 mL/min (interquartile range [IQR]: 88 - 105).

Table 1. Characteristics of patients treated with pemetrexed (N = 106)

	Cycle-PK N = 106	24h-PK N = 15
Age, mean (SD)	63.3 (9.3)	64.3 (9.7)
Sex, male	58 (54.7)	12 (80.0)
Ethnicity		
Caucasian	97 (91.5)	14 (93.3)
ECOG performance score		
0 or 1	91 (85.8)	13 (86.7)
≥ 2	14 (15.4)	2 (13.3)
unknown	1 (0.9)	
Weight (kg), mean (SD)	72.5 (12.8)	73.9 (15.0)
BSA (m^2), mean (SD)	1.8 (0.2)	1.9 (0.2)
eGFR (ml/min/1.73m^2), median (IQR)	98 (88-105)	100 (92-109)
Type of tumor		
Adenocarcinoma	102 (96.2)	15 (100)
Large cell carcinoma	4 (3.8)	0
Stage of disease		
Locally advanced (IIIB)	16 (15.1)	5 (33.3)
Metastatic (IV)	90 (84.9)	10 (66.7)
Line of therapy		
First line	95 (89.6)	15 (100)
Second line	11 (10.4)	0
Treatment combination		
Cisplatin	71 (67.0)	15 (100)
Carboplatin	33 (31.1)	0
Monotherapy	2 (1.9)	0
Pemetrexed dosage (mg), mean (SD)	910.9 (87.3)	938.3 (88.1)
Number of chemotherapy cycles, median (IQR)	3.2 (2.0-4.0)	3.1 (2.0-4.0)

Data are expressed as numbers (%), unless otherwise stated. Abbreviations: PK, pharmacokinetics; BSA, body surface area; SD, standard deviation; IQR, interquartile range; eGFR, estimated glomerular filtration rate according to CKDEPI

Model development

The parameter estimates of the final popPK model are demonstrated in Table 2. A two-compartment model (population estimate (%standard error of the estimate) in terms of pemetrexed CL (4.58 L/h (3.1%)), V_c (15.9 L (3.3%)), V_p (21.6 L (5.0%)) and intercompartmental clearance (Q; 0.05 L/h (4.7%)) fitted PK data appropriately. Between-patient variability was included on CL (16.7%) and residual unexplained variability between observed and predicted measurements could be described using an additional error model.

Table 2. Estimation of pemetrexed pharmacokinetic and covariate parameters in final population PK model

Parameters	Units	Estimate	RSE (%)	Shrinkage (%)	Bootstrap estimate	Bootstrap 95% CI
Population parameter						
Clearance (*CL*)	L/h	4.58	3.0		4.60	4.08-5.27
Parameter for effect of creatinine clearance (*CrCL*) on clearance (*CL*)[a]	L/h	0.46	12.1		0.46	0.33-0.58
Central volume of distribution (V_c)	L	15.9	3.0		16.0	14.4-17.8
Parameter for effect of body surface area (*BSA*) on central volume of distribution (V_c)[b]	L	1 FIX			1 FIX	
Intercompartimental clearance (*Q*)	L/h	0.05	4.5		0.0464	0.0354-0.061
Peripheral volume of distribution (V_p)	L	21.6	4.9		22	16.0-29.5
Between-subject variability						
Clearance (*CL*)[c]	CV%	16.7	9	8	16.7	13.7-19.6
Residual unexplained variability						
Additional residual error	ng/mL	0.36	1.5	5	0.35	0.32-0.39

[a] $\mathrm{CL} = 4.58 * \frac{eGFR^{0.461}}{91.7}$ where 91.7 is the median eGFR during all cycles.

[b] $Vc = 15.9 * \frac{BSA^{1}}{1.84}$ where 1.85 is the median BSA at baseline.

[c] Between subject variability was included on CL using the following formula: $CL_i = CL * \exp(\eta_i)$; where CL_i represents the individual parameter estimate for individual *i*, CL is the eGFR corrected mean value for Clearance and η_i represents the between subject variability distributed following N (0, ω^2).
Abbreviations: RSE, relative standard error; CV%, percentage coefficient of variation; CI, confidence interval.

Supplemental Table S1 lists all tested covariables. A power model $(\mathrm{CL} = 4.58 * \frac{eGFR^{0.461}}{91.7})$ described the relation between pemetrexed CL and eGFR (Supplemental Figure S4). The addition of covariable eGFR significantly reduced between-patient variability in CL from 20.2% to 16.7% ($P < 0.005$), while BSA did not influence pemetrexed clearance significantly.

Clinical outcomes

Effectiveness

Median estimated AUCs during 4 cycles of chemotherapy were 201 mg·h/L (IQR 179-224), 203 mg·h/L (IQR 176-223), 208 mg·h/L (IQR 179-233) and 208 mg·h/L (IQR 178-234) respectively for cycle 1 (N = 106), cycle 2 (N = 90), cycle 3 (N = 73) and cycle 4 (N = 56). In the 56 patients who underwent 4 cycles of pemetrexed treatment, the AUC of pemetrexed was significantly higher during cycle 4 compared to cycle 1 (210 mg·h/L vs 196 mg·h/L, $P < 0.001$). The median OS and PFS in treatment-naive patients (N = 95, 89.6%) was 9.0 months (IQR: 3.9-25.7) and 4.9 months (IQR: 2.4-10.4), respectively. AUC_1 did neither univariably predict for OS/PFS, nor when adjusted for prognostic factors sex, disease stage and ECOG performance score (OS, HR = 1.05, 95% confidence interval [95%CI] 1.00-1.11; PFS, HR = 1.03, 95%CI 0.98-1.08) (Table 3). Mean AUC_1 was also not significantly different between patients with a partial response, stable disease or progressive disease as best response during 4 treatment cycles (Supplemental Table S2). For patients who experienced grade 3 / 4 toxicities (*N* = 55) compared to patients without severe toxicities (*N* = 51), the mean number of cycles was not significantly different (3.2 ± 1.1 vs 3.1 ± 1.1, p=0.69) during induction treatment.

Table 3. Multivariable analyses of total systemic exposure to pemetrexed and prognostic factors associated with overall and progression-free survival

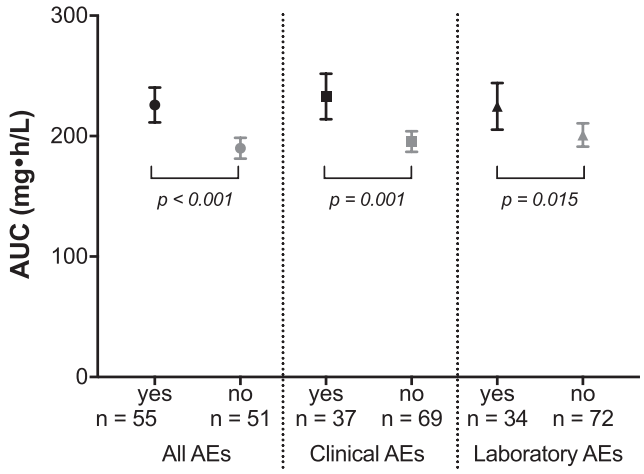
	Overall survival		Progression-free survival	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Sex				
Male vs Female	1.63 (1.02, 2.59)	0.04	1.37 (0.88, 2.12)	0.16
Disease stage				
Stage IV vs IIIB	2.96 (1.37, 6.40)	0.006	2.88 (1.46, 5.68)	0.002
ECOG PS				
1 vs 0	3.0 (1.68, 5.37)	<0.001	1.80 (1.08, 2.99)	0.024
≥2 vs 0	9.91 (4.45, 22.07)	<0.001	7.34 (3.43, 15.72)	<0.001
AUC_1[a]	1.05 (1.00, 1.11)	0.058	1.03 (0.98, 1.08)	0.31

HR, hazard ratio; CI, confidence interval; ECOG PS, ECOG performance score; AUC_1, area under the curve of pemetrexed during chemotherapy cycle 1. [a] per unit 10 mg·h/L

Toxicity

For the analyses of associations between total systemic exposure to pemetrexed and treatment toxicities, all patients with cycle-PK measurements were included (N = 106). Detailed information about treatment-related clinical and laboratory AEs is provided in Table 4. Compared to patients without severe chemotherapy-related AEs (N = 51), patients with these AEs (N = 55) had significantly higher AUC_1 values (190 mg·h/L ± 31 vs 226 mg·h/L ± 53, $P < 0.001$). When separated into clinical and laboratory AEs, identical results were found (Figure 3). Patients with severe chemotherapy-related AEs during chemotherapy had a significantly higher BSA than patients without these AEs (1.88 ± 0.18 m^2 vs. 1.81 ± 0.18 m^2, $P = 0.042$). Furthermore, eGFR before start of chemotherapy was lower in patients who would experience severe AEs throughout treatment compared who would not (91.2 ± 14.9 mL/min vs 98.1 ± 21.0 mL/min, $P = 0.053$). For severe laboratory AEs, the difference in eGFR was significant between patients who did not and did experience them (86.2 ± 20.7 mL/min vs 98.4 ± 16.2 mL/min, $P = 0.004$).

Figure 3. Differences in AUC_1 between patients with and without chemotherapy-related adverse events ≥ grade 3 during 4 cycles chemotherapy.



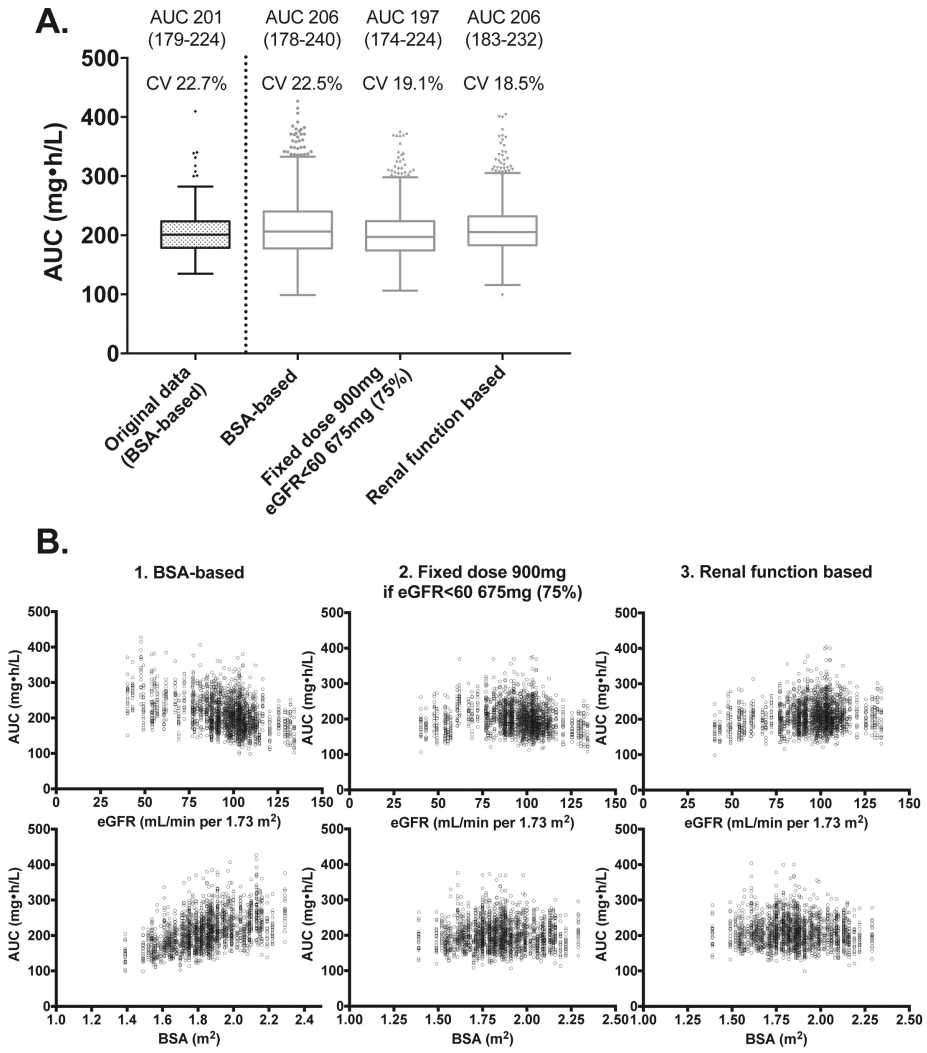
Means and error bars representing 95% confidence intervals. AUC, area under the curve; AE, adverse event.

Table 4. Adverse events in all patients with cycle-PK measurements (N = 106)

	Frequency (%)	
Adverse event	**All grades**	**Grade ≥3**
Treatment-related[a]		
Any	105 (99)	55 (52)
Clinical		
Fatigue	86 (81)	11 (10)
Anemia	85 (80)	9 (8)
Nausea	66 (62)	2 (2)
Decreased appetite	62 (58)	7 (7)
Oral mucositis	45 (42)	3 (3)
Constipation	41 (39)	1 (1)
Taste alteration	38 (36)	0
Dry skin	35 (33)	0
Dry eyes/watering eyes	34 (32)	0
Neuropathy sensory	28 (26)	0
Dysphagia	25 (24)	1 (1)
Diarrhea	20 (19)	2 (2)
Vomiting	20 (19)	0
Dizziness	17 (16)	0
Alopecia	16 (15)	0
Rash	16 (15)	0
Weight loss	15 (14)	0
Dyspepsia	12 (11)	0
Laboratory		
Decreased white cell count	75 (71)	17 (16)
Decreased neutrophil count	67 (63)	28 (26)
Decreased thrombocyte count	53 (50)	12 (11)
Alanine aminotransferase elevation	43 (41)	0
Aspartate aminotransferase elevation	30 (28)	0
Blood creatinine level elevation	26 (25)	1 (1)
Alkaline phosphatase elevation	19 (18)	0

Listed are adverse events that are reported in at least 10% of the patients. [a]Adverse events were scored as treatment-related if investigator defined relatedness as probably or definitely.

Figure 4. Pemetrexed exposure (AUC) and interindividual variability for each of three dosing strategies.



A. Boxplots representing medians and interquartile ranges of total exposure in BSA-based dosing in original (unsimulated) data and three different dosing regimens after simulations. Whiskers represent minimum and maximum 1.5 interquartile range (Tukey). AUC, area under the curve; CV, coefficient of variation; BSA, body surface area; eGFR, estimated glomerular filtration rate.
B. Simulations of total exposure to pemetrexed (AUC) by body surface area or renal function for dosing strategies: Panel 1. BSA-based; Panel 2. Fixed dose 900 mg q3w, if eGFR <60 than 675 mg (75%) q3w; Panel 3. Renal function based. As illustrated, BSA-based dosing results in increased variability in total exposure; larger patients and those with a decreased renal function have higher pemetrexed exposure (1). BSA, body surface area; eGFR, estimated glomerular filtration rate.

Dosing strategies

Compared to BSA-based dosing (CV 22.5%, AUC 206 mg·h/L (IQR: 178-240)), simulation of eGFR-based dosing (CV 18.5%, AUC 206 mg·h/L (IQR 183-232)) and fixed dose of 900 mg with 25% dose reduction if eGFR<60 (CV 19.1%, AUC 197 mg·h/L (174-224)) both showed less interindividual variability of AUC, while median AUC was comparable to the estimated AUC in our population (Figure 4A).

BSA-based dosing strategy leads to an overcorrection as large patients have a higher total exposure to pemetrexed than smaller patients. Since this dosing strategy does not adjust dose to renal function, a main predictor of clearance and therefore AUC, patients with a decreased renal function are also exposed to a higher AUC (Figure 4B, Panel 1). A fixed dose with 25% dose reduction if eGFR <60 ml/min or renal function based dosing results in a more stable exposure independent of body size and renal function (Figure 4B, Panel 2 and 3).

DISCUSSION

In a real-world setting, we developed and internally validated a popPK model for patients with advanced NSCLC treated with pemetrexed, using sparse data sampling during their total treatment period in addition to 24hPK data. Total exposure to pemetrexed did not predict for clinical effectiveness, while the occurrence of severe chemotherapy-related toxicity was significantly associated with higher exposure.

Previous PK analyses demonstrated that the AUC of pemetrexed increases linearly with dose.[5,11] However, Cullen *et al* and Ohe *et al* showed that higher doses (900 mg/m^2) - and thus higher AUCs - were not associated with an additional survival benefit in patients receiving pemetrexed as second-line or third-line treatment.[12,13] There might be a threshold dose at which the dose-response curve levels off. The absence of an exposure-response relation might also be explained by limitations in transport capacity, variable intracellular formation of more effective polyglutamate metabolites or dose-dependent gene-expression of target enzymes above a certain dose.[14–16]

Severe hematological and clinical chemotherapy-related adverse events were more common in patients with a higher AUC of pemetrexed. The toxicities observed in our study were comparable to findings in phase III trials and the incidence was similar.[12,17] The correlation between total exposure to pemetrexed and the occurrence of hematological toxicity has been demonstrated in previous research.[5,7,12,13] Early data in phase I trials were contradicting with regard to the association between baseline renal function and the development of severe toxicity.[5,18] Supporting our findings, a recent study of our group showed that the occurrence of renal toxicity during maintenance treatment with pemetrexed was associated with decreased renal function at baseline or deterioration of renal function during induction.[19] Importantly, the significantly higher

AUC in patients after 4 cycles suggests that patients are more prone to toxicity after a higher number of cycles. In a by-cycle analysis, Langer *et al.* already reported an increase in treatment-related clinical and hematological toxicities during induction treatment combined cisplatin/pemetrexed and a decrease in renal function during pemetrexed maintenance.[20]

There is a lack of rationale to use BSA-based dosing if BSA is not an important predictor of the interpatient variability of total exposure.[21–23] Our findings suggest that eGFR-based dosing reduces interpatient variability while similar population median AUC values are maintained compared to the current BSA-based dosing schedule. Therefore, this dosing strategy might prevent severe toxicity with preservation of effectiveness. These results are supported by an earlier large popPK analysis by Latz *et al.*, who already suggested that dose adjustments based on renal function might be considered favorable as total exposure to pemetrexed was dependent on renal function and the primary determinant of neutropenic response was AUC and not the peak concentration (C_{max}).[7,8] However, the best substitute for the current pemetrexed dosing schedule in our view is a flat-fixed dosing of 900 mg q3w of pemetrexed with a dose reduction of 25% if eGFR <60 mL/min. This schedule reduced interindividual variability to the same extent as eGFR based dosing in our simulation study and may have additional safety and economic benefits as it is less prone to errors and single dose vials can be used.[21,24]

Our findings are of even more importance in the light of current developments of systemic treatment of NSCLC, where combination chemotherapy with pemetrexed and anti-PD-1 pembrolizumab or anti-PD-L1 atezolizumab are new standard treatments in all patients without sensitizing mutations regardless of PD-L1 status of the tumor.[2,3] Since the combination treatment led to more severe toxicities and withdrawal of treatment in these trials,[2,25] focus on minimizing adverse effects of pemetrexed is warranted.

A dosing schedule with a 3-weekly fixed dose of 900mg as tested in our simulation would indicate that approximately half of the population would receive a dose reduction compared to the currently used dosing regimen. Since our data were not suitable to elaborate further on the role of C_{max}, its impact on treatment effectiveness remains unclear. Additionally, it remains questionable whether the established differences between interindividual variation of dosing strategies in simulations are associated with clinical relevance. Covariable eGFR only reduced the between-patient variability of pemetrexed CL by approximately 20% and thus the larger part of this variability is still unexplained. Although BSA did not affect pemetrexed CL, body composition might influence drug clearance and thus exposure and toxicity.[26,27] Other factors might affect CL and therefore AUC even more, such as genetic polymorphisms in metabolizing enzymes and drug transporters. At last, confounding of the association between AUC of pemetrexed and toxicity by the nephrotoxic platinum compound cannot be excluded without a pemetrexed monotherapy comparator arm. However, it is unlikely that the

increased toxicity in patients with higher exposure to pemetrexed is solely a cisplatin effect as the pharmacokinetics of cisplatin and pemetrexed are not significantly influenced by each other.[28,29] Additionally, creatinine clearance is not a main predictor of cisplatin clearance in contrast to pemetrexed clearance.[30,31]

In conclusion, total systemic exposure to pemetrexed does not predict for clinical effectiveness, but is significantly associated with more frequent occurrence of severe hematological and clinical AEs. Although we currently dose pemetrexed based on BSA, our data show better rationale for a 3-weekly flat-fixed dose of 900 mg (with 25% dose reduction if eGFR <60). However, benefit of this alternative dosing strategy should be confirmed in a randomized clinical trial with direct comparison to the current BSA-based dosing strategy.

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SUPPLEMENTAL MATERIAL

Standard of care treatment

Pemetrexed was dosed at 500 mg/m^2 and cisplatin 75 mg/m^2. Carboplatin dosage was calculated using the Calvert formula with a target AUC of 5 or 6.[1] If the chemotherapy schedule involved cisplatin, pre- and post-hydration treatment was given per protocol according to local guidelines. Dose adjustments (i.e. reductions) at the start of subsequent courses of therapy were based on nadir counts (neutrophils, platelets) or maximal non-hematologic toxicity from the preceding cycle of therapy.

Pharmacokinetic measurements: assay and validation

For logistic reasons, all patients with 24h-PK were treated in combination with cisplatin as they were admitted overnight for hydration per protocol.

For the quantitative determination of pemetrexed in human plasma, a rapid and sensitive liquid chromatography/tandem mass spectrometry (UPLC-MS/MS) method has been developed and validated. Aliquots of 25 µL of human lithium heparinized plasma samples were deproteinized, after the addition of 100 µL of Internal Standard (pemetrexed-d5) in acetonitrile. After vigorously mixing for 5 seconds and centrifugation for 10 minutes at 18,000*xg*, the supernatant was evaporated. Hereafter the residue was resuspended in 50 µL methanol/water/formic acid (10:90:0.1, v/v/v). Chromatographic separations were achieved on a reversed phase C18 column eluted at a flow-rate of 0.400 mL/min on a gradient of acetonitrile. The overall cycle time of the method was 4 min, with pemetrexed eluting at 1.5 min. The multiple reaction monitoring transitions were set at 428>163 (m/z), and 433>163 (m/z) for pemetrexed and the internal standard, respectively. For extended pharmacokinetic sampling, a sensitive method was developed with a linear function of the concentration from 1.00 to 50.0 ng/mL, with the lower limit of quantitation (LLQ) validated at 1.00 ng/mL. The within-run precisions and between-run precisions including the lower limit of quantification did not exceed 6.15%, while the average accuracy ranged from 101.3 to 108.0%. For quantification of high concentrations (>50.0 ng/mL) of pemetrexed, calibration curves were prepared ranging 20.0 to 5,000 ng/mL with lower limit of quantitation validated at 20.0 ng/mL. The within-run and between-run precisions, also at the level of the LLQ, were within 15.0%, while the accuracy ranged from 93.1% to 104.0%. The method was successfully applied to samples derived from the clinical study.

Pharmacokinetic model development

Plasma concentration-time data were analyzed using the nonlinear mixed effect modeling software, NONMEM (version 7.1; ICON, Ellicott City, MD). The first-order conditional estimation method with interaction was used for the parameter estimation, Piraña was used as the modeling environment and data were further handled in R version 2.13.0 (http://cran.r-project.org).

A two-compartment model for pemetrexed was structured based as schematically shown in supplemental Figure S1. The selection of this model was based on earlier results from population pharmacokinetic modelling,[2, 3] and our own data as pemetrexed pharmacokinetics were adequately described by a two-compartment model parametrized in terms of clearance (CL), central volume of distribution (V_c), inter-compartmental clearance (Q) and peripheral volume of distribution (V_p). The base PK model was designated based on objective function value (Δ OFV) > 3.84; $P < 0.05$), successful minimization and covariance estimation and graphical evaluation performed by goodness-of-fit plots.

Covariable analyses

First, a univariable forward-inclusion step was conducted in which the improvement of the model fit relative to the base model is compared when each of the covariables was added univariably. The threshold of this step was set at $P < 0.01$ (likelihood ratio test, Δ OFV >6.64, df = 1). In the next step, all potentially related covariables were included in the full model. Next, a backward elimination procedure was started, during which covariables were removed one at a time from the full model again if the fit of the model did not decrease significantly ($P < 0.005$) tested using the likelihood ratio test (Δ OFV >7.88, df = 1).

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SUPPLEMENTAL TABLES AND FIGURES

Table S1. Covariate analysis on the base model using stepwise forward inclusion and backward elimination

PK parameter	Tested covariate - PK parameter relationship	OFV	⊿OFV[a]
	Structural base model[b]	-661.6	
Forward inclusion			
CL	Age on CL	-661.6	0
	Sex on CL	-661.7	-0.1
	eGFR on CL	*-723.4*	*-61.7*
	Cisplatin effect on CL	-662.3	-0.6
	Time effect of cisplatin on CL	-663.3	-1.6
	Time effect on CL	-661.3	0.4
	BSA on CL	-646.8	14.8
	Weight on CL	-661.8	-0.2
V_c	*Age on V_c*	*-674.4*	*-12.8*
	Sex on V_c	-660.1	1.5
	BSA on V_c[c]	*-679.2*	*-17.5*
	Weight on V_c	-672.7	-11.1
V_p	Age on V_p	-661.6	0
	Sex on V_p	-661.7	-0.1
	BSA on V_p	-664.4	-2.7
	Weight on V_p	-663.2	-1.6
	Full model including eGFR on CL and age/BSA on Vc	-745.6	
Backward elimination			
Step 1	- eGFR on CL	-683.6	62
	- BSA on Vc	-731.4	14.2
	- age on Vc	-743.8	1.8
Step 2	Full model - age on Vc	-743.8	
	-eGFR on CL	-679.2	64.6
	-BSA on Vc	-723.4	20.4
Final model	**Structural base model + eGFR on CL and BSA on Vc**	**-743.8**	

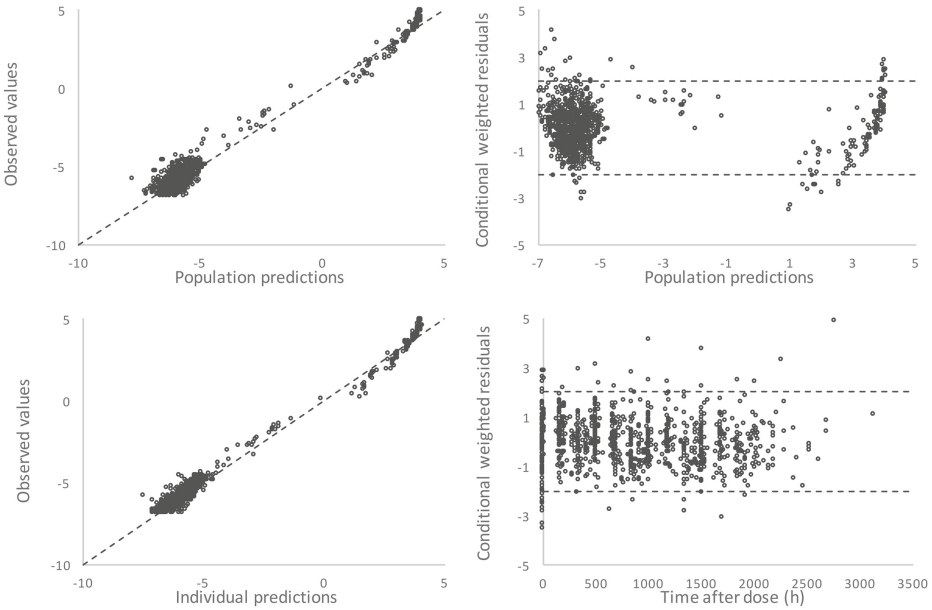
[a] To determine model fit, ⊿OFV was used according to the likelihood ratio test following a Chi-square distribution. In the stepwise forward inclusion, the threshold for significant improvement of the model was set at $P < 0.01$ (Δ OFV > 6.64, df =1). In the backward elemination significant worsening of the model was set at $P < 0.005$ (Δ OFV > 7.88, df =1).
[b] Structural model: Two-compartment model in terms of pemetrexed clearance (CL), central distribution volume (Vc), intercompartimental clearance (Q) and peripheral volume of distribution (Vp), including between-patient variability on CL and proportional error model describing between-patient variability
[c] BSA and weight were highly correlated. As BSA resulted in larger decrease of OFV, it was kept in the full model
Abbreviations: OFV, objective function value; CL, pemetrexed clearance; Vc, central volume of distribution; Vp, peripheral volume of distribution; BSA, body surface area

Table S2. Association between total systemic exposure to pemetrexed and treatment response

		AUC_1	
Best treatment response	*N*	**Mean (SD)**	**95% CI**
partial response	30	196.9 (37.9)	188.6 - 215.0
stable disease	40	199.2 (52.2)	189.5 - 220.8
progressive disease	10	197.3 (34.3)	179.1 - 222.8
not evaluable[a]	15	240.4 (51.7)[b]	211.7 - 269.0

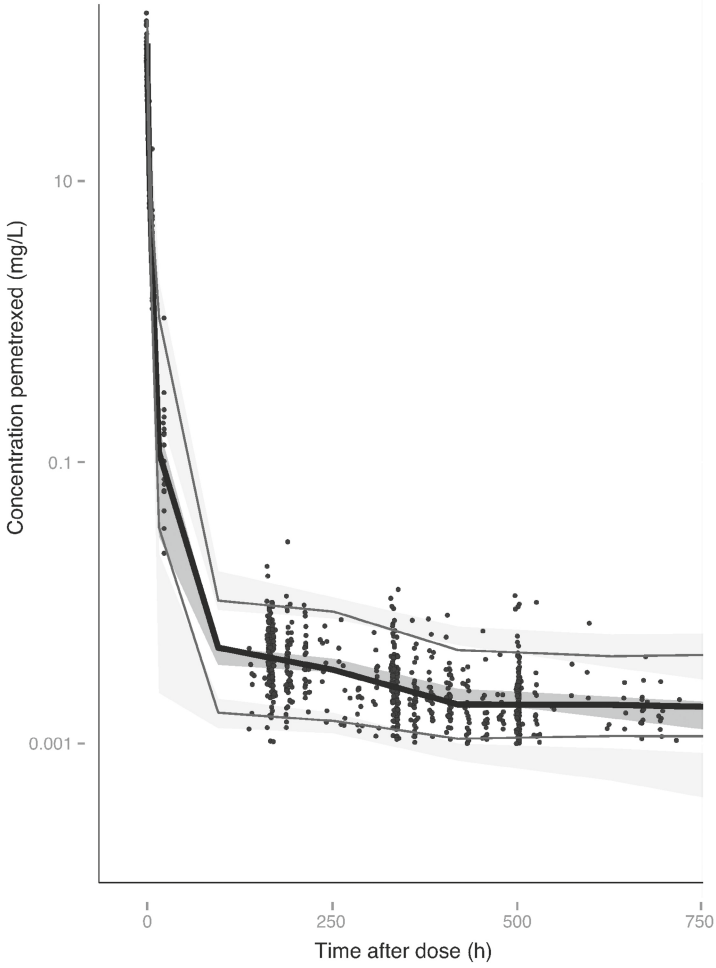
[a] reasons for non evaluable treatment response: early death/systemic deterioration due to toxicity or early disease progression. [b] one-way ANOVA $P = 0.005$. Abbreviations: AUC_1, area under the curve of pemetrexed during cycle 1; SD, standard deviation; CI, confidence interval

Figure S1. Goodness of fit plots.



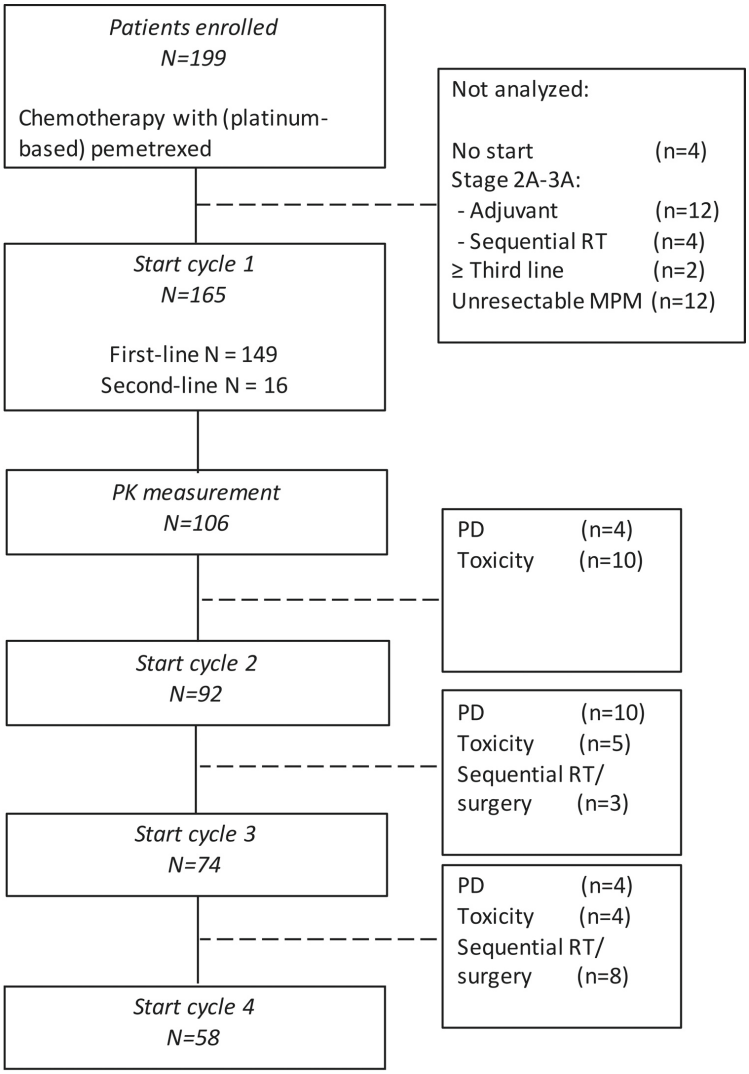
Left: Log predictions (upper) and log individual predictions (bottom) versus log observed concentrations. *Right:* Log predictions (upper) and time after dose (bottom) versus conditional weighted residuals. Observed and predicted concentrations are in mg/L (log-transformed).

Figure S2. Visual predictive check of the final popPK model of pemetrexed.



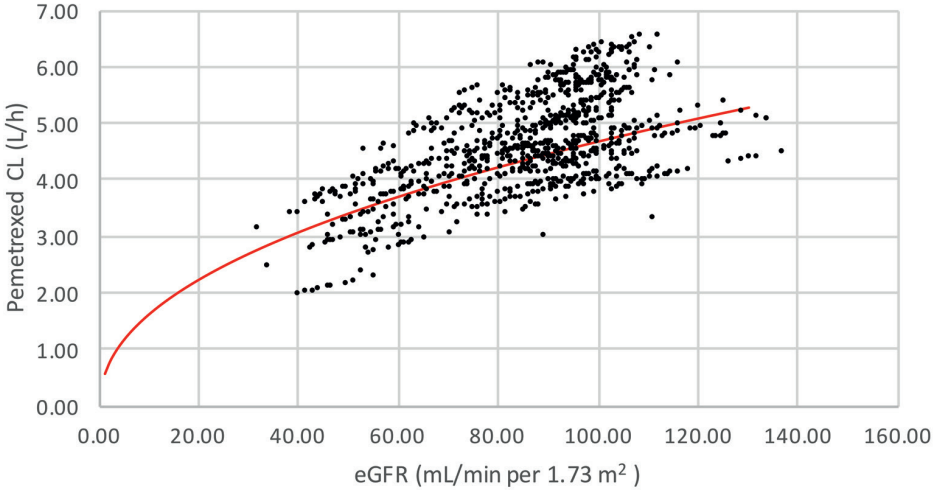
Dark grey and light grey surfaces are 90% confidence areas of the predicted median and 5th and 95th percentile of the prediction interval. The bold black line is the observed median and the grey lines are the 5th and 95th percentile of the observed data.

Figure S3. Flowchart of patients with reasons of withdrawal during chemotherapy (4 cycles).



Abbreviations: MPM, malignant pleural mesothelioma; PD, progressive disease; RT, radiotherapy.

Figure S4. Predicted clearance of pemetrexed and its association with renal function.



Dots represent observed patient values. Red line represents model predicted clearance of pemetrexed.

CHAPTER 7

A limited sampling schedule to estimate individual pharmacokinetics of pemetrexed in patients with varying renal functions

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ABSTRACT

Purpose

Pemetrexed is a widely used cytostatic agent with an established exposure-response relationship. Although dosing is based on body surface area (BSA), large interindividual variability in pemetrexed plasma concentrations is observed. Therapeutic drug monitoring (TDM) can be a feasible strategy to reduce variability in specific cases leading to potentially optimized pemetrexed treatment. The aim of this study was to develop a limited sampling schedule (LSS) for the assessment of pemetrexed pharmacokinetics.

Methods

Based on two real-life datasets, several limited sampling designs were evaluated on predicting clearance using NONMEM, based on mean prediction error (MPE %) and normalized root mean squared error (NRMSE %). The predefined criteria for an acceptable LSS were: a maximum of 4 sampling time points within 8 hours with an MPE and NRMSE ≤20%.

Results

For an accurate estimation of clearance, only 4 samples in a convenient window of 8 hours were required for accurate and precise prediction (MPE and NRMSE of 3.6% and 5.7% for dataset 1 and of 15.5% and 16.5% for dataset 2). A single sample at t = 24hrs also met the criteria with MPE and NRMSE of 5.8% and 8.7% for dataset 1 and of 11.5% and 16.4% for dataset 2. Bias increased when patients had lower creatinine clearance.

Conclusions

We presented two limited sampling designs for estimation of pemetrexed pharmacokinetics. Either one can be used based on preference and feasibility.

INTRODUCTION

Pemetrexed is an anti-folate drug which is widely used as first and second line treatment of non-small-cell lung cancer and mesothelioma.[1,2] There is a relationship between pemetrexed pharmacokinetics and toxicity.[3-5] Despite the introduction of prophylactic use of folic acid and vitamin B12 to reduce the risk of haematological toxicity, neutropenia remains a main exposure-related and treatment-limiting adverse reaction.[3] Latz *et al.* (2006) showed that higher exposure relates with both decrease in neutrophil count and a longer recovery time after neutropenia.[3]

Currently, pemetrexed is dosed based on body surface area (BSA) and this introduces large interindividual variability in exposure.[6] There are several other factors which can contribute to variability in exposure, such as change in renal function or drug interactions.[6-9] Since pemetrexed exposure correlates well with toxicity,[3, 10] pharmacokinetically (PK) guided dosing may be a feasible strategy to optimize treatment. Previously, the proposed target for safe and effective treatment is an AUC of 164 mg*h/L ± 25%.[3, 6] A prerequisite to validate this target for PK-guided dosing is the availability of an accurate, precise and clinically feasible limited sampling strategy (LSS) to assess the AUC.

From a patient's perspective, a minimally invasive strategy is desired in a short time window. Therefore, our aim was to develop a limited sampling schedule (LSS) for the assessment of pemetrexed pharmacokinetics to use in clinical practice.

METHODS

Limited sampling design evaluation

The predictive performance of several limited sampling designs to predict the pemetrexed clearance were evaluated. To assess individual exposure, the AUC can be calculated from clearance and the administered dose ($AUC = \frac{Dose}{Clearance}$). The previously developed and validated pharmacokinetic model by Latz *et al*. (2006) was used to obtain the empirical Bayesian estimates for clearance using the post-hoc option in the software package NONMEM v7.4.3 [Icon, Ireland]. First, the full pharmacokinetic curves were fitted and obtained clearances were assumed to be 'true values'. Subsequently, individual clearances were estimated from several limited sampling strategies based on the original dataset with certain timepoints removed.

The predictive performance was assessed with the mean relative prediction error (MPE %) for precision and normalized root mean squared error (NRMSE %) for accuracy, respectively. For MPE, confidence intervals were calculated as described by Sheiner *et al.* (1981).[11] For NRMSE, relative uncertainty was determined according the distribution-free approach of Faber (1999).[12] Subsequently, corresponding confidence intervals were calculated.

Taking both patient's perspective and statistical considerations into account, the pragmatic criteria for an acceptable LSS were defined as: a maximum of 4 sampling time points within 8 hours with an MPE and NRMSE ≤20%. The value of acceptable precision, and therefore bias of clearance, depends on multiple factors such as expected analytical error, therapeutic range of the drug and the purpose of the LSS. For pemetrexed, we found this performance acceptable for the estimation of pemetrexed clearance.

Datasets

Two separate datasets were used to evaluate several sampling designs. The first set contained pharmacokinetic data of 15 pemetrexed patients (from Visser *et al.* 2019) with adequate renal function (range creatinine clearance according Cockcroft-Gault (CrCl-CG) 60-166 ml/min).[5] Patients were treated according to standard of care with a pemetrexed dose of 500 mg/m^2 over a 10 minutes intravenous infusion. For dataset 1, the following sampling times were available 0.17, 0.5, 1, 2, 4, 8 24h after the start of administration. The second set included rich pharmacokinetic data of 47 individuals from the JMAW phase I trial of Eli Lilly, with varying renal function (range CrCl-CG 17-200 ml/min.). These data were obtained through www.clinicalstudydatarequest.com.[13] The dose varied between patients but was administered over a 10 minutes intravenous infusion. The sampling times were 0.17, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72h after the start of administration. Since the used model of Latz *et al.* (2006) was designed based on sampling up to 36 hours after administration of pemetrexed, datapoints after 36 hours were excluded from the analysis.

RESULTS

Table 1 presents the relevant baseline characteristics of the patients that were included in the two datasets and the results of the two best performing limited sampling designs. The second dataset contains patients with a wider range of both creatinine clearance and pemetrexed dose. For both datasets, several designs were tested based on the available sampling times. For an adequate estimation of pemetrexed clearance, within a sampling window of 8 hours, 4 sampling times were required to reach acceptable precision and accuracy (MPE and NRMSE <20%) in both datasets (not all data shown). As can be seen in table 1, sampling at 0.5, 2, 4, 8 hours after administration resulted in an MPE and NRMSE of 3.6% and 5.7% for dataset 1. Using the second dataset, the performance of this sampling strategy was slightly lower but still within the acceptable range, with an MPE and NRMSE of 15.5% and 16.5%, respectively. Table 1 also shows the performance for a single sample at t = 24h. This strategy performed more or less equal to multiple sampling within 8 hours, with imprecision and inaccuracy in the same order of magnitude. For all sampling designs the MPE confidence interval did not include zero in both datasets, indicating a structural bias.

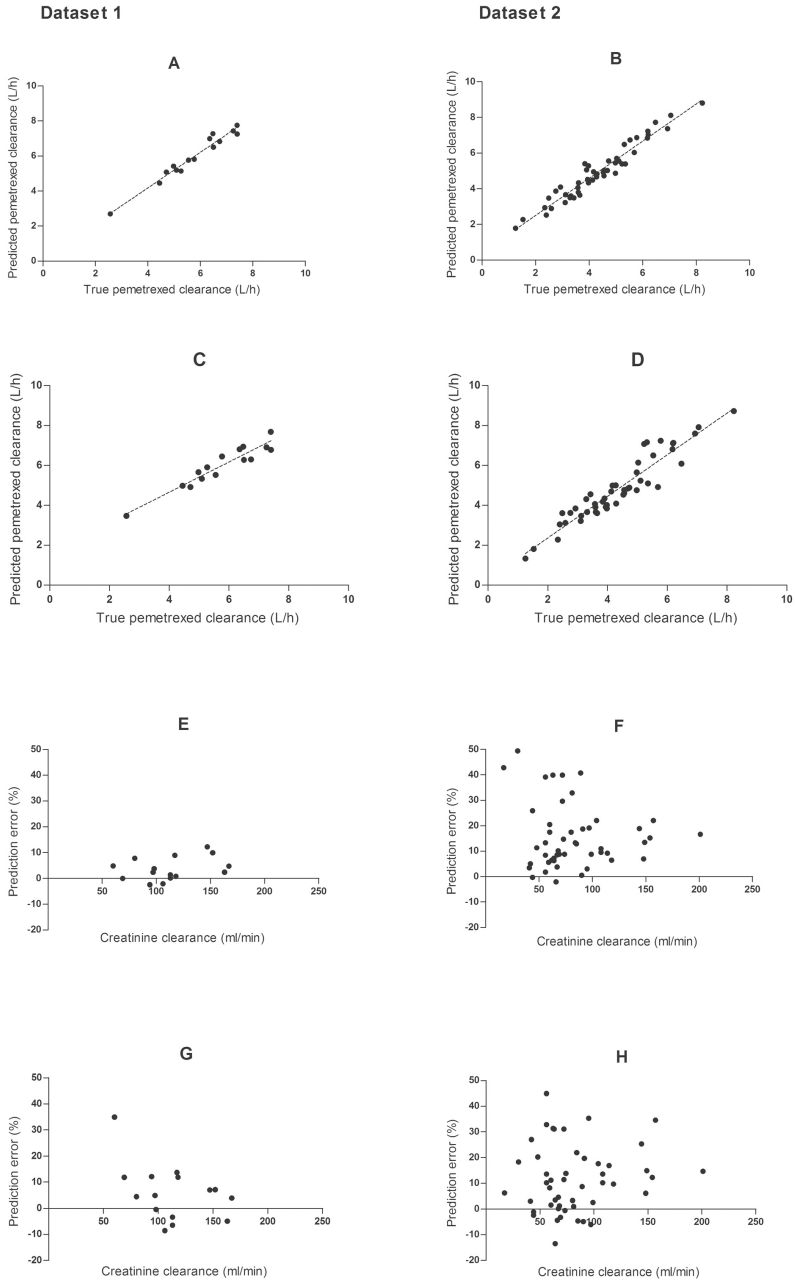
Figure 1 - panels A-D show true versus predicted pemetrexed clearance for the two proposed limited sampling designs. There is an acceptable correlation between the predicted and true clearances. Single sampling at t=24h for dataset 1 (see panel C) apparently introduces a slight overprediction of pemetrexed clearance. This is not observed in the second dataset. In figure 1 – panels E-H, creatinine clearances versus bias (MPE %) are visualized. Generally, for lower creatinine clearances in dataset 2, a larger prediction error (MPE %) was observed.

Table 1. Baseline characteristics and predictive performance of best performing limited sampling designs.

	Dataset 1		Dataset 2	
Total	**N = 15**		**N =48**	
Sex				
Male	12		36	
Female	3		12	
Age [yrs] *Median [range]*	68 [43 – 77]		62 [25 – 79]	
Weight [kg] *Median [range]*	72.9 [53.8 – 104.4]		79.3 [48.1 – 124.3]	
BSA [m2] *Median [range]*	1.91 [1.54 – 2.22]		1.95 [1.44 – 2.47]	
Creatinine clearance [ml/min] *Median [range]*	112.8 [60.5 – 166.5]		72.0 [16.7 – 201.2]	
Pemetrexed dose [mg/m²] *Mean [range]*	400 [463 – 519]		500 [150 – 600]	
No. of datapoints per curve	7		11	
	MPE [%]	**NRMSE [%]**	**MPE [%]**	**NRMSE [%]**
Sampling design				
0.5 – 2 – 4 – 8 hrs	3.6 ± 2.2	5.7± 0.2	15.5 ± 3.5	16.5± 0.6
24 hrs	5.8 ± 5.3	8.7± 0.3	11.5 ± 3.6	16.4± 0.6

MPE = mean percentage error NRMSE = normalized root mean squared error hrs = hours CrCl = creatinine clearance

Figure 1



A: True pemetrexed clearance versus predicted pemetrexed clearance for dataset 1, LSS 0.5, 2, 4, 8hrs. B: True pemetrexed clearance versus predicted pemetrexed clearance for dataset 2, LSS 0.5, 2, 4, 8hrs. C: True pemetrexed clearance versus predicted pemetrexed clearance for dataset 1, LSS 24rs. D: True pemetrexed clearance versus predicted pemetrexed clearance for dataset 2, LSS 24hrs. E: Creatinine clearance versus relative prediction error for dataset 1, LSS 0.5, 2, 4, 8hrs. F: Creatinine clearance versus relative prediction error for dataset 2, LSS 0.5, 2, 4, 8hrs. G: Creatinine clearance versus relative prediction error for dataset 1, LSS 24hrs. H: Creatinine clearance versus relative prediction error for dataset 2, LSS 24hrs.

DISCUSSION

Our aim was to develop a patient-friendly limited sampling strategy for pemetrexed to assess the exposure in clinical practice and for research purposes. We found that two approaches resulted in acceptable estimation of clearance (which serves as a proxy for the exposure). We propose two possible sampling schedules: the first consists of 4 sampling times at 0.5, 2, 4, 8h after pemetrexed administration. The second approach is a single sample at t = 24h. These sampling schedules can be used for dose optimization and therapeutic drug monitoring, in specific cases as proposed earlier. Either one can be chosen based on preference and practical feasibility.

In general, the selected LSSs seemed to slightly overpredict clearance in both datasets and both sampling strategies. Overprediction of clearance could possibly result in unwarranted dose adjustments resulting in toxic exposure. However, taking the proposed target AUC of 164 mg*h/L ± 25% in mind, this structural overprediction is not considered relevant, because it is still well within the therapeutic range. Especially for dataset 2, bias increased with decreasing creatinine clearance. An explanation for the observation of increasing bias is that the used model of Latz et al. (2006) was developed using patients with adequate renal function. In renal impairment, larger variability may be introduced, which is not observed in patients with adequate renal function. Also, with decreasing clearance, early datapoints in the pharmacokinetic curve become less informative. For dataset 2, removing the 8 h timepoint resulted in unacceptable loss of accuracy and precision. Additionally, the result of the t = 24 strategy in dataset 2 showed that at a later sampling time there may be less bias in patients with extremely low creatinine clearance. Altogether, a single sample at t = 24 may a feasible strategy for clinical practice, but it may require an extra hospital visit for the patient instead of a short prolongation of stay.

Our limited samplings strategy aimed to accurately predict pemetrexed AUC. Although Latz et al. have previously suggested that pharmacokinetically-guided dosing using the AUC may result in improved treatment,[3] there is currently no conclusive evidence that the AUC is the best pharmacokinetic parameter to predict efficacy and toxicity. For example, the cytotoxicity of other drugs from the antifolate class, like methotrexate, is concentration threshold driven.[14] Prospective studies should confirm the utility of AUC-guided dosing before implementing this in clinical practice.

Altogether, we presented two patient-friendly and reliable limited sampling designs for estimation of pemetrexed pharmacokinetics. We are now using the 4-point LSS for development of personalized dosing strategies for pemetrexed in ongoing clinical studies.[15-17]

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CHAPTER 8

Renal toxicity from pemetrexed and pembrolizumab in the era of combination therapy in patients with metastatic nonsquamous cell NSCLC

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ABSTRACT

The combination of chemotherapy and immune checkpoint inhibition (ICI) therapy is the current standard of care for the majority of patients who are fit to undergo treatment for metastatic non-small-cell lung cancer (NSCLC). With this combination, renal toxicity was slightly higher than with chemotherapy alone in initial clinical trials, but in recent real-world data kidney function loss is reported to be quite more frequent. Both chemotherapy and ICI therapy can induce renal impairment, although the mechanism of renal damage is different. Renal injury due to chemotherapy is often ascribed to acute tubular injury and necrosis (ATN), while the main mechanism of injury due to ICI therapy is acute tubulointerstitial nephritis (ATIN). In case of concomitant use of chemotherapy and ICI therapy, it is a challenge to distinguish the cause of the renal failure. Discriminating between these etiologies is of utmost importance for assessing which drug can be safely continued and which drug must be halted. The aim of this review is to describe the underlying mechanisms of the renal side effects caused by chemotherapy and ICI therapy, leading to a suggested diagnostic and treatment algorithm based on clinical, laboratory, radiographical and pathological parameters. This algorithm may be a supportive tool for clinicians to diagnose the underlying cause of the acute kidney injury in patients treated with combination chemo- and immunotherapy.

INTRODUCTION

For many years, first-line treatment for advanced non-small-cell lung cancer (NSCLC) was platinum-based combination chemotherapy. Based on the Keynote-024 study, in patients with stage IV NSCLC without EGFR mutation or ALK translocation and programmed death-ligand 1 (PD-L1) expression of greater than or equal to 50%, pembrolizumab became the standard first-line therapy because of a significantly longer progression-free and overall survival compared to chemotherapy.[1] Recently, the phase 3 Keynote-189 trial reported that in previously untreated patients with advanced nonsquamous NSCLC without EGFR mutation or ALK translocation, the progression-free and overall survival were significantly longer with the addition of pembrolizumab to platinum-pemetrexed chemotherapy than with chemotherapy alone, irrespective of PD-L1 expression of the tumor.[2] This combination therapy is now considered a standard of care for the majority of patients, who are fit to undergo treatment for advanced nonsquamous NSCLC.

One of the major concerns about combination treatment with different antitumor drugs is toxicity, as this may have major impact on quality of life and may lead to the withdrawal of effective treatment in patients. Although the overall reported frequency is still low, renal toxicity seems more frequent in the setting of the chemotherapeutic agent pemetrexed in combination with the immune checkpoint inhibitor (ICI) pembrolizumab. According to Common Terminology Criteria for Adverse Events version 4.0 (CTCAE version 4.0), in the Keynote-24 trial comparing pembrolizumab to standard chemotherapy in the first-line setting, nephritis grades 3 to 5 were seen in 0.6% of the patients receiving immunotherapy.[1] In addition, an increased creatinine was reported in 1.9% of these patients. In the Keynote-189 study, acute kidney injury (AKI), defined according to CTCAE version 4.0, was observed in 5.2% of the patients in the pembrolizumab-combination group compared to only 0.5% in the placebo-combination group. A total of 12.2% of patients treated with pembrolizumab and carboplatin-pemetrexed revealed all-grade increased blood creatinine, of which 0.7% were grades 3 to 4. Renal adverse events in the pembrolizumab-combination group led to treatment discontinuation in 2% of the patients. The majority of patients in this trial received chemotherapy with carboplatin as the platinum compound, and only about 25% received the more nephrotoxic cisplatin. Although initial clinical trials reported a low incidence of immunotherapy-related nephrotoxicity, emerging data suggest a higher incidence rate between 13.9% and 29%, especially when chemo- and immunotherapy are combined.[3]

Discrepancies between results of clinical trials and real-world data are also present with regard to pemetrexed-induced nephrotoxicity. In the pivotal PARAMOUNT trial, only less than 10% of patients treated with pemetrexed maintenance therapy experienced renal impairment, and less than 5% had to discontinue treatment due to nephrotoxicity.[4] Several retrospective studies already described a higher incidence (17-21%) of renal impairment with pemetrexed.[5,6] In a recent prospective cohort study by our group,

frequencies of approximately 30% acute kidney disease (AKD) and up to 20% treatment discontinuation were reported during pemetrexed maintenance treatment.[7]

As platinum, pemetrexed and pembrolizumab are now often combined, it is a challenge to distinguish between chemotherapy- and pembrolizumab-induced renal adverse events. However, discriminating between these causes is of utmost importance as misdiagnosis of the causative agent may lead to inappropriate interventions, which potentially lead to further deterioration of renal toxicity, interruption or even cessation of an effective treatment. This review aims to describe the mechanisms of the renal side effects caused by the frequently used combination of platinum, pemetrexed and pembrolizumab, leading to a suggested diagnostic and treatment algorithm. Other oncological therapeutic agents will not be covered in this article.

DEFINITION OF RENAL TOXICITY

Estimations of the frequency of kidney injuries in clinical studies depend on how kidney injury has been defined. In the field of oncology, (renal) adverse events are reported according to the descriptive terminologies of CTCAE, providing a grading (severity) scale for each adverse event (Table 1).[8] In CTCAE version 4.0, an important adjustment has been made that takes into account the absolute increase of creatinine and its relative increase from baseline. Notably, in the newest version (version 5.0) lower grades (1/2) AKI are not anymore defined and severe AKI (grade ≥3) only depends on the need of hospitalization or dialysis and not on measured kidney function. The Acute Kidney Injury Working Group of Kidney Disease: Improving Global Outcomes (KDIGO) proposed the most commonly used definitions of kidney disease nowadays and they divided renal injury into three categories based on the duration of renal function deterioration: AKI, AKD and chronic kidney disease (CKD) (Table 1).[9] All individuals, including the elderly, with a glomerular filtration rate (GFR) <60 mL/min are considered to have CKD.[9] Although some decline of GFR is expected with age, most healthy older individuals do not necessarily have a decreased GFR.[9] Moreover, among older individuals, decreased GFR is associated with increased risk of mortality and kidney failure.[10] In an earlier study by our group, renal adverse events were graded according to CTCAE 4.03 as well as to CTCAE 3.0, to allow for comparison with data from the registration trial of pemetrexed maintenance treatment.[7] Among patients who developed AKD during maintenance pemetrexed therapy according to KDIGO definitions, 77% had all grades renal adverse events according to CTCAE 4.03 but only 54% using CTCAE 3.0. Hence, using CTCAE 3.0 we found only 16% of patients experienced renal adverse events in contrast with 30% when using the KDIGO definitions. This study illustrates the probable underestimation of renal toxicity by using the CTCAE 3.0 and 4.03 compared to AKD (KDIGO). By taking into account absolute increases of creatinine and its relative increase from baseline, the results of the updated version CTCAE 4.03 corresponded better with the AKD results.

Table 1. Definitions and classifications of kidney injury according to CTCAE and KDIGO

CTCAE						
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**		
version 3.0						
creatinine	>ULN-1.5 x ULN	>1.5-3.0 x ULN	>3.0-6.0 x ULN	>6.0 x ULN		
GFR	<75-50% LLN	<50-25% LLN	<25% LLN, chronic dialysis not indicated	Chronic dialysis or renal transplant indicated		
version 4.03						
AKI	Creatinine level increase of >0.3 mg/dL (26.5 µmol/L); creatinine 1.5-2.0 x above baseline	Creatinine 2-3 x above baseline	Creatinine >3 x baseline or >4.0 mg/dL (354 µmol/L); hospitalization indicated	Life-threatening consequences; dialysis indicated		
version 5.0						
*AKI**	-	-	Hospitalization indicated	Life-threatening consequences; dialysis indicated		
KDIGO						
AKI	Increase in serum creatinine by 50% within 7 days *OR* Increase in serum creatinine by 0.3mg/dL (26.5 mmol/L) within 2 days *OR* Oliguria†					
	Stage 1		Stage 2		Stage 3	
	Creatinine: 1.5-1.9 x baseline OR ³ 0.3mg/dL (26.5 mmol/L)		Creatinine: 2.0-3.0 x baseline		Creatinine: > 3.0 x baseline OR ³ 4.0 mg/dL (354 mmol/L)	
AKD	AKI *OR* GFR <60mL/min per 1.73m^2 for <3 months *OR* Decrease in eGFR by >35% *OR* Increase in serum creatinine >50% for <3 months					
CKD	GFR <60 mL/min per 1.73 m^2 for >3 months					
	G1 (normal)	G2‡	G3A	G3B	G4	G5 (renal failure)
	GFR ³90	GFR 60-89	GFR 45-59	GFR 30-44	GFR 15-29	GFR <15

* A disorder characterized by the acute loss of renal function (within 2 weeks). † Oliguria is also used in staging of AKI, but not further discussed here. ‡ GFR 60-89 mL/min is considered to be mildly decreased, but the threshold of GFR <60mL/min (G3a-G5) is chosen for CKD. Abbreviations: (e)GFR, glomerular filtration rate; AKI, acute kidney injury; AKD, acute kidney disease; CKD, chronic kidney disease

MECHANISMS OF RENAL TOXICITY

Antitumor drugs can cause renal toxicity by different mechanisms. Renal injury owing to chemotherapy is often ascribed to acute tubular injury and necrosis (ATN) while the main mechanism of injury due to immunotherapy is acute tubulointerstitial nephritis (ATIN).[11,12] AKI is associated with immediate and long-term unfavorable outcomes and the development of CKD.[13] Therefore, it is of utmost importance to rapidly identify the cause and start the appropriate management. Uncovering the underlying mechanisms can be key in the management of AKI during combination treatment of chemotherapy and immunotherapy. In the case of ATIN, timely administration of steroids can salvage kidney tissue by reducing the amount of tubulointerstitial fibrosis that may ultimately develop.[14]

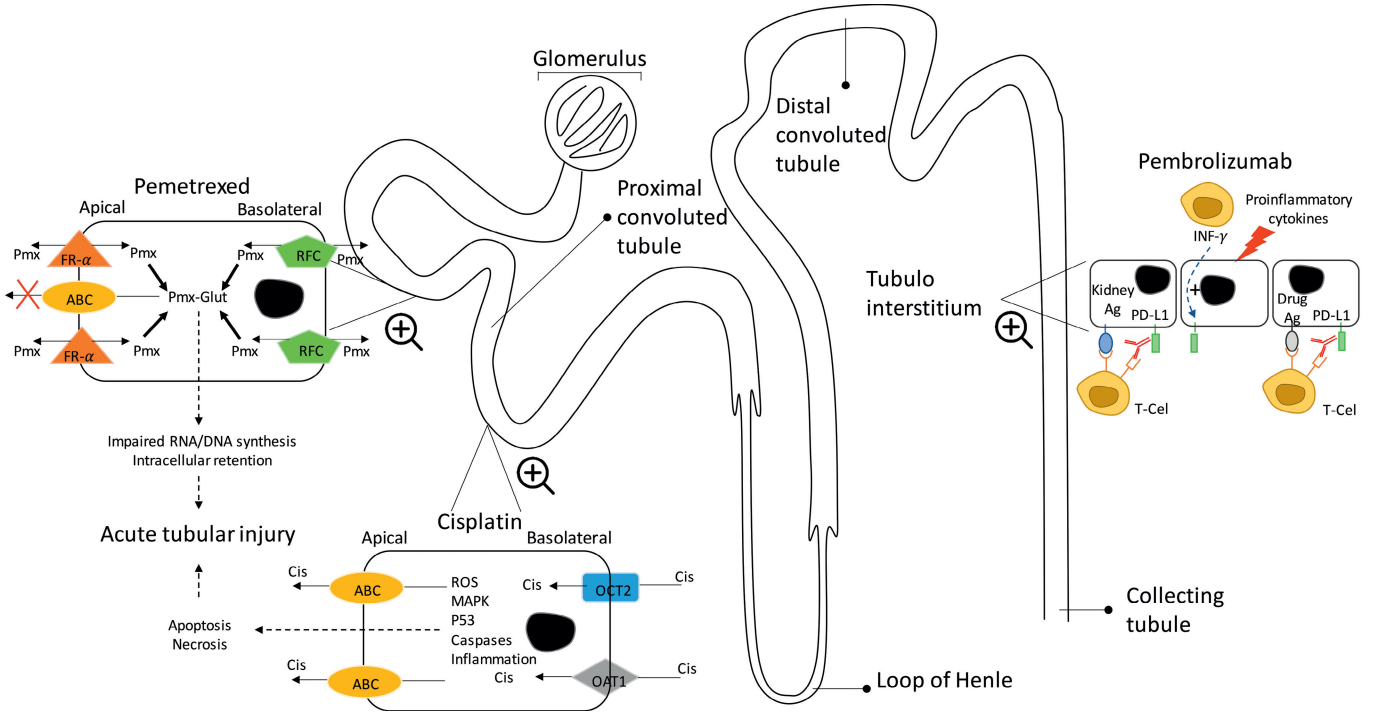
Below, we discuss several separate chemotherapeutic agents used in the treatment of NSCLC in the Keynote-189 trial, followed by ICI.

Cisplatin

Cisplatin is a platinum compound that is widely used as a cornerstone of chemotherapeutic therapy for many carcinomas, sarcomas and lymphomas. One of its major adverse events is nephrotoxicity, which is often (partially) reversible but may be permanent.[15] Cisplatin is principally excreted by the kidneys and thus its concentrations in the renal cortex are high compared to plasma and other organs.

A key role in the development of cisplatin-mediated nephrotoxicity might be ascribed to basolateral drug transporters, as the expression of proximal tubule organic cation transporter-2 (OCT2) has been reported to influence intracellular accumulation.[16] After cisplatin enters the tubular cell, multiple intracellular injury pathways including inflammation, oxidative stress, apoptotic pathways, cytoplasmic organelle dysfunction and DNA damage can contribute to kidney injury.[17] The renal tubular cell injury ultimately leads to clinical AKI by ATN and apoptosis (Figure 1). Another usually observed manifestation of nephrotoxicity is hypomagnesemia by decreased renal tubular reabsorption, which occurs in 40-100% of patients.[18] Less common manifestations of nephrotoxicity are thrombotic microangiopathy (TMA), Fanconi like syndrome, distal tubular acidosis and renal concentrating defect.[17] Despite renoprotective strategies using hydration and diuresis, magnesium supplementation and mannitol, approximately one-third of patients treated with cisplatin still develop renal impairment after the initial dose. Cisplatin-induced nephrotoxicity is dose-dependent and also increases with recurrent drug administration.[19] In patients with thoracic malignancies (mostly NSCLC), cisplatin induced AKI was observed in 21% of the patients.[15] In this study by our group, the frequency of acute kidney disease accumulated from 20% during cycle 1 to 50% during cycle 4 in patients treated with combined cisplatin-pemetrexed treatment.[7]

Figure 1. Mechanisms underlying chemotherapy- and immune checkpoint-induced kidney injury.



After entrance of tubular cells, polyglutamation leads to entrapment of pemetrexed in the cell as these polyglutamates are no substrate for ABC transporters. Impaired RNA and DNA synthesis leads to acute tubular injury. Cisplatin induces multiple intracellular injury pathways including inflammation, oxidative stress, apoptotic pathways and DNA damage mediating renal tubular cell injury. Immune activation by checkpoint inhibitors leads to the development of autoimmunity, reactivation of memory T-cells previously primed by exogenous drug exposure and increase in proinflammatory cytokines/chemokines in kidney tissue. FR-a, folate receptor alpha; ABC, ATP binding cassette transporter; Pmx, pemetrexed; Pmx-glut; pemetrexed polyglutamates; RFC, reduced folate carrier; OCT2, organic cation transporter 2; OAT 1, organic anion transporter 1; Cis, cisplatin; ROS, reactive oxygen species; MAPK, mitogen-activated protein kinase; PD-L1, programmed cell death- ligand 1; INF-g, interferon gamma

Carboplatin

Carboplatin has a lesser nephrotoxic profile than cisplatin, despite the fact that the elimination of carboplatin is primarily renal through glomerular filtration. Its lower nephrotoxic potential can most likely be explained by a lack of cell transport by OCT2, thereby reducing proximal tubular intracellular accumulation. In addition, the chloride at cis-position in cisplatin is replaced by carboxylate which is thought to further reduce toxicity.[11] Another explanation for the lower incidence of renal toxicity of carboplatin is the fact that dosing is based on the renal clearance of the patient. Thus, in case of declining kidney function the dose of carboplatin will be adapted, which is not the case with cisplatin treated patients. Nevertheless, renal adverse events are observed during carboplatin-based chemotherapy with direct tubular injury as the most common primary mechanism, followed by magnesium wasting. A meta-analysis based on individual patient data from phase II and III trials showed a significantly higher incidence of grade 3 to 4 nephrotoxicity in patients treated with various combinations of chemotherapy combined with cisplatin compared with carboplatin (1.5% vs 0.5%, $P = 0.018$).[20] In a real-life setting approximately 20% of the patients having carboplatin-pemetrexed treatment developed AKD.[7]

Pemetrexed

Pemetrexed is an antifolate agent that inhibits multiple enzymes involved in the synthesis of purine and thymidine nucleotides. After cell entrance, pemetrexed undergoes rapid intracellular polyglutamation resulting in polyglutamates that are more potent inhibitors of the enzymatic processes involved in de novo DNA synthesis. Pemetrexed does not undergo significant metabolization and the unchanged parent compound is primarily eliminated via the kidneys, with 70% to 90% of the administered drug excreted unchanged into urine within 24 hours.[21] Although pemetrexed is often combined with cisplatin or carboplatin, pemetrexed monotherapy can also cause renal failure. While the pathogenic mechanism of renal injury of pemetrexed is not fully understood, histopathology in several case reports described distinct patterns of tubular toxicity.[11] Reduced folate carrier (RFC) is the main entrance transporter of pemetrexed and is expressed on basolateral membranes of kidney tubules, while the folate receptor-alfa (FR-α) provides drug uptake at the apical site.[11] Pemetrexed polyglutamation results in prolonged retention of polyglutamates intracellularly, which in turn may lead to an increase of impaired RNA and DNA synthesis and ultimately tubular injury (Figure 1). Cumulative systemic dose of pemetrexed might play a role in the development of nephrotoxicity.[22] Permanent impairment of the kidney function after discontinuation of pemetrexed maintenance therapy has been reported.[23]

Immune checkpoint inhibitors (ICI)

ICI are monoclonal antibodies targeted at a specific receptor, either PD-1 or PD-L1, to counteract the blockade of cytotoxic T cells by PD-L1 upregulating tumor cells. Using this mechanism, the inhibition of T cells is released and the immune system can effectively

kill the cancer cells. However, PD-L1 is also constitutively expressed on renal cells, and is upregulated by IFN-γ.[24] By administrating an anti-PD-1 or anti-PD-L1 antibody, the PD-1 receptor will be blocked causing proliferation of T-cells and cytotoxic injury of the kidney. It has been speculated that PD-L1 inhibitors potentially lead to less autoimmune toxicity due to diminished blockade of the negative inhibitory signal, caused by the persistent interaction between PD-1 and and its other ligand PD-L2. A systematic review showed similar incidence of adverse events in patients treated with PD-1 and PD-L1 inhibitors.[25] Although renal toxicity was not described separately, there was a trend towards higher incidence of the overall rate of immune related adverse events (irAE) with PD-1 inhibitors, but the number of grades greater than or equal to 3 irAEs was comparable.

Thus, kidney injury might be caused by loss of peripheral tolerance of self-reactive T-cells against endogenous kidney antigens leading to an auto-immune variant of interstitial nephritis.[26] Alternatively, ICI may induce reactivation of drug-specific T-cells primed by nephrotoxic drugs (e.g. NSAIDs).[12] As associations between drug-specific T-cells and ATIN have been described, it is plausible that ICI may reactivate these latent drug-specific T-cells.[27] Another hypothesis-driven explanation is that the increase of proinflammatory cytokines or chemokines may mediate inflammatory injury in the kidney tissue.[28] In contrast with the pharmacokinetics of mentioned chemotherapeutic agents, ICI are not eliminated by the kidneys but cleared primarily by proteolytic degradation in plasma and peripheral tissues.[29]

Renal parenchymal damage due to ICI can be subdivided into two types: ATIN and more rarely, glomerular diseases.[3] In addition one case report described thrombotic microangiopathy (TMA) as a result of checkpoint inhibition.[30] However, TMA is also associated with malignancies in general, which makes it uncertain if TMA can be caused by checkpoint inhibition.[31] TMA is characterized by hemolytic anemia due to red blood cell fragmentation, thrombocytopenia due to platelet consumption, and end-organ damage due to microvascular thrombi.[32] Drug-induced TMA has also been reported after treatment with a number of chemotherapeutic agents, including gemcitabine and the already mentioned cisplatin.[33] The exact incidence of drug-induced TMA is difficult to estimate because cases are underreported, and the clinical presentation is sometimes confused with other causes. The mechanism by which the chemotherapeutic agent induces TMA can either be non-dose dependent (immune-related) or, more frequently, dose-related (toxic).[34] In a patient with severe acute renal failure after treatment with nivolumab/ipilimumab combination therapy a combination of acute interstitial nephritis and TMA-like lesions were found in the renal biopsy.[35]

ATIN induced by ICI is caused by migration of T-cells into the kidneys, resulting in severe inflammatory cell infiltrates with or without granuloma. This mechanism can occur as early as days after treatment initiation, but a considerable delay in development of AIN is often observed with a median time of 3 months and even as late as 12 months in some

cases.[12,36] Immune-mediated kidney involvement is relatively rare compared with other organs such as the skin, gastrointestinal tract, endocrine glands, and liver; however, when ICI causes nephrotoxicity, it can be severe and treatment must be initiated quickly. Timely administration of steroids can salvage kidney tissue by reducing the amount of tubulointerstitial fibrosis that may ultimately develop.[14]

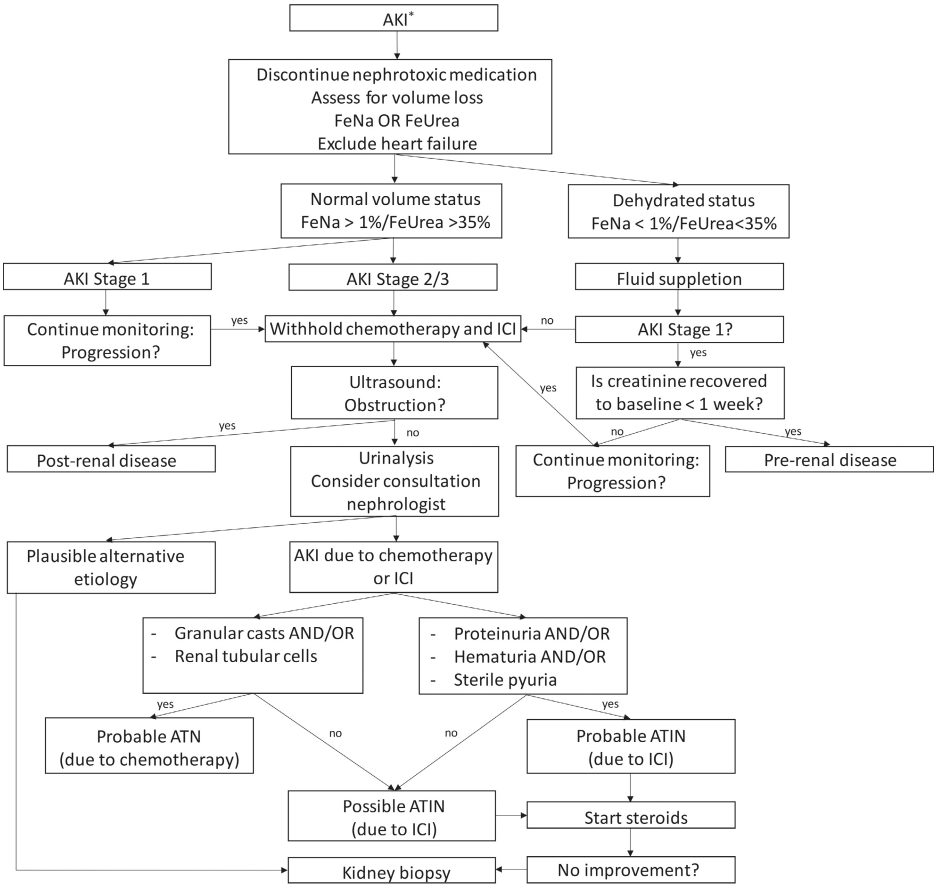
EVALUATION AND MANAGEMENT OF ACUTE KIDNEY INJURY

As described above, renal impairment during both treatment with chemotherapy and ICI is common, but their pathophysiologic mechanisms are different. The presence of CKD (eGFR <60 mL/min) prior to treatment is a known risk factor for AKI. Baseline renal function should be measured before the start of platinum-pemetrexed treatment and immunotherapy, as even mildly (eGFR 60-90 mL/min) decreased renal function can predispose to chemotherapy-induced nephrotoxicity.[7,37] In addition to a baseline values of creatinine and eGFR, monitoring these parameters during treatment before each (next) administration is needed. Some important pitfalls with regard to measuring renal function must be addressed. First, eGFR is only reliable when plasma creatinine is in steady state, which is not the case in AKI. Therefore KDIGO states that only an absolute or relative change of creatinine within 48-hours and 7 days respectively (or loss of urine output) can be used for diagnosis of AKI (Table 1). The AKD definition takes into account changes in both creatinine and eGFR. In clinical practice, using the AKD definition is more convenient as it allows for comparsion between these values with a time interval up until 3 months. Second, estimations of GFR are dependent on creatinine values. In patients with advanced age, muscle wasting and poor nutritional status, the use of eGFR may lead to an overestimation of actual renal function.

Before starting chemotherapy in combination with ICI, withdrawal of potential nephrotoxic comedication should be considered. The use of high-dose NSAIDs are (relatively) contra-indicated in the days before and after pemetrexed administration, and contra-indicated in patients with impaired renal function at baseline (Food and Drug Administration-labeled pemetrexed). Besides NSAIDs, interruption of the use of diuretics, angiotensin-converting enzyme inhibitors, or angiotensin II receptor blockers should be considered, as different studies have revealed an association between nephrotoxicity and the use of these agents during platinum chemotherapy.[38,39] Among patients treated with ICI, 60% were taking drugs known to potentially cause ATIN[40]; thus, discontinuation of these drugs should be considered.

A diagnostic algorithm for AKI during treatment of chemotherapy in combination with immunotherapy has been developed based on clinical, laboratory, radiographical and pathological parameters (figure 2).

Figure 2. Diagnostic and treatment algorithm for renal injury during combination chemotherapy/ immunotherapy



*AKI is defined and staged according to the KDIGO guideline[9] but decreases in eGFR and a longer time interval (<3months) for renal injury to develop based on the AKD definition should be taken into account. Abbreviations: AKI, acute kidney injury; FeNa, fractional excretion of sodium; FeUrea, fractional excretion of urea; ICI, immune checkpoint inhibitor; ATN, acute tubular necrosis; ATIN, acute tubulointerstitial nephritis

Clinical evaluation

When AKI is observed during treatment, it is important to critically evaluate again whether all potential nephrotoxic medication has been withdrawn, if possible. Another mechanism which may contribute to renal failure in patients treated with systemic therapy for lung cancer is intravenous contrast administration during imaging procedures. These agents cause contrast-induced acute kidney injury by direct and indirect nephrotoxic effects. [41] Patients treated with chemotherapy and immunotherapy are frequently exposed to contrast agents, since they undergo follow-up CT scans regularly to evaluate response to treatment. The KDIGO working group defined contrast-induced AKI (definition table 1) as

AKI after exposure to a contrast medium. Preexisting CKD is the strongest independent risk factor for contrast-induced AKI.[41] For this reason the use of intravenous contrast must be carefully considered in each patient, especially in patients with preexisting kidney disease. Although increments of plasma creatinine levels meeting the AKI criteria are not uncommon the incidence of severe AKI due to contrast-enhanced CT is low with a rate of 0.3% post-procedure dialysis.[42]

Therefore, in the context of the frequently detected decreasing renal function in patients undergoing systemic treatment for lung cancer, the risk of intravenous contrast should be carefully weighted against the benefit and should not be a routine procedure when a CT scan is ordered.

Symptoms may be observed with ATIN, like generalized malaise, fatigue, weakness, fever and anorexia. Obviously, it will be impossible to distinguish the cause of these non-specific symptoms in the presence of malignant disease. Interestingly, in 60% of the patients in this case series reporting on clinical features of immuno-therapy induced AKI, at least one extrarenal immune-related adverse events was documented prior or concurrently with AKI onset.[37] In addition, the time of onset of AKI seems to be delayed with a median of 91 days (IQR 60-183 days) and patients could still develop ATIN two months after treatment discontinuation.[12] Thus, concomitant extrarenal irAEs at the time of AKI may raise the suspicion of immunotherapy-related renal toxicity. Timing of AKI is unlikely to be helpful in distinguishing between immunotherapy- or chemotherapy-related renal toxicity during combination treatment, except for patients who have a very rapid onset of renal impairment after initiation of treatment, which is suggestive of chemotherapy-related toxicity.

Blood testing

None of the blood tests is helpful in pointing the differential diagnosis of AKI toward ATIN. Serum eosinophils may be moderately or highly elevated (up to 50%-75% of the total white blood cell count).[43] However, in a case-series on renal failure only one of 12 (8.3%) patients treated with ICI had eosinophilia.[12] Eosinophilia is also associated with NSCLC and the use of immunotherapy and therefore is not a specific marker.[44]

Blood tests in combination with urine chemistry may be helpful to distinguish prerenal renal injury from ATN. Fractional excretion of sodium (FeNa) and urea (FeUrea) can be calculated and are measures of tubular resorption of sodium and urea, respectively. A FeNa <1% in patients with volume depletion is suggestive of prerenal acute kidney injury; however, its value is unreliable during the use of diuretics.[45] In such cases, FeUrea is more accurate, with the FeUrea usually <35% in prerenal disease.[46] Patients with ATIN may have FeNa values <1% and >1%; therefore, FeNa is useless for diagnosing ATIN.[45] FeUrea has not been properly examined in this population.

As mentioned above, it is important to take into account the kidney function before treatment, as a decreased creatinine clearance at baseline may be predictive of sensitivity to kidney dysfunction during treatment. In our previous study we also established that a decline in renal function during treatment is predictive for developing renal failure.[7] Additionally, the trend of renal function during treatment should be noted. Although values may still be within a normal range, a decreasing renal function during induction treatment may predict the occurrence of AKI during maintenance treatment.[7]

Urinalysis

Urinalysis is a simple test but is the most important noninvasive test in the workup of AKI in general (table 2).

Table 2. Urinalysis in acute tubulointerstitial nephritis (ATIN) and acute tubular necrosis (ATN)

	ATIN	ATN
WBC	+*	0
WBC casts	+	0
RBC	+	0
Protein	+	+/-
Renal tubular cell casts	+-	+
Granular casts	0	+

* eosinophiluria may be present. Abbreviations: WBC, white blood cells; RBC, red blood cell

In ATIN sterile pyuria is present in most cases, as well as microscopic hematuria without casts suggesting non-glomerular disease. Proteinuria is mild, generally revealing protein concentrations <2 g/d. White blood cell casts may be observed, but the sensitivity is low.[47] ATN is characterized by the presence of (deeply-pigmented) granular and/or renal tubular epithelial cell casts with or without free renal tubular epithelial cells.[48]

PD-1 related ATIN seems to present similarly to other causes of ATIN, with evidence of pyuria and subnephrotic-range proteinuria in 60% and 50% of the patients, respectively.[12] Red blood cells were also detected in approximately 60% of the patients. Urinary cytokine IL-9 and tumor necrosis factor-a effectively distinguished ATIN from other renal lesions in patients treated with ICI, but these biomarkers still need validation.[49]

Imaging

If prerenal disease is excluded or severe AKI is present, an ultrasound should be performed to rule out postrenal disease by urinary tract obstruction. A CT may be performed when hydronephrosis or urinary tract obstruction cannot be reliable excluded by ultrasound. Kidney imaging with gallium-67 scintigraphy has been proposed in the evaluation of ATIN, as positive enhancement is seen if administered gallium-67 binds to lactoferrin, which is released by leukocytes within the kidney interstitium. However,

sensitivity (58%-100%) shows a large variety and specificity (50%-60%) is low.[50] The role of imaging during the workup of AKI during chemotherapy/immunotherapy combination is limited to excluding postrenal disease. However, when imaging procedures are requested, the use of intravenous contrast must be carefully considered to prevent further decrease of kidney function.

Renal biopsy

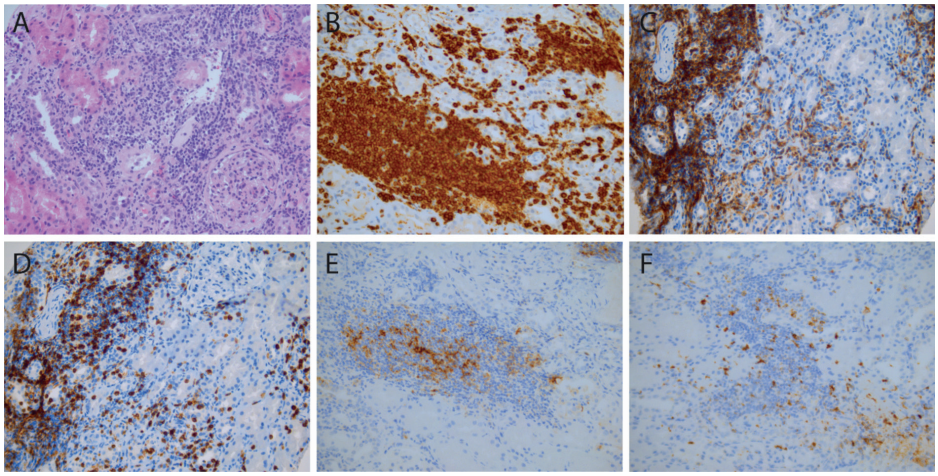
The regular procedure for distinction between chemotherapy- or immunotherapy-induced renal toxicity is a renal biopsy. Renal toxicity caused by chemotherapy shows ATN, while renal toxicity as a consequence of immunotherapy shows ATIN (figure 3). ATIN is characterized by marked mononuclear cell infiltration and a variable number of lymphoid follicles and tubulitis. There is a strong infiltration of mainly $CD3^+$ T cells, many of which are $CD4^+$ T helper cells with a mild infiltrate of $CD8^+$ cytotoxic T cells and CD20+ B lymphocytes (Fig. 3B-D).[12] $CD68^+$ and $CD163^+$ macrophages are also seen, together with $CD1c^+$ dendritic cells. More uncommon mechanisms of immunotherapy-induced renal disease have previously been published as case reports and these include TMA, minimal change disease, immune complex glomerulonephritis, as well as drug-induced lupus nephritis.[51–53] While TMA can be diagnosed histomorphologically, minimal change disease can only be diagnosed with confidence using electron microscopy, and the latter two require confirmation by demonstration of a characteristic immunofluorescence staining pattern.

The timing of a kidney biopsy is disputable and often depends on the subjective judgement of the clinician. Empirical treatment with steroids after ruling out pre- and postrenal causes of renal injury is recommended for most patients. A renal biopsy is indicated directly for patients who are likely to have an alternative cause of renal injuries, such as glomerulonephritis (i.e., not ICI-related), and for patients who do not recover even with high doses of steroids.

Management

In grade 1 AKI, it is recommended to continue ICI and monitor closely; whereas in grade 2 to 4 AKI, discontinuation of treatment should be done with prompt initiation of steroids, while at the same time exploring the exact cause of AKI.[54] In patients with grade 4 AKI, immunotherapy should not be restarted. This review of observational studies revealed that most patients (80%) received corticosteroids and that immunotherapy was discontinued (90%) if ATIN was considered during treatment with ICI; however, the approach with regard to dose and length of corticosteroid treatment was highly variable.[40] Only one-third of these patients had a complete recovery of their kidney function and 10% of the patients needed renal replacement therapy. There is a need for better immunopathophysiologic knowledge and biomarkers to develop more personalized therapeutic drug regimens for severe and refractory irAEs.[55]

Figure 3. *Kidney biopsy with tubulointerstitial nephritis*



A. Hematoxylin-eosin stain, demonstrating extensive immune cell infiltration in the kidney parenchyma, affecting and displacing tubules but not encroaching on glomeruli (bottom right). B. Immunohistochemical stain for CD3, demonstrating aggregates of T lymphocytes, and tubulitis. C. CD4 stain, positive in histiocytes and helper T cells in interstitial stroma, but not present in tubules. D. CD8, positive in cytotoxic T cells in stroma, and present in intratubular lymphocytes. E. PD-L1, limited to lymphoid aggregates, likely positive in dendritic / antigen presenting cells. F. PD-1 stain, positive in lymphocytes, within and outside of aggregates / follicles.

In the case of severe kidney injury most likely to be caused by chemotherapy, dose reductions or discontinuation should be considered although extensive data supporting such recommendations are lacking.[56,57] According to Kintzel et al., in patients treated with cisplatin a dose reduction of 25% is suggested for creatinine clearance (CrCl) 46-60 mL/min and a 50% dose reduction for CrCl 30-45 mL/min[56], whereas Aronoff et al. still recommend cisplatin administration in patients with more severe renal impairment. [57] Substituting cisplatin by carboplatin is a pragmatic approach in most patients with advanced NSCLC. For carboplatin renal function-based dose adjustments, using the Calvert formula, are recommended, capping the maximum carboplatin dose based on target area under the curve. In patients treated with pemetrexed, dose adjustment is not necessary in patients with a CrCl ≥ 45 mL/min and it is not recommended to use the drug in patients with a CrCl <45 ml/min, although data about these patients are scarce.[58] Pemetrexed dosing is based on the body surface area of the patient; however, increasing evidence suggests renal function is a main predictor of pemetrexed clearance and thus exposure.[59] Therefore, a renal-based dosing may result in a more stable exposure and less toxicity. Currently, a phase II study is assessing the feasibility of renal function-based dosing of pemetrexed in patients with an impaired renal function CrCl <45 ml/min (IMPROVE-I, ClinicalTrials.gov Identifier: NCT03656549).

DISCUSSION

Combination chemotherapy and immunotherapy with PD-1/PD-L1 inhibition improves survival in patients with NSCLC. The hypothesis is that chemotherapy increases the responsiveness to ICI, causing some synergistic effects with outcomes superior to the administration of both therapies in a sequential way. This also holds true for the maintenance phase, in which it is recommended to continue treatment with pemetrexed in combination with pembrolizumab.

The gain in survival benefit due to combination of chemotherapy and immunotherapy probably increases the willingness of patients to undergo treatment. This will lead to a larger treatment population in clinical practice, including patients who are frail and more prone to treatment adverse effects. Given the advanced age and the cardiovascular comorbidities often seen in lung cancer patients, renal side effects are more frequently seen in a general population then reported in clinical trials.[7]

Some important challenges are encountered in clinical practice when dealing with renal injury during the combination with chemotherapy and ICI treatment. We need to be aware of not only the underestimation of kidney injuries in clinical trials, but also of the large variations in incidence that may be reported owing to the use of different definitions. In particular, the latest CTCAE (version 5.0) may falsely report low numbers, as only kidney disease leading to hospitalization will be scored. Additionally, rather than using single eGFR and creatinine measurements alone, we emphasize looking at the trend during total treatment period. Further complications during maintenance treatment may be predicted not only by the absolute value of kidney function but also its decrease during treatment. For this reason, defining (sub)acute renal injury according to the AKD definition seems most appropriate.

Proper diagnosis of the causes of the adverse effects in these patients is of upmost importance to preclude worsening of adverse effects and decrease in quality of life. The algorithm described in this article may help clinicians to diagnose acute kidney injury in patients treated with a combination of chemotherapy and ICI.

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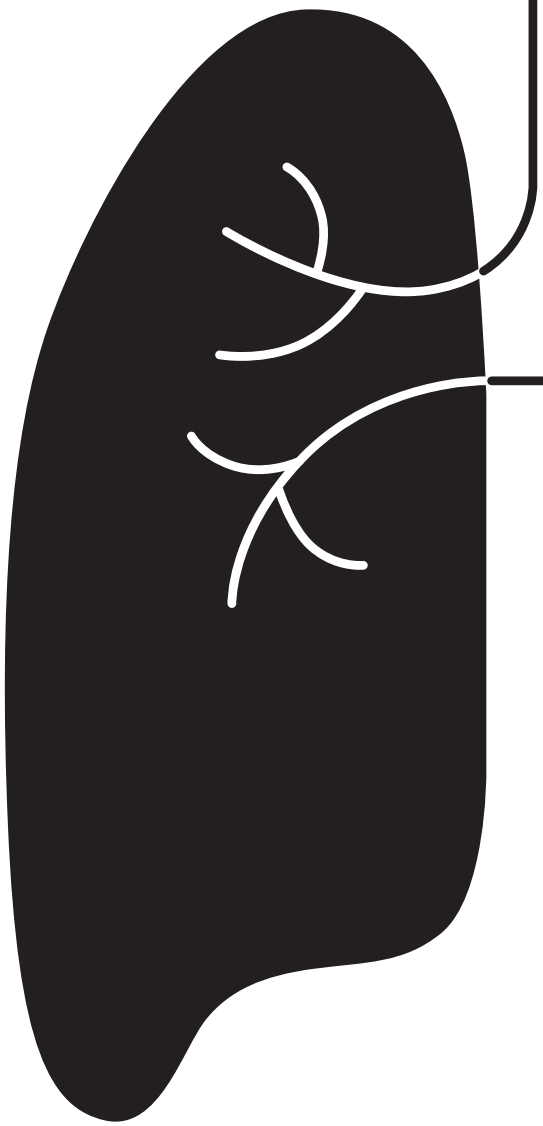

PART 3

PATIENTS' VIEW ON PEMETREXED

CHAPTER 9

Reliability and validity of the Cancer Therapy Satisfaction Questionnaire in Lung Cancer

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ABSTRACT

Purpose

To test the reliability and validity of the Cancer Therapy Satisfaction Questionnaire (CTSQ), to assess its relation with quality of life (QoL), and to assess the interpretability of the domain scores in patients with lung cancer receiving intravenous chemotherapy.

Methods

Patients with stage IIIB and IV nonsquamous non-small- cell lung carcinoma treated with pemetrexed were enrolled in our study. They completed the 16-item CTSQ and two other (HR)QoL questionnaires. Information about sociodemographic characteristics, cancer stage, the Eastern Cooperative Oncology Group performance status, and the experience of adverse events was collected. Internal consistency, construct validity, and clinical interpretability were calculated.

Results

Fifty-five patients completed the CTSQ. Correlations of the CTSQ items with its domain were all above 0.40. A high correlation between item 8 and the Expectations of Therapy and Satisfaction with Therapy domain was observed (0.50 and 0.48, respectively). The CTSQ domains demonstrated good internal consistency and low to moderate correlations of the CTSQ with the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-C30 and World Health Organization Quality of Life-BREF. No significant differences in mean domain scores were observed in relation to the number and severity of different adverse events and chemotherapy-related adverse events.

Conclusions

The Dutch version of the CTSQ was found to be a reliable and valid instrument to assess satisfaction and expectations of treatment in patients with lung cancer receiving intravenous chemotherapy. Furthermore, the CTSQ proved to be of additional informative value as not all of its domains correlated positively with the various domains of the existing HRQoL instruments.

INTRODUCTION

Anticancer therapies mostly offer modest improvements in survival, making the occurrence of adverse events an important outcome parameter in studies and clinical practice. It is well established that adverse events impair health-related quality of life (HRQoL) [1] and that (change of) HRQoL acts as a prognostic factor in (lung) cancer patients.[2-7] Questionnaires evaluating HRQoL offer valuable information about the impact of cancer and therapy related adverse events. However, they do not address patients' satisfaction, expectations and preferences concerning the occurrence and management of adverse events, the choice and type of therapy, and the efficacy of treatment. Such information provides opportunities for physicians to improve therapy compliance, personalize the course of treatment and to develop interventions designed to prevent or effectively treat adverse events and thus improve HRQoL. Certainly in diseases with a poor prognosis (e.g. advanced lung cancer) where the treatment is associated with only limited increases in survival and elevated risks for adverse events, insight into patients' expectations and satisfaction is of upmost importance.

In 2005, the Cancer Therapy Satisfaction Questionnaire (CTSQ) was developed to assess patients' opinions and feelings concerning their cancer therapy and associated adverse events.[8] A psychometric validation study of this questionnaire was performed, which resulted in an optimized and more brief version ensuring its reliability for research purposes.[9] Since then, the CTSQ has only been validated in a Korean study in which just four patients were treated with chemotherapy.[10]

Given these considerations the objective of our study was focused on three main aspects of the CTSQ: (1) to test the reliability and validity of the CTSQ in patients with lung cancer intravenous chemotherapy, (2) to assess its relation with (HR)QoL and (3) to assess the interpretability of the domain scores.

MATERIALS AND METHODS

Study population

This study was approved by the Institutional Review Board of the Erasmus University Medical Center in Rotterdam, The Netherlands. Patients were recruited from a university hospital (Erasmus University Medical Center) and a large teaching hospital (Amphia hospital) specialized in lung cancer care located in the western part of the Netherlands. Patients were enrolled in our study if they met the following criteria: they provided written informed consent, were aged eighteen years or older, and were treated with at least four cycles of pemetrexed combined with cisplatin or carboplatin as first-line therapy or pemetrexed single agent as second-line therapy. Patients were excluded if they met the following criteria: they were not able to read Dutch or could not complete the questionnaire because of a physical or mental condition (which prohibited participation

in the study). A sample size of at least 50 patients was needed in order to perform a validation study.[11]

Study measures

The CTSQ contains three domains covering 16 items: Expectations of Therapy (ET; 5 items), Feelings about Side Effects (FSE; 4 items) and Satisfaction with Therapy (SWT; 7 items). Each item was scored on a scale from one to five with a value of one corresponding with the worst response and a value of five representing the best response. Four items are reverse coded. The domain score was calculated by the formula: (mean of completed item scores -1) x 25. This results in a domain score ranging from 0 to 100, with a higher score representing a better outcome on each domain.

The original CTSQ was translated into Dutch by TransPerfect Translations Inc. according to the forward/backward methodology following international guidelines.[12] Questions were translated in a forward manner (English to Dutch) by two independent native-speaking linguists of the target language experienced in the translation of quality of life instruments. A third independent native speaker reviewed these translations and selected the most appropriate translation of the items or provided an alternative version. Discrepancies, linguistic limitations or cultural differences were addressed. Back translation was performed by a fourth independent native-speaker with proficiency in English. An oncologist determined whether the Dutch translation was in line with the medical terminology as used in the Netherlands. Finally, five respondents who received cancer treatment in the past 18 months were asked to provide feedback on the Dutch CTSQ during an interview. The respondents' overall impression of the instrument was that it was ''easy to complete''. The respondents' answers corresponded with the intended meanings of the items. During the translation process some questions were slightly changed (i.e. not literally translated) to ensure conceptual equivalence and cultural relevance to facilitate correct use of Dutch grammar. Permission of use was granted by Pfizer Inc. the current owner of the intellectual rights of the CTSQ. A pre-assessment of the Dutch version was conducted in 14 patients with lung cancer (not included in this study) to assess whether the questions were understandable and acceptable for use in the study.

The European Organization for Research and Treatment of Cancer-Quality of Life Questionnaire- Core 30 (EORTC-QLQ-C30) is a cancer-specific HRQoL instrument with demonstrated psychometric properties.[13] It consists of 30 items and incorporates a global health status/ quality of life scale, five functional scales and a number of single items assessing additional symptoms or difficulties. Each of the QLQ-C30 domains is scored on a 0-100 scale, with higher scores on the functional scales being indicative of better HRQoL, whereas higher scores on the symptom scales are reflective of worse symptoms.[14, 15]

The World Health Organization Quality of Life-BREF (WHOQoL-BREF) is a shorter version of the original WHOQoL-100 questionnaire. It is a generic QoL instrument and comprises 26 items divided over 4 domains: physical health, psychological health, social relationships, and environment and one facet: overall quality of life and general health. The WHOQoL-BREF domains are scored on a 4-20 scale and the facet on a 2-10 scale with higher scores indicating a better quality of life.[16] The WHOQoL-Bref is a well-established instrument that was developed for use in a wide range of disease areas and health problems.[17]

All questionnaires were completed after patients finished their four-cycle therapy of chemotherapy. In addition to completing the instruments, respondents were asked to provide information about the frequency and severity of adverse events they have experienced (cancer or therapy related). We also collected sociodemographic information (age, gender, educational level, ethnicity, smoking status and clinical history) and information about cancer stage, hospitalization (due to cancer or adverse effect of therapy), and the ECOG performance status.

Statistical analysis

Floor and ceiling effects were calculated in our study and were considered to be present if more than 15% of the respondents achieved the lowest (floor effect) or highest (ceiling effect) possible domain score.[11] Construct validity was evaluated using Pearson's rank correlation coefficient between the questionnaire items and domains. Correlations of 0.40 or higher indicate a good correlation between items and domains.[11] Internal consistency reliability measures to which extent items within a domain correlate with each other to form a (multi-item) domain. Reliability coefficients for the CTSQ domains were estimated using Cronbach's coefficient alpha where a reliability coefficient of 0.70 or higher was considered to be acceptable.[11]

Known-groups validity comparisons were made for the CTSQ domains in relation to the number of different adverse events and its severity. Also the impact of therapy related adverse events compared to cancer related adverse events on CTSQ domain score was evaluated. For this analysis, the one-way ANOVA was used to determine whether there were any significant differences between the means of two or more independent groups.

The association between the CTSQ domains with domains of the EORTC QLQ-C30 and WHOQoL-BREF was assessed using Spearman's correlation coefficients.

We assessed interpretability, which is defined as the degree to which one can assign qualitative meaning to quantitative scores. For each CTSQ domain, the minimal clinically important difference (MCID) was calculated using the approach of 0.5 standard deviation (SD) [18] and 1 standard error of measure (SEM).[19-21] MCID is the smallest change in an outcome that a patient would identify as important. The 0.5 SD benchmark of an

outcome measure entails that patients improving more than 0.5 of the outcome score's SD have achieved a minimally clinically important difference.[22] For the 1 SEM approach we have used the internal consistency reliability estimates. In addition, results of the known-groups comparison were used to derive the MCID using the number of adverse events with Common Terminology Criteria for Adverse Events (CTCAE) grade 3 or 4 as an anchor. A *P*-value below 0.05 was considered to be statistically significant.

All analyses were performed using SPSS version 21.0 (IBM Corporation, Armonk, NY).

RESULTS

Patient characteristics

Table 1 describes the characteristics of our study population. A total of 55 patients completed the questionnaires. The age of these patients ranged from 45 to 79 years, with a mean of 55.0 (SD 8.6). Forty-four patients indicated they had received a low level of education (80.0%), and 32.7% stated to be employed. The majority of these patients were diagnosed with adenocarcinoma of the lung (94.5%) and 85.5% had stage IV NSCLC. Almost all patients (98.2%) had a good ECOG performance score (grade 0 or 1). The majority of patients received pemetrexed chemotherapy as a first line treatment (85.5%).

Table 1. Characteristics of study population

Characteristic	Overall sample (N = 55)
Age, years	
Mean (SD)	55 (8.6)
Min, max	45, 79
Sex	
Male	27 (49.1)
Ethnicity	
White / Caucasian	52 (94.5)
Asian	1 (1.8)
Negroid	1 (1.8)
Other	1 (1.8)
Education[a]	
Low	44 (80.0)
High	8 (14.5)
Unknown	3 (5.5)
Employment	
Yes	18 (32.7)

Table 1. Characteristics of study population (continued)

Characteristic	Overall sample (N = 55)
Marital status	
Married/ cohabiting	44 (80.0)
Unmarried partners/ not cohabiting	3 (5.5)
Divorced/ separated	2 (3.6)
Widowed/ partner died	4 (7.3)
Single	1 (1.8)
Unknown	1 (1.8)
Cancer stage[b]	
Locally advanced (IIIB)	4 (7.3)
Metastatic (IV)	47 (85.5)
Other	4 (7.3)
Type of tumor[b]	
Adenocarcinoma	52 (94.5)
Large cell carcinoma	1 (1.8)
Mesothelioma	1 (1.8)
Large cell neuroendocrine carcinoma	1 (1.8)
Line of therapy	
First line	47 (85.5)
Second line	5 (9.1)
Adjuvant	3 (5.5)
ECOG performance status[a]	
Grade 0	17 (30.9)
Grade 1	38 (69.1)

Values are given in numbers (percentages) unless stated otherwise. [a]Low education: persons whose highest level of education is primary education, lower general education or lower vocational education. High education: persons whose highest level of education is higher general education, higher vocational education or university. [b]Measured at baseline. Abbreviations: N, number of patients; SD, standard deviation; ECOG, Eastern Cooperative Oncology Group (ECOG)

Mean scores and floor and ceiling effects

The mean scores of the ET and FSE domain were 55.6 (SD 22.5) and 52.2 (SD 23.8), respectively. The SWT domain had a mean score of 79.7 (SD 13.9), which was much higher compared to the mean scores of the other domains. No patients demonstrated the lowest possible domain score of 0.0. The floor effects for all domains were therefore zero. The FSE domain did not reach the highest possible score of 100, resulting in a negligible ceiling effect for this domain. For the ET and SWT domain we observed a ceiling effect of 5.5% and 9.1% respectively, which is below the common accepted limit of 15% (Table 2).

Table 2. Summary statistics for CTSQ domains

CTSQ domain	N	Mean (SD)	Median	Observed range (min, max)	Floor effect N(%)	Ceiling effect N(%)
Expectations of Therapy (ET)	55	55.6 (22.5)	55.0	15.0, 100.0	0 (0.0)	3 (5.5)
Feelings about Side Effects (FSE)	54	52.2 (23.8)	56.3	12.5, 93.8	0 (0.0)	0 (0.0)
Satisfaction with Therapy (SWT)	55	79.7 (13.9)	82.1	42.9, 100.0	0 (0.0)	5 (9.1)

Abbreviations: SD, standard deviation; N, number of patients; CTSQ, cancer therapy satisfaction questionnaire

Construct validity

Construct validity was supported for all 16 items as we observed a correlation of 0.40 or higher with their own hypothesized domain. However, we found that item 8, (chemotherapy would help you live longer) had a good correlation with its own hypothesized domain ET (0.50), and with the competing SWT domain (0.48). All other comparisons showed good results, as these items correlated better with their own hypothesized domain than with competing domains (Table 3).

Internal consistency

The internal consistency of the CTSQ domains is shown in Table 4. All three domains met the reliability coefficient of 0.70 or higher. Cronbach's alpha of the ET and FSE domains were both above 0.80 (0.83), except for the SWT domain (0.77). As presented in Table 3, we observed that item 8 had a similar correlation with the SWT domain as with the ET domain. For this reason, we decided to move item 8 from the ET domain to the SWT domain and calculated Cronbach's alpha for the revised CTSQ domains. We found a slight increase of the alpha coefficients of both domains (ET: 0.86, SWT: 0.79).

Table 3. Construct validity of the CTSQ (n=55)

Item number	Description	ET correlation coefficient (sig.)	FSE correlation coefficient (sig.)	SWT correlation coefficient (sig.)
Expectations of therapy (ET)				
1	CT would help you to return to a normal life	**0.73** (<0.001)	-0.20 (0.16)	-0.04 (0.77)
2	CT would get rid of the cancer	**0.87** (<0.001)	0.07 (0.61)	-0.006 (0.97)
3	CT would help prevent the cancer from coming back	**0.89** (<0.001)	0.13 (0.33)	0.20 (0.15)
4	CT would stop the cancer from spreading	**0.81** (<0.001)	-0.04 (0.80)	0.34 (0.01)
8	CT would help you live longer	**0.50** (<0.001)	0.15 (0.39)	**0.48** (<0.001)
Feelings about side effects (FSE)				
5R*	CT limited your daily activities	0.002 (0.99)	**0.68** (<0.001)	0.23 (0.09)
6R*	Upset about side effects	0.02 (0.91)	**0.80** (<0.001)	0.14 (0.30)
11R*	Overall, was taking CT as difficult as expected	-0.05 (0.70)	**0.91** (<0.001)	0.20 (0.14)
13	Overall, were side effects as expected	0.12 (0.38)	**0.87** (<0.001)	**0.41** (0.002)
Satisfaction with therapy (SWT)				
7	CT was worth taking even with side effects	0.37 (0.006)	0.08 (0.56)	**0.70** (<0.001)
9R*	How often did you think about stopping CT	-0.08 (0.56)	0.30 (0.03)	**0.42** (0.002)
10	Overall, how worthwhile was your CT	0.29 (0.03)	0.02 (0.89)	**0.63** (<0.001)
12	Overall, how well did the benefits of CT meet your expectations	0.27 (0.05)	0.25 (0.06)	**0.79** (<0.001)
14	How satisfied were you with the form of your CT	-0.11 (0.45)	0.19 (0.17)	**0.57** (<0.001)
15	How satisfied were you with your most recent CT	0.09 (0.51)	**0.40** (0.003)	**0.64** (<0.001)
16	If given choice again, would you decide to take this CT treatment	0.02 (0.87)	0.28 (0.04)	**0.74** (<0.001)

Correlations of CTSQ domains with CTSQ items of 0.40 or larger are in bold.
*These items were reverse-coded by subtracting the original value from 6, where a value of 1 represents the worst response and a value of 5 represents the best response.
Abbreviations: sig., significance (2-tailed); CT, chemotherapy; CTSQ, cancer therapy satisfaction questionnaire

Table 4. Internal consistency of CTSQ domains

	Internal consistency Cronbach's alpha	Internal consistency (revised) Cronbach's alpha*
CTSQ domain	**N = 55**	**N = 55**
Expectations of Therapy (ET)	0.83	0.86
Feelings about Side Effects (FSE)	0.83	0.83
Satisfaction with Therapy (SWT)	0.77	0.79

*Item 8 was moved from the ET domain to the SWT domain
Abbreviations: N, number of patients who completed the CTSQ questionnaire; CTSQ, cancer therapy satisfaction questionnaire

Known-groups comparisons

Table 5 shows the known-groups validity comparisons for the CTSQ domains in relation to the number of different adverse events, its severity and chemo-related adverse events. None of these results were found to be significant. We observed that an increasing number of grade 3 and 4 adverse events corresponded with a decreasing mean score of the FSE domain. The same observation was found in the analysis where we looked at the percentage of adverse events that were related to chemotherapy. Also, frequency and severity of adverse events were not related to satisfaction with therapy.

Table 5. Known-groups comparisons (n=55)

Description	CTSQ Expectations of Therapy			CTSQ Feelings about Side Effects			CTSQ Satisfaction with Therapy		
	N	Mean (SD)	*P*-value (effect size)*	N	Mean (SD)	*P*-value (effect size)*	N	Mean (SD)	*P*-value (effect size)*
Number of different adverse events[a]									
0-10	27	56.2 (24.7)	0.86	26	55.3 (22.9)	0.36	27	79.1 (13.2)	0.77
more than 10	28	55.1 (20.6)		28	49.3 (24.7)		28	80.2 (14.7)	
Number of adverse events with CTCAE grade 3 or 4[a]									
0	25	57.1 (22.7)	0.17	24	53.6 (23.6)	0.41	25	77.5 (14.4)	0.47
1	10	42.3 (16.3)	0.35	10	51.9 (23.0)	0.56	10	80.0 (14.4)	0.65
2 or 3	12	63.3 (27.2)		12	57.8 (26.1)		12	85.1 (11.0)	
more than 3	8	56.3 (16.4)		8	39.8 (21.6)		8	77.7 (15.8)	
% of adverse events that are related to chemotherapy									
0-25	6	63.3 (23.2)		6	56.3 (22.7)		6	84.5 (14.0)	
26-50	11	61.6 (23.8)		10	55.0 (22.6)		11	76.0 (9.5)	
51-75	23	49.5 (21.4)		23	54.9 (25.7)		23	80.7 (14.1)	
76-100	15	57.7 (22.6)		15	44.6 (22.5)		15	78.8 (16.5)	

*Effect sizes were only shown where one-way ANOVA was significant (P<0.05)
[a]reported adverse events: 2 weeks prior to last chemo until 4 weeks after last chemo
Abbreviations: CTSQ, cancer therapy satisfaction questionnaire; SD, standard deviation; N, number of patients who completed the questionnaire; CTCAE, Common Terminology Criteria for Adverse Events

Minimal clinically important differences

The estimates of the MCIDs are given in table 6. Estimates of the MCID for the ET and FSE domain were almost the same (0.5 SD: 11.25; 1 SEM: 9.28 and 0.5 SD: 11.9; 1 SEM: 9.81, respectively). The calculated estimates using the 0.5 SD approach were higher for both domains compared to the estimates using the 1 SEM approach. We observed a much lower estimate for the SWT domain (0.5 SD: 6.95; 1 SEM: 6.37) and a smaller difference between the estimates of the 0.5 SD and 1 SEM. The anchor-based MCID was estimated by calculating the average change in CTSQ score. For the ET domain, the estimate that was obtained using the number of grade 3 or 4 adverse events as an anchor was higher than the observed estimates using the 0.5 SD and 1 SEM approach (14.3). For the other two domains, we observed lower estimates when using the anchor- based method (SE: 8.5 and SWT: 5).

Table 6. Estimates of minimally clinically important differences on CTSQ domains

CTSQ domain	0.5 SD[a]	1 SEM[b]	Known-groups differences[c]
Expectations of Therapy	11.25	9.28	A difference of 14.8 points between 0 and 1 AE, 21 points difference between 1 and 2/3 AEs and a difference of 7 points between 2/3 and >3 AEs. The average difference is 14.3 points
Feelings about Side Effects	11.9	9.81	A difference of 1.7 points between 0 and 1 AE, 5.9 points difference between 1 and 2/3 AEs and a difference of 18 points between 2/3 and >3 AEs. The average difference is 8.5 points.
Satisfaction with Therapy	6.95	6.37	A difference of 2.5 points between 0 and 1 AE, 5.1 points difference between 1 and 2/3 AEs and a difference of 7.4 points between 2/3 and>3 AEs. The average difference is 5 points.

[a]0.5 SD of CTSQ domain scores
[b]using internal consistency reliability estimates
[c]using the known-group 'number of adverse events with CTCAE grade 3 or 4'
Abbreviations: CTSQ, cancer therapy satisfaction questionnaire; AE, adverse events; SD, standard deviation; SEM, standard error of measure

Correlation of CTSQ domains with quality of life

The correlation between the CTSQ domains and domains of the EORTC QLQ-C30 is shown in Table 7. We found the FSE domain correlated more strongly with the EORTC QLQ-C30 domains than the other two CTSQ domains. The highest correlations (r ≥ 0.40) were observed with global health status, role functioning, emotional functioning and the symptom domains fatigue, nausea and vomiting, and appetite loss. No correlation of 0.40 or higher was observed between the ET domain and the HRQoL domains. The SWT domain only significantly correlated with nausea and vomiting (r = -0.41). The negative correlations between the CTSQ and HRQoL domains indicate that higher scores of the CTSQ domains are associated with worse symptoms.

Table 7. Correlations of CTSQ with EORTC QLQ-C30 domains

N=55	CTSQ domains		
	Expectations of Therapy	Feelings about Side Effects	Satisfaction with Therapy
EORTC QLQ-C30 domains			
Global health status/ Quality of Life	0.01	**0.40****	0.27*
Physical functioning	0.18	0.34*	0.20
Role functioning	0.13	**0.48****	0.09
Emotional functioning	-0.011	**0.51****	0.17
Cognitive functioning	0.006	0.18	-0.03
Social functioning	-0.080	0.32*	0.02
Fatigue	-0.10	**-0.52****	-0.22
Nausea and vomiting	-0.04	**-0.53****	**-0.41****
Pain	-0.006	-0.26	-0.17
Dyspnea	0.018	-0.23	0.07
Insomnia	-0.16	0.10	-0.06
Appetite loss	-0.07	**-0.60****	-0.30*
Constipation	-0.20	-0.39**	-0.11
Diarrhea	-0.15	-0.11	0.04
Financial difficulties	-0.09	-0.04	0.04

Spearman correlations. Correlations of CTSQ domains with EORTC QLQ-C30 domains of r ≥ 0.40 or larger are in bold.
*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).
Abbreviations: CTSQ, cancer therapy satisfaction questionnaire; EORTC QLQ-C30, European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30; N, number of patients who completed the questionnaire

Results of the association between the CTSQ and WHOQoL-BREF domains are presented in Table 8. The domains of WHOQoL-BREF had the strongest correlations with the FSE domain. However, only the psychological domain had a correlation above 0.40 (r = 0.52).

Table 8. Correlations of CTSQ with WHOQoL-Bref domains

N=55	CTSQ domains		
	Expectations of Therapy	Feelings about Side Effects	Satisfaction with Therapy
WHOQoL-Bref domains			
Overall Quality of Life and General Health	0.20	0.28*	0.14
Physical Health	0.10	0.36**	0.10
Psychological Health	0.21	**0.52****	0.24
Social Relationships	0.07	0.12	0.12
Environment	0.04	0.15	0.04

Spearman correlations. Correlations of CTSQ domains with WHOQoL-Bref domains of r ≥ 0.40 or larger are in bold.
*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).
Abbreviations: CTSQ, cancer therapy satisfaction questionnaire; WHOQoL-Bref, World Health Organisation Quality of Life -Bref; N, number of patients who completed the questionnaire

DISCUSSION

Although (HR)QoL questionnaires inform health care professionals about the well-being of their patients, they do not address patients' expectations and satisfaction with therapy. Brown et al. demonstrated that expectations of therapy and adverse events are important determinants for patient compliance.[1] In addition, satisfaction is likely to express contentment with therapy and may also be influenced by the occurrence of adverse events. The CTSQ could be used as a tool to monitor the management of therapy and adverse events to improve HRQoL. Especially in cancer patients with a limited prognosis, this may be of importance. Therefore, our objective was to evaluate the reliability and validity of the CTSQ. Our study showed good results and hence supports the construct validity and internal consistency reliability of the CTSQ.

The previous psychometric validation study demonstrated a positively skewed score distribution of the ET domain with a substantial ceiling effect (20.5).[9] Even higher ceiling effects were observed in the study by Park et al. for the ET and FSE domains (21.6 and 36.3, respectively).[10] No floor or ceiling effects were found in our study, which indicates that no extreme items are missing in the lower or upper end of the scale. This might be explained by the fact that all patients in our study had advanced stage lung cancer of whom all have a limited survival compared to those with a curable disease. As lung cancer patients in general demonstrate information seeking behavior to cope with their disease [23] and the patients in our study were already informed about their limited survival prior to the start of therapy, we assume that the patients enrolled in our study did not have such high expectations. Moreover, disease stage may also influence the FSE and SWT domains. Simultaneously with disease progression, patients may

experience more and severe cancer related adverse events. These adverse events may be attributed by patients to chemotherapy probably resulting in a lower FSE domain score and decreased satisfaction with therapy.

All items correlated better with their own domains than with the other domains, which is in line with the results of the psychometric validation study. However, the correlations between the items and domains were found to be higher in our study compared with the previous study, which might be explained by the homogeneity of the population in our study. We observed that item 8 of the CTSQ (cancer therapy would help you live longer) had strong correlations with the SWT domain and with its own ET domain. Moreover, when we moved item 8 from the ET to the SWT domain, it resulted in a slight increase of alpha coefficients for both the ET and SWT domains. Although our results are in line with the results of the previous CTSQ studies,[9, 10] the sample size in our study was small. Therefore, we suggest further research to be conducted in a larger population to confirm this finding.

In 2004, a validation study of another patient satisfaction questionnaire (TSQM) was performed and showed significant differences in patient satisfaction and convenience of treatment between different treatment modalities (e.g. oral, topical, injectable, inhaler).[24] As patients in our study received only intravenously administered chemotherapy, we expect this may have affected the generalizability of our results. In addition, all patients in our study were diagnosed with advanced lung cancer whereas patients with various diseases were included in the TSQM validation study.[24] This may also hamper broad application of the CTSQ. However, when we compare our study with the study of Trask et al., which was conducted in a more heterogeneous population, we observed similar results with respect to construct validity and internal consistency reliability. Therefore, we assume that the single route of administration and the disease stage of the included patients in our study did not have a major impact on our results in terms of generalizability.

As for the estimates of the MCIDs, we observed similar results for the FSE and SWT domains when we compare our results using 0.5 SD and 1 SEM (FSE 11.9, 9.81; SWT 6.95, 6.37) with the results of the previous psychometric validation study (FSE 11.0, 10.55; SWT 6.88, 5.84). However, we found a clear difference of the MCIDs of the ET domain between both studies as in our study a larger change of domain score is needed for it to be considered clinically relevant (MCIDs in our study estimated based on 0.5 SD and 1 SEM, respectively: 11.25, 9.28; Trask et al.: 9.59, 6.92). A possible explanation for this is the ceiling effect of 20.5%, which was observed in the study by Trask, which was not observed in our study.[9] Consequently, they were not able to detect such a difference, because this change would then exceed the range of the scale.

We observed that an increasing number of severe and chemotherapy-related adverse events corresponded with a decreasing mean FSE domain score. According to Grutters et al. this may be due to the impact of adverse events on HRQoL as they showed in their study that already moderate adverse events resulted in a significant decrease in HRQoL.[25] To assess this relation between patient satisfaction and expectations regarding treatment and HRQoL in more detail, we correlated the CTSQ domains with the HRQoL domains and items. No positive correlations were found between the ET domain and any of the HRQoL domains or items indicating that not all concepts of the CTSQ are identified by HRQoL questionnaires. As argued before, expectations of therapy are likely to be influenced by the information patients have received. However, satisfaction seems also to be influenced by patients' opinions regarding the received information as several studies investigating patient satisfaction reported increased satisfaction when adequate information was provided by health care professionals.[26-28] Moreover, satisfaction with information has been associated with better HRQoL.[29] Therefore, we assume the CTSQ may give additional clinically relevant information that is not provided by HRQoL questionnaires regarding patients' expectations and satisfaction with information provision and possibly also other aspects of cancer care.

Terwee et al. suggested that a sample size of at least 50 patients would be sufficient for a validation study.[11] Nevertheless, for the clinical interpretation of the scores, a larger sample size may be needed to get more reliable results as we were not able to calculate the effect size in the known-groups comparison. For this reason, the small sample size may be considered as a limitation in our study. We were not able to evaluate test-retest reliability since the questionnaire was only given once after the fourth cycle of chemotherapy. If patients fill in the CTSQ a second time after the first completion, it will be hard to define an appropriate interval between those two completions as we included patients who have a relatively poor prognosis. If the interval between these completions is too short, the difficulty may be that they recall their earlier answers upon filling in the CTSQ for a second time. Moreover, when the interval is too long, patients may have progressed in their disease experiencing more adverse events, which may bias our results. However, we do not expect this to be a major problem as this part has already been validated in the psychometric validation study, showing good results.[9]

In conclusion, we were able to support the internal consistency reliability and construct validity of the Dutch version of the CTSQ in lung cancer patients treated with intravenous chemotherapy. Only a few aspects of HRQoL were significantly correlated to items of the CTSQ, indicating the need of using the CTSQ in studies evaluating satisfaction and expectations of patients on cancer chemotherapy. Since patients with disseminated cancer often have a limited prognosis, considering patients' motivations and needs is of importance to improve HRQoL. We therefore believe that our results may encourage researchers to use the CTSQ to investigate patients' expectations and satisfaction with therapy in future studies.

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CHAPTER 10

Treatment satisfaction of patients with advanced non-small-cell lung cancer receiving platinum-based chemotherapy: results from a prospective cohort study (PERSONAL)

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ABSTRACT

Introduction

In patients with advanced non-small-cell lung cancer (NSCLC) treatment benefits and risks need to be constantly weighed. We explored patient-reported satisfaction with therapy (SWT) and assessed its added value alongside quality of life (QoL) and adverse events (AEs).

Patients and methods

In a prospective multi-center cohort study, patients with stage IIIB/IV NSCLC received platinum-pemetrexed chemotherapy. They completed the World Health Organization Quality of Life-BREF (WHOQoL-BREF) and European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30 (EORTC QLQ-C30) before and during chemotherapy. After the last cycle, patients reported on SWT, expectations of therapy (ET) and feelings about side effects (FSE) using the Cancer Therapy Satisfaction Questionnaire (CTSQ). Explained variance (R^2) of QoL after treatment by SWT was calculated. Using (multivariable) linear regression, we examined the association of SWT with patient- and treatment-related variables, FSE and AEs.

Results

Eighty-nine patients finished four cycles of chemotherapy, of whom 65 completed the CTSQ. Fifty-six patients (86.2%) would probably/definitely decide to undergo the same treatment again, regardless of a deterioration/improvement of QoL or high/low frequency of AEs during chemotherapy. Explained variance of QoL by SWT was highest for the EORTC QLQ C-30 global health status/QoL scale ($R^2 = 0.170$). Patient's age ($\beta = 0.43$; 95%CI 0.05-0.82), FSE ($\beta = 0.17$; 95%CI 0.06-0.29) and tumor response ($\beta = 7.93$; 95%CI (1.64-14.22) were independently associated with SWT.

Conclusion

SWT may provide important supplemental information besides QoL and treatment toxicities. Tumor response, advanced age and FSE score were associated with better SWT. These insights may impact decision-making during palliative chemotherapy.

INTRODUCTION

Chemotherapy has shown to improve overall survival and quality of life (QoL) of patients with advanced non-small-cell lung cancer (NSCLC).[1,2] However, survival gain remains limited and treatment is often accompanied by adverse events (AEs) varying in number and severity depending on the different chemotherapy regimens and patients' individual characteristics.[1,3] Therefore, decisions whether to start or continue with treatment are complex and require that patients' expectations, preferences, and values with regard to benefits and risks are taken into account.

The implementation of patient reported outcomes in clinical practice has shown to improve assessment of and communication about symptoms and QoL.[4] AEs can have a considerable impact on health-related (HR)QoL.[5] In turn, change of (HR)QoL provides prognostic information with regard to (lung) cancer survival.[6–9] HRQoL has gained importance in treatment decision-making in addition to clinical effectiveness of treatment, since it incorporates the influence of AEs (treatment- or cancer-related) and acts as a prognostic factor for survival. However, considering treatment decisions in this manner ignores patients' reflection on treatment harms and benefits.

Another challenge in clinical decision-making is the considerable variability in how patients value the importance of survival benefit and symptom relief offered by chemotherapy.[10–12] In general, patients with metastatic lung cancer consider even a small increase in life expectancy as worthwhile, yet 10-25% of patients would not choose chemotherapy if additional survival is < 12 months.[13] Younger patients tend to accept a much smaller treatment benefit compared to older patients.[13,14] Patients' preferences are also affected by their understanding of prognosis. Many patients receiving chemotherapy for metastatic (lung) cancer overestimate their life expectancy, which might explain the discordance between the treatment decisions they make and their actual preferences.[15–17]

To date, there is little insight into which patient- or treatment-related factors are associated with treatment satisfaction. Taking into account patients' perceptions of prognosis and treatment satisfaction could offer a patient-centered view on the impact of negative and positive treatment effects and therefore may have added value in decision-making.

In this prospective multi-center study from a real-world's perspective, we explored the association between SWT and patient- and treatment-related factors and (feelings about) AEs in patients with advanced NSCLC treated with chemotherapy and we aimed to assess the added value of SWT alongside generally accepted clinical outcomes (HR)QoL and AEs.

PATIENTS AND METHODS

PEmetrexed and biomaRkerS: an observatiONAL study (PERSONAL) is a prospective multi-center cohort study of adult patients with locally advanced or metastatic (stage IIIB/IV) nonsquamous NSCLC and unresectable mesothelioma receiving platinum-combined pemetrexed as first-line and pemetrexed monotherapy as second-line treatment. Patients were recruited from October 2012 until November 2014 from a university hospital (Erasmus University Medical Center), two large teaching hospitals specialized in lung cancer care (Amphia hospital; Franciscus Gasthuis) and a regional hospital (Bravis hospital) located in the southwestern part of the Netherlands. Patients with unresectable mesothelioma were excluded from analyses in the present study. All patients provided written informed consent. This study was approved by the Institutional Review Board of the Erasmus University Medical Center in Rotterdam, The Netherlands.

Data collection

The validated Cancer Therapy Satisfaction Questionnaire (CTSQ) consists of 16 items covering three domains: satisfaction with therapy (SWT; seven items), feelings about side effects (FSE; four items) and expectations of therapy (ET; 5 items).[18] Items were scored on a scale from one (worst score) to five (best score). Four items were reverse coded. Each domain score was calculated by linear transformation of the mean of the corresponding item scores, resulting in a domain score range from 0 to 100. A higher score represents a better outcome on each domain, for instance a higher domain score of SWT corresponds with better treatment satisfaction. Items of special interest from the ET and SWT domain were the following: "How often do you think the chemotherapy can cure the disease?" (ET domain) and two items from the SWT domain; "The chemotherapy was worth it, even with side effects?"; "Would you decide to take the chemotherapy again, if given the choice?".

The World Health Organization Quality of Life-BREF (WHOQoL-BREF) is a generic QoL instrument developed to use in a wide range of disorders and health problems, including oncological diseases.[19] The questionnaire comprises 26 items covering four domains (Physical health, Psychological Health, Social Relationships and Environment) and one facet, including one item to assess overall QoL and one item to measure general health. The domain scores range between 4 to 20 and the facet is scored on a 2 to 10 scale, with a higher score indicating a better QoL.

The European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30 (EORTC QLQ-C30) is a HRQoL questionnaire, which is internationally used in clinical studies.[20] The questionnaire consists of 30 items and incorporates a global health status/QoL scale and five functional scales. Each of the QLQ-C30 scales is scored on a 0 to 100 scale, with a higher score being representative of a better HRQoL.

Patients completed (HR)QoL questionnaires before the start of chemotherapy (baseline). Follow-up assessments were performed during the 2nd cycle (day 7-14) and during the 4th cycle (day 14-21) of chemotherapy. At the latter moment, patients were also requested to complete the CTSQ. We collected sociodemographic information (age, sex, ethnicity), ECOG performance status, comorbidities, disease stage and treatment. After the start of chemotherapy, all clinical and laboratory AEs (cancer- or therapy-related) were weekly registered according to Common Terminology Criteria for Adverse Events (CTCAE 4.0). Tumor response measurements were obtained according to RECIST 1.1 after the 2nd and 4th cycle of chemotherapy.

Statistical analysis

Sociodemographic and clinical variables were compared between patients who completed the CTSQ questionnaires and patients who did not. We used the independent-samples *t*-test and the χ^2-test or Fisher's exact test for continuous and categorical variables, respectively.

Patients were categorized into 2 groups using the median number of all grades clinical AEs and into 3 groups with regard to alteration of QoL during treatment, based on known minimal clinically important differences (MCID) of the EORTC QLQ-C30 and WHOQoL-BREF.[21–23] These included deterioration, no change or improvement (Supplemental material). Using the Mann-Whitney *U* and Kruskal-Wallis test, differences in response distributions to individual items of the SWT domain were examined across mentioned groups according to the alterations of QoL and frequency of AEs. Differences in mean SWT domain scores were described for the three groups based on alteration of QoL. The Pearson's correlation coefficients (*r*) were used to calculate the explained variance (R^2) of QoL after four cycles of chemotherapy by SWT.

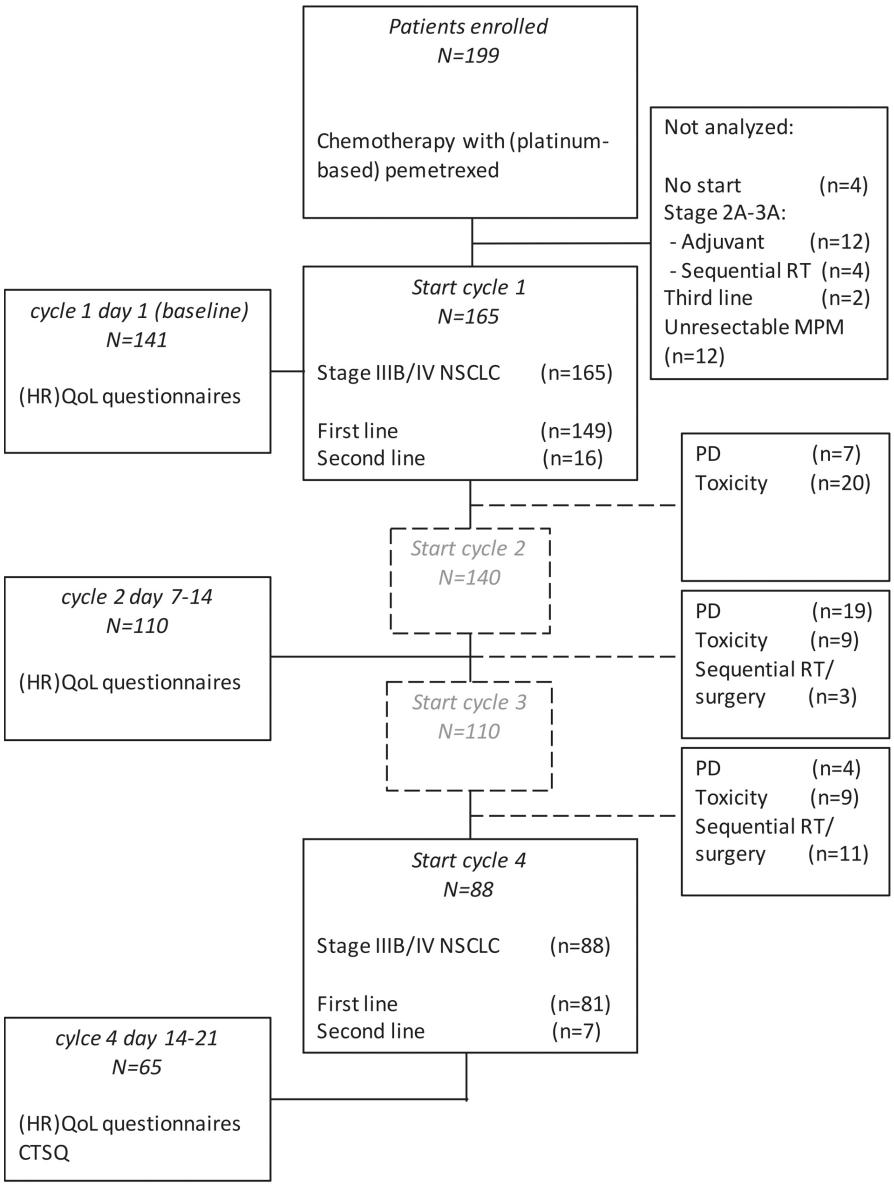
Patient- and treatment-related variables and (feelings about) AEs associated with SWT ($P < 0.05$) in univariable analyses, were analyzed with the use of multivariable linear regression (method: Enter). These regression analyses were restricted to patients treated with first-line platinum-based treatment to ensure a more homogeneous population. All statistical analyses were performed with the use of SPSS, version 22.0 (IBM Corporation, Armonk, NY).

RESULTS

In total, 165 patients with advanced NSCLC were enrolled in this study (Figure 1). All patients included in the analyses received pemetrexed-based chemotherapy as first- or second-line treatment. Of these patients, 85.5% completed the (HR)QoL questionnaires at baseline. Eighty-nine (53.9%) patients finished four cycles of chemotherapy, of whom 73.0% completed the CTSQ and (HR)QoL questionnaires. Reasons for non-completion of the questionnaires are reported in Supplemental Table S1. Seventy-six patients stopped

chemotherapy preliminary due to intolerable toxicities (42.1%), progressive disease (38.2%) or preplanned sequential radiotherapy or surgery (19.7%).

Figure 1. Flowchart



Abbreviations: (HR)QoL, (health-related) quality of life; NSCLC, non-small cell lung cancer; MPM, malignant pleural mesothelioma; CTSQ, Cancer Therapy Satisfaction Questionnaire; PD, progressive disease; RT, radiotherapy.

Patient characteristics

Patient characteristics are outlined in Table 1. The mean age in this population was 63.3 ± 9.2 years and slightly more than half of the patients were male (50.9%). The majority of patients had metastatic NSCLC (87.3%) and received pemetrexed as first-line treatment (90.3%), mostly combined with cisplatin (61.8%) or carboplatin (36.4%). The patients who completed the CTSQ questionnaires after four cycles of chemotherapy had a significantly lower ECOG performance score at baseline ($P = 0.001$), a better tumor response ($P < 0.001$), and a higher frequency of treatment- or cancer-related AEs ($P < 0.001$) than patients who did not complete the CTSQ.

Treatment satisfaction

The median domain scores of SWT, FSE and ET were 82.1 (Interquartile range [IQR]: 71.4 - 89.3), 56.3 (IQR: 37.5 - 75.0), and 55.0 (IQR: 40.0 - 78.8), respectively. Of the patients who completed the CTSQ, 26.1% often or always expected chemotherapy could cure their disease. During treatment, patients experienced 20.5 ± 5.0 all grades AEs, 13.5 ± 3.7 all grades clinical AEs, and 1.8 ± 1.7 grade ≥ 3 AEs, both treatment- and cancer-related. Detailed information about treatment-related clinical and laboratory AEs is provided in Table 2.

Responses to individual items within the SWT domain are shown in Figure 2. Of the patients who completed the item whether chemotherapy was worth taking even with side effects (N = 64), 81.3% answered positively. Twelve patients responded negatively (N = 3, 4.7%) or were in doubt (N = 9, 14.1%). Fifty-six of the 65 patients (86.2%) would probably or definitely decide to undergo the same treatment again. Distributions of the answers of both items were not significantly different for patients with a deterioration in QoL compared to patients with no change or improvement of QoL and between patients with a high (≥ 14) or low (< 14) frequency of clinical AEs (both treatment- and cancer-related).

Table 3 shows the distribution of SWT scores across different groups based on alteration of QoL during treatment. Patients with an improved WHOQoL-BREF facet score had a statistically higher ($P = 0.008$) SWT domain score (84.1 ± 10.5) than patients without change (71.2 ± 17.1) or a deterioration (75.8 ± 9.5). The SWT domain scores did not differ between groups across the WHOQoL-BREF domains. No significantly different SWT domain scores were found between groups based on EORTC QLQ C-30 global health status/QoL scale and the other scales (Supplemental Table S2). Likewise, the SWT scores did not differ across QoL groups between the 2nd and 4th chemotherapy cycle assessed with the WHOQoL-BREF and EORTC QLQ-C30 respectively (Supplemental Table S3 and S4). The explained variance of (HR)QoL after chemotherapy by SWT ranged from 0.002 (Cognitive scale) to 0.170 (Global health status/QoL scale) using the EORTC QLQ C-30 and from .009 (Social relationships) to 0.125 (Psychological health) assessed with the WHOQoL-BREF (Table 4).

If we restricted all above mentioned analyses to patients with first-line treatment, no significantly different results were found (data not shown).

Table 1. Characteristics of patients who started treatment with pemetrexed

Characteristic	Total (N = 165)	Completion CTSQ questionnaire (N = 65)	No completion CTSQ questionnaire (N = 100)	*P*-value
Age, mean (SD)	63.3 (9.2)	62.1 (7.9)	64.1 (9.8)	0.174
Gender, male	84 (50.9)	34 (52.3)	50 (50.0)	0.874
Ethnicity, Caucasian	155 (93.9)	60 (92.3)	95 (95.0)	0.814
ECOG performance score				
0 or 1	145 (87.8)	64 (98.5)	81 (81.0)	0.001
≥ 2	20 (12.2)	1 (1.5)	19 (19.0)	
Type of tumor				
Adenocarcinoma	160 (97.0)	63 (96.9)	97 (97.0)	0.577
Large cell carcinoma	5 (3.0)	2 (3.1)	3 (3.0)	
Cancer stage				
Locally advanced (IIIB)	21 (12.7)	5 (7.7)	16 (16.0)	0.153
Metastatic (IV)	144 (87.3)	60 (92.3)	84 (84.0)	
Combination therapy				
Cisplatin	102 (61.8)	39 (60.0)	63 (63.0)	0.665
Carboplatin	60 (36.4)	24 (36.9)	36 (36.0)	
Monotherapy	3 (1.8)	2 (3.1)	1 (1.0)	
Line of therapy				
1st line	149 (90.3)	60 (92.3)	89 (89.0)	0.595
2nd line	16 (9.7)	5 (7.7)	11 (11.0)	
Best tumor response				
PR	44 (26.7)	24 (36.9)	20 (20.0)	<0.001
SD	76 (46.1)	40 (61.5)	36 (36.0)	
PD	17 (10.3)	0 (0.0)	17 (17.0)	
Not evaluable*	28 (16.9)	1 (1.5)	27 (27.0)	
Mean number of adverse events per cycle (SD)†				
All grades	7.4 (4.1)	5.1 (1.2)	8.7 (4.6)	<0.001
Grade 1 and 2	6.3 (3.2)	4.7 (1.2)	7.2 (3.5)	<0.001
Grade 3 and 4	1.0 (1.4)	0.5 (0.4)	1.4 (1.7)	<0.001
Comorbidity				
Cardiovascular disease	71 (43.0)	25 (38.5)	46 (46.0)	0.421
COPD	25 (15.2)	7 (10.8)	19 (18.0)	0.268
Diabetes	22 (13.3)	5 (7.7)	17 (17.0)	0.217

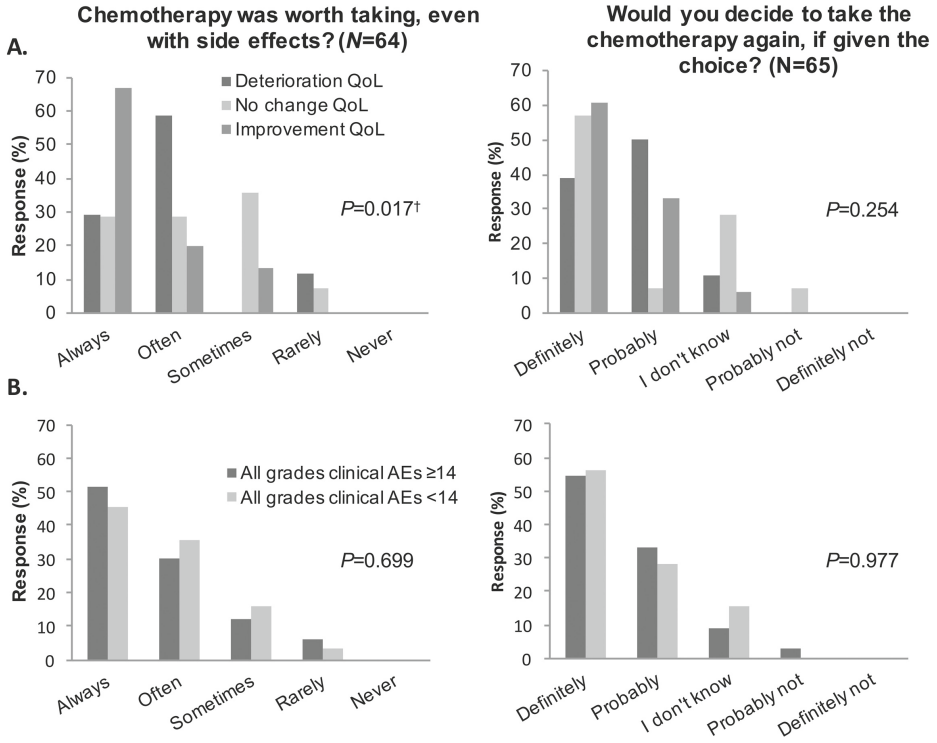
Data are expressed as numbers (%) unless stated otherwise. *Not evaluable due to early progression/death or systemic deterioration. † Distinct treatment- or cancer-related adverse events according to CTCAE 4.0. Abbreviations: CTSQ, Cancer Therapy Satisfaction Questionnaire; PR, partial response; SD, stable disease; PD, progressive disease.

Table 2. Adverse events in patients who completed the CTSQ (N=65)

	Frequency (%)	
Adverse event	**All grades**	**Grade≥ 3**
Treatment-related*		
Any	69 (100)	32 (49)
Clinical		
Fatigue	61 (94)	7 (11)
Anemia	57 (88)	8 (12)
Nausea	46 (71)	3 (5)
Decreased appetite	44 (68)	2 (3)
Taste alteration	37 (57)	0
Oral mucositis	33 (51)	1 (2)
Dry eyes/watering eyes	31 (48)	0
Dry skin	29 (45)	0
Constipation	26 (40)	1 (2)
Rash	19 (29)	0
Diarrhea	15 (23)	1 (2)
Vomiting	13 (20)	0
Dizziness	13 (20)	0
Alopecia	13 (20)	0
Dysphagia	12 (18)	0
Dyspepsia	10 (15)	0
Pruritus	10 (15)	0
Abdominal bloating	9 (14)	0
Weight loss	8 (12)	0
Laboratory		
Decreased white cell count	43 (66)	9 (14)
Decreased neutrophil count	42 (65)	18 (28)
Decreased thrombocyte count	33 (51)	6 (9)
Alanine aminotransferase elevation	32 (49)	0
Aspartate aminotransferase elevation	25 (38)	0
Alkaline phosphatase elevation	22 (34)	0
Blood creatinine level elevation	15 (23)	0

Listed are adverse events that are reported in at least 10% of the patients. *Adverse events were scored as treatment-related if investigator defined relatedness as probably or definitely.

Figure 2. A. Distribution of responses to two items of the SWT domain across patients with a deterioration, no change and improvement of the facet score (global QoL/general health) of the WHOQoL-BREF using minimal clinical important differences.



†Distribution of answers to this item was significantly different between patients with no change and an improvement of QoL (P = 0.010). B. Distribution of responses to two items of the SWT domain across patients with more (≥14) or less (<14) clinical adverse events. Abbreviations: AE, adverse event; QoL, quality of life.

Table 3. Mean SWT domain scores across groups regarding change in WHOQoL-BREF Q facet and domain scores between baseline and after 4th cycle of pemetrexed treatment (N = 62)

WHOQoL-BREF facet/ domains	Δ QoL group	N (%)	Mean change in facet/ domain scores†	Domain score SWT	*P*-value*
Overall QoL/General health	Deterioration	17 (27)	-2.1 (1.1)	75.8 (9.5)	*0.008*
	No change	14 (23)	0.0 (0.0)	71.2 (17.1)	
	Improvement	31 (50)	1.7 (0.9)	84.1 (10.5)	
Physical health	Deterioration	16 (26)	-4.2 (2.6)	76.3 (15.9)	0.455
	No change	24 (39)	0.0 (0.7)	78.1 (13.1)	
	Improvement	20 (32)	3.7 (2.1)	82.0 (10.9)	
	Missing	2 (3)			
Psychological health	Deterioration	26 (42)	-3.0 (1.6)	78.0 (12.7)	0.853
	No change	25 (40)	0.0 (0.5)	79.2 (14.5)	
	Improvement	10 (16)	2.7 (1.3)	80.0 (11.8)	
	Missing	1 (2)			
Social relationships	Deterioration	29 (47)	-3.2 (2.3)	78.6 (13.0)	0.309
	No change	17 (27)	0.0 (0.2)	75.6 (15.1)	
	Improvement	15 (24)	2.2 (1.8)	82.9 (10.6)	
	Missing	1 (2)			
Environment	Deterioration	18 (29)	-2.4 (1.2)	81.3 (9.8)	0.428
	No change	28 (45)	-0.1 (0.8)	76.0 (15.4)	
	Improvement	14 (23)	2.8 (1.3)	82.1 (11.2)	
	Missing	2 (3)			

Data are expressed as means (SD). †Minimal clinical important differences were used to determine deterioration, no change and improvement of QoL per domain/facet. *Distributions of SWT scores across change in QoL groups were compared using the Kruskal-Wallis test. Abbreviations: WHOQoL-BREF, World Health Organization Quality of Life-BREF; SWT, satisfaction with therapy.

Table 4. QoL scores after 4 cycles of chemotherapy and its explained variance by SWT domain score of the CTSQ

WHOQoL-BREF					EORTC QLQ-C30				
Facet/domains	**N**	**Mean (SD)**	**ρ**	**R^2**	**Scales**	**N**	**Mean (SD)**	**ρ**	**R^2**
Overall QoL/General health	65	6.3 (1.6)	0.203	0.041	Global health status/QoL	63	58.6 (23.8)	0.412	0.170
Physical health	63	13.7 (3.0)	0.240	0.058	Physical functioning	65	65.7 (21.8)	0.279	0.078
Psychological health	64	14.2 (2.2)	0.354	0.125	Role functioning	65	54.9 (33.6)	0.155	0.024
Social relationships	64	15.5 (2.5)	0.094	0.009	Emotional functioning	64	76.0 (21.5)	0.191	0.036
Environment	63	16.2 (2.1)	0.179	0.032	Cognitive functioning	64	78.1 (23.9)	-0.042	0.002
					Social functioning	63	75.4 (26.8)	0.128	0.016

ρ is the Pearson's correlation coefficient between the SWT domain score and the QoL score. Abbreviations: WHOQoL-BREF, World Health Organization Quality of Life-BREF; EORTC QLQ-C30, European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30; QoL, quality of life

Factors associated with satisfaction with therapy

Results of the regression analyses performed in patients treated with first-line platinum-based pemetrexed treatment (N = 60) with the SWT domain score as dependent variable and patient- and treatment-related factors as independent variables are shown in Table 5. In the univariable analyses patients' age (*P* = 0.042), tumor response (*P* = 0.014), sex (*P* = 0.048) and the domain score FSE (*P* = 0.004) were significantly related to SWT. In the multivariable analysis (R^2=0.326), only age (β = 0.43; 95% CI 0.05-0.82), FSE (β = 0.17; 95% CI 0.06-0.29) and tumor response (β = 7.93; 95% CI 1.64-14.22) showed independent relations with SWT. No associations were found between SWT and the frequency of grade 1/2 or grade 3/4 AEs. Similarly, recent AEs (within four weeks before completion of CTSQ) and clinical AEs were not related with SWT (data not shown).

Table 5. Linear regression analyses of factors associated with satisfaction with therapy (N = 60)

	Univariable analysis		Multivariable analysis	
	β coefficient (95% CI)	*P*-value	β coefficient* (95% CI)	*P*-value
Age	0.51 (0.10, 0.93)	*0.042*	0.43 (0.05, 0.82)	*0.028*
Sex female vs. male	-6.74 (-13.42, -0.06)	*0.048*	-3.90 (-9.98, 2.17)	0.203
ECOG performance score 0 vs. ≥1	1.61 (-5.92, 9.14)	0.670		
Tumor response (4th cycle) PR vs. SD or PD	8.94 (1.90, 15.99)	*0.014*	7.93 (1.64, 14.22)	*0.014*
No. of grade 1/2 AEs†	-0.13 (-0.86, 0.60)	0.731		
No. of grade 3/4 AEs†	1.27 (-0.77, 3.30)	0.217		
FSE domain score	0.19 (0.06, 0.32)	*0.004*	0.17 (0.06, 0.29)	*0.005*

*Adjusted for all factors statistically significant $P < 0.05$ in the univariable model. † cancer- or treatment related adverse events during total treatment period. Abbreviations: PR, partial response; SD, stable disease; PD, progressive disease; QoL, quality of life; AE, adverse event; FSE, feelings about side effects.

DISCUSSION

As shared decision-making becomes increasingly important nowadays, the need for clinically useful patient reported outcomes increases likewise. It has been recently demonstrated that shared decisions were positively associated with a higher patient-reported quality of care,[24] which may be particularly important in cancer patients with poor prognosis. Therefore, our objective was to assess the value of patient reported SWT alongside widely accepted clinical outcomes of therapy. To our knowledge, this is the first study to have extensively assessed and characterized patients' satisfaction with chemotherapy. Our results propose that SWT covers different aspects of patient-centered and -reported impact of treatment effects than QoL and adverse events; therefore, SWT could be useful in decision-making, as it offers important supplemental information from

a patients' perspective. SWT described < 10% of the variance of the functional scales and domains from both (HR)QoL questionnaires, except for global health status/QoL (17%) and the psychological domain (13%). Accordingly, our group[18] already suggested the additional informative value of patients' SWT as the different aspects of (HR)QoL showed a low correlation (< 0.3) to items of the CTSQ. Although symptomatic adverse events may substantially contribute to QoL in NSCLC,[5] the frequency of (severe) treatment- and cancer related adverse events was not associated with treatment satisfaction. However, patients with better feelings about these side effects appeared to be more content with therapy. Therefore, patients' education about and management of adverse events may have added value in maintaining patients' well-being during chemotherapy, ultimately resulting in higher treatment satisfaction.

In our study, >80% of the patients valued pemetrexed- and platinum-based chemotherapy as worth taking and would probably or definitely decide to take the chemotherapy again regardless of the presence of chemotherapy-related adverse events or deterioration in QoL. Because ~75% of the patients correctly expected no or unlikely cancer cure, expressed satisfaction with therapy in our study is not solely a reflection of inaccurate expectations. Previous studies evaluating treatment preferences in a variety of oncological populations have reported that patients value even small benefits greatly and judge toxicity as less important.[10,25] More recently, Peeters et al.[12] and Pacchiana et al.[26] assessed patients' perceptions on future maintenance treatment for advanced NSCLC and they showed a generally favorable attitude towards treatment continuation at foresight, even if the expected gain of overall survival would be minimal. In agreement with our findings, mild-to-moderate side effects would be accepted by most patients.[12] Blinman et al[10] noticed that smaller benefits were judged sufficient for metastatic compared to locally advanced NSCLC. Furthermore, pemetrexed has been shown to be associated with relatively mild toxicity profiles and is generally well tolerated.[3,27] In our study population, these considerations may have contributed to the highly valued merits of treatment despite side effects and the large willingness to undergo treatment again at hindsight.

Older patients showed a higher treatment satisfaction than younger patients, which offers no support to restrained prescription of pemetrexed- and platinum-based chemotherapy in the elderly. Although recent studies have shown that palliative platinum-based doublet treatments result in improved survival rates comparable to younger patients, they often receive no chemotherapy or only single-agent regimens resulting in risk of undertreatment.[28–30] However, adequate information about other important treatment outcomes as toxicity, symptom relief and costs are scarce. In general, younger patients are more socially active compared to elderly. Moreover, it is commonly accepted that senescence is associated with an increased risk of morbidity and mortality. Therefore, higher hopes and demands of chemotherapy and worse coping

with a shorter life expectancy may explain the finding in our study that younger patients are less easily satisfied with treatment.

Importantly, patients in our study represent a real-life study population which significantly differs from populations generally included in clinical trials. Many patients in our population had (multiple) comorbidities, which occurs more frequently in unselected cancer populations.[31] However, this is in contrast to earlier clinical trials where patients with significant comorbidities or organ dysfunction were excluded from enrolment.[3,32] Additionally, an older median age and the inclusion of patients with a high (≥2) ECOG performance score compared to previous clinical trials could have led to lower tolerability of treatment and higher number of (severe) adverse events. Grutters et al. already showed that (even mild) adverse events might negatively influence QoL outcomes.[5]

A major limitation of our study is imposed by the study design, which prevented us to evaluate treatment satisfaction (and its relation with change of QoL) in patients who did not complete the full treatment of four cycles chemotherapy. Therefore, our results were obtained in a group of patients who had a good performance score and who mainly established disease stabilization. These factors could have led to an overestimation of the level of treatment satisfaction and underestimation of the associations between SWT with QoL and (feelings about) adverse events and between treatment response and SWT. In future research, we would recommend to assess SWT earlier during therapy to increase knowledge with respect to treatment satisfaction in patients with clinically important toxicities, poor treatment response and worse QoL. Finally, we cannot exclude the possibility of unmeasured false hope and social desirability bias in our results. Since patients completed the questionnaires by self-report and the questionnaires were collected by their care providers, it is possible patients responded with greater optimism than they actually felt.

CONCLUSION

In conclusion, the CTSQ is a useful tool to extensively assess SWT in research as well as in daily clinical decision-making. The results of this study justify further exploration of SWT in patients with advanced NSCLC treated with chemotherapy. In shared decision-making regarding palliative treatment, knowledge about patients' treatment satisfaction could provide important supplemental information, in addition to patients' QoL and treatment toxicities.

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SUPPLEMENTAL MATERIAL

USE OF MINIMAL CLINICALLY IMPORTANT DIFFERENCES

We divided patients into three groups with respect to the change of quality of life after the 2nd and 4th cycle of chemotherapy compared to baseline QoL. For EORTC QLQ C-30, minimal clinical important differences (MCID) have been established earlier using distribution-based and anchor-based methods, which had comparable results [1,2]. When we categorized patients according to change in EORTC QLQ-C30 scores, patients with a change of ≥ 5 of the functional scale scores in positive or negative direction were considered as having an improvement or deterioration in QoL respectively, while patients who showed a difference of <5 were considered to have no significant change in QoL. Using the WHOQoL-BREF, we used recently established MCIDs based on distribution-based methods in the same study population [3]. In our study, we applied the 0.5 standard deviation (SD) estimates of MCID on WHOQoL-BREF domains (listed in table below). When we categorized patients according to change in WHOQoL-BREF scores, patients with a change of ≥ 0.5 SD of the domains in positive or negative direction were considered as having an improvement or deterioration in QoL respectively, while patients who showed a difference of <0.5 SD were considered to have no significant change in QoL.

Domains WHOQoL-BREF	0.5 SD
General Facet	0.876
Physical Health	1.545
Psychological Health	1.259
Social Relationships	1.274
Environment	1.142

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SUPPLEMENTAL TABLES

Table S1. Reasons for non-completion of questionnaires by patients who started chemotherapy

	baseline N=165 *EORTC QLQ-C30 WHOQoL-BREF*	**2nd cycle (day 7-14) N=141** *EORTC QLQ-C30 WHOQoL-BREF*	**4th cycle (day 14-21) N=89** *EORTC QLQ-C30 WHOQoL-BREF CTSQ*
Non-completion, total	24 (14.5)	31 (21.9)	24 (27.0)
Not able to read Dutch	1 (0.6)	1 (0.7)	1 (1.1)
Physical disabilities	3 (1.8)	1 (0.7)	1 (1.1)
Poor condition	8 (4.2)	6 (4.3)	2 (2.2)
Mental burden	7 (4.2)	4 (2.8)	4 (4.5)
Stop study, death	N/A	3 (2.1)	1 (1.1)
Stop study, PD	N/A	3 (2.1)	1 (1.1)
Stop study, toxicity	N/A	1 (0.7)	0
Logistic failure	5 (3.0)	10 (7.1)	10 (11.2)
Unknown	1 (0.6)	2 (1.4)	4 (4.5)

Data are expressed as frequencies (percentage). Abbreviations: EORTC QLQ-C30, European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30; WHOQoL-BREF, World Health Organization Quality of Life-BREF; CTSQ, Cancer Therapy Satisfaction Questionnaire; PD, progressive disease; N/A, not applicable

Table S2. CTSQ domains in relation with change in EORTC QLQ C-30 QoL and functional scale scores between before and after treatment with pemetrexed (*N*=63)

EORTC QLQ C-30 scales	Δ QoL group	N (%)	Mean change in scale score†	Domain score SWT	*P*-value*
Global health status/QoL	Deterioration	21 (33)	-24.6 (24.4)	78.4 (13.2)	0.801
	No change	11 (17)	-0.76 (24.6)	77.3 (14.8)	
	Improvement	31 (49)	13.9 (22.5)	80.0 (12.5)	
Physical functioning	Deterioration	36 (57)	-23.3 (17.0)	76.6 (14.5)	0.346
	No change	6 (10)	0.0 (0.0)	83.8 (8.6)	
	Improvement	21 (33)	20.3 (17.8)	81.8 (10.5)	
Role functioning	Deterioration	33 (52)	-38.9 (120.2)	78.7 (12.4)	0.965
	No change	9 (14)	0.0 (0.0)	77.0 (20.0)	
	Improvement	21 (33)	41.3 (22.7)	80.4 (10.6)	
Emotional functioning	Deterioration	21 (33)	-22.4 (18.2)	77.9 (14.5)	0.818
	No change	8 (13)	0.0 (0.0)	79.9 (19.6)	
	Improvement	32 (51)	22.4 (18.3)	79.2 (10.6)	
	Missing	2 (3)			
Cognitive functioning	Deterioration	21 (33)	-29.4 (22.3)	76.0 (13.9)	0.441
	No change	28 (45)	0.0 (0.0)	80.4 (11.6)	
	Improvement	12 (19)	25.0 (16.7)	80.1 (15.4)	
	Missing	2 (3)			
Social functioning	Deterioration	21 (33)	-30.2 (25.6)	78.5 (13.1)	0.904
	No change	19 (31)	0.0 (0.0)	78.2 (15.0)	
	Improvement	20 (32)	33.3 (18.7)	80.0 (12.1)	
	Missing	3 (5)			

Data are expressed as means (SD). †Minimal clinical important difference=5, > 5 in positive or negative direction were considered as having an improvement or deterioration in QoL respectively *Distributions of data across groups were compared using the Kruskal-Wallis test. Abbreviations: EORTC QLQ-C30; European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30; QoL, quality of life; SWT, satisfaction with therapy.

Table S3. CTSQ domains in relation with change in WHOQoL-BREF QoL and domain scores between 2nd and 4thcycle of chemotherapy (after treatment) with pemetrexed (*N*=55)

WHOQoL-BREF facet/domains	Δ QoL group	N (%)	Mean change in overall QoL/ domain score†	Domain score SWT	*P*-value*
Overall QoL/General health	Deterioration	15 (27)	-2.2 (1.2)	78.1 (15.0)	0.230
	No change	16 (29)	0.0 (0.0)	76.1 (12.4)	
	Improvement	24 (44)	1.8 (0.9)	83.2 (9.8)	
Physical health	Deterioration	15 (27)	-3.6 (1.8)	78.5 (15.3)	0.883
	No change	22 (40)	0.0 (0.7)	79.2 (11.3)	
	Improvement	16 (29)	3.0 (1.1)	81.7 (11.7)	
	Missing	2 (4)			
Psychological health	Deterioration	20 (36)	-2.4 (1.2)	75.0 (12.1)	0.132
	No change	20 (36)	0.1 (0.6)	81.8 (13.8)	
	Improvement	14 (25)	2.3 (0.7)	83.2 (9.0)	
	Missing	1 (2)			
Social relationships	Deterioration	22 (40)	-2.6 (2.2)	81.1 (11.0)	0.404
	No change	15 (27)	0.0 (0.2)	75.7 (12.5)	
	Improvement	17 (31)	2.1 (0.9)	81.1 (14.0)	
	Missing	1 (2)			
Environment	Deterioration	13 (24)	-2.8 (1.1)	81.8 (10.2)	0.847
	No change	31 (56)	0.0 (0.6)	79.6 (13.1)	
	Improvement	9 (16)	1.9 (0.6)	78.2 (13.5)	
	Missing	2 (4)			

Data are expressed as means (SD). †Minimal clinical important differences were used to determine deterioration, no change and improvement of QoL per domain/facet. *Distributions of data across groups were compared using the Kruskal-Wallis test. Abbreviations: WHOQoL-BREF, World Health Organization Quality of Life-BREF; QoL, quality of life; SWT, satisfaction with therapy.

Table S4. CTSQ domains in relation with change in EORTC QLQ C-30 QoL and functional domain scores between 2nd and 4thcycle of chemotherapy (after treatment) with pemetrexed (*N*=60)

EORTC QLQ C-30 scales	Δ QoL group	N (%)	Mean change in scale score†	Domain score SWT	*P*-value*
Global health status/ QoL	Deterioration	21 (35)	-26.2 (15.0)	78.2 (11.9)	0.191
	No change	11 (18)	0.0 (0.0)	86.0 (11.8)	
	Improvement	21 (35)	18.3 (13.3)	78.1 (12.9)	
	Missing	7 (12)			
Physical functioning	Deterioration	29 (48)	-20.2 (16.2)	76.9 (15.7)	0.855
	No change	12 (20)	0.0 (0.0)	81.3 (7.2)	
	Improvement	20 (33)	21.0 (11.9)	81.4 (11.6)	
Role functioning	Deterioration	26 (43)	-32.7 (16.0)	78.9 (12.8)	0.980
	No change	19 (32)	0.0 (0.0)	89.1 (13.4)	
	Improvement	15 (25)	36.7 (16.9)	80.2 (14.1)	
Emotional functioning	Deterioration	21 (35)	-22.2 (14.8)	77.9 (14.8)	0.699
	No change	15 (25)	0.0 (0.0)	78.3 (10.0)	
	Improvement	18 (30)	15.7 (7.5)	82.7 (11.2)	
	Missing	6 (10)			
Cognitive functioning	Deterioration	19 (32)	-26.3 (12.8)	77.6 (14.4)	0.670
	No change	21 (35)	0.0 (0.0)	79.6 (10.8)	
	Improvement	14 (23)	26.2 (12.6)	82.4 (12.2)	
	Missing	6 (10)			
Social functioning	Deterioration	20 (33)	-30.0 (23.3)	77.3 (14.3)	0.807
	No change	17 (28)	0.0 (0.0)	80.2 (11.7)	
	Improvement	16 (27)	34.4 (15.5)	82.4 (10.9)	
	Missing	7 (12)			

Data are expressed as means (SD). †Minimum clinical important difference=5, >5 in positive or negative direction was considered as having an improvement or deterioration in QoL, respectively. *Distributions of data across groups were compared using the Kruskal-Wallis test. Abbreviations: EORTC QLQ-C30, European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30; QoL, quality of life; SWT, satisfaction with therapy.

CHAPTER 11

Frequency of low-grade adverse events and quality of life during chemotherapy determine patients' judgement about treatment in advanced stage thoracic cancer

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ABSTRACT

Purpose

In lung cancer, the preservation of well-being is warranted given the limited prognosis. Chemotherapy may negatively influence Health Related Quality of Life (HRQoL) due to adverse events. However, patients' judgement about this negative impact is not well understood. We examined the relationship between expectations, feelings about side effects and satisfaction with therapy and (HR)QoL in advanced stage thoracic cancer and investigated which of these factors has the highest impact on (HR)QoL.

Methods

69 patients completed the Cancer Therapy Satisfaction Questionnaire (CTSQ), the World Health Organization Quality of Life-BREF (WHOQOL-BREF), and the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30 (EORTC QLQ-C30). Multiple regression analyses were performed to investigate the relation of the CTSQ domains (i.e., Expectations of Therapy, Feelings about Side Effects, Satisfaction with Therapy) with (HR)QoL and simple regression analyses to identify clinical and sociodemographic factors associated with the CTSQ domain that was most often associated with (HR)QoL.

Results

Feelings about Side Effects were associated with the (HR)QoL domain/scale scores, (i.e., WHOQOL-BREF domains: $\beta = 0.36$ to 0.58; EORTC QLQ-C30 scales: $\beta = 0.33$ to 0.61) except Social Relationships of the WHOQOL-BREF. Low grade adverse events were related to Feelings about Side Effects ($\beta = -0.326$; $P = 0.007$).

Conclusions

Patients experiencing negative feelings about side effects have worse (HR)QoL. Additional care should be provided to prevent low grade adverse events.

INTRODUCTION

In patients with advanced stage lung cancer, the preservation of their well-being is warranted given their, in general, limited prognosis.[1,2] Chemotherapy may have a negative impact on patients' Health Related Quality of Life (HRQoL) due to side effects.[3] However, it is not well understood what aspect of chemotherapy causes this potential negative effect on QoL. The Cancer Therapy Satisfaction Questionnaire (CTSQ) is an instrument that assesses patients' expectations, their feelings about side effects and their satisfaction with therapy. Application of this questionnaire gives more insight in patients view on treatment.

Although several publications reported about patients' satisfaction with care,[4-6] patients' opinions related to side effects were not evaluated in these studies. Moreover, in a study by Rha et al. it was observed that clinicians underestimated the impact of side effects compared to patients. In addition, physicians rated different symptoms (i.e., nausea and vomiting) as most problematic than patients (i.e., fatigue and anorexia) did.[7] The CTSQ assesses the feelings patients have about treatment.[8] As such, the CTSQ could inform physicians about patients' treatment related opinions, which may facilitate the management of (HR)QoL. For instance, if a patient scores low on the Feelings about Side Effects domain of the CTSQ, this is a clear indicator that they are bothered by side effects. Subsequent identification and adequate management of the experienced side effects may offer opportunities to maintain (HR)QoL at an acceptable level.

However, the CTSQ may also be useful in the process of clinical decision-making. In many patients with advanced cancer, a physician's decision to start with treatment is related to a patient's functional status, comorbidity and potential toxicity,[9,10] whilst patients often focus on survival benefits [10,11] and may accept a decrease in QoL.[12] Moreover, patients with cancer would like to be involved in treatment decisions.[13] A considerable proportion (38.3%; N = 49) of patients with lung cancer preferred to have some input in treatment decision-making or would like shared treatment decisions. However, this was achieved in only 46.9% (N = 23) of the 49 cases.[14] Therefore, exploring a patient's treatment-related opinion is important as they could have a different understanding of survival rates and the impact of side effects on (HR)QoL than their physicians.

In previous studies, we and others have shown that the domains of the CTSQ (i.e., Expectations of Therapy, Feelings about Side Effects, Satisfaction with Therapy) are related to (HR)QoL.[15,16] In this study, we investigate which of the CTSQ domains are associated with (HR)QoL at the end of treatment in patients with advanced stage lung cancer and mesothelioma. In addition, we assess which underlying factors (i.e., sociodemographic and clinical variables) are associated with the CTSQ domain that is most often significantly related with (HR)QoL.

METHODS

Study population

PERSONAL is a prospective observational multi-center cohort study of patients with locally advanced or metastatic (i.e., stage IIIB or IV) nonsquamous non-small cell lung carcinoma (NSCLC) and unresectable mesothelioma treated with pemetrexed. Patients were recruited from October 2012 to November 2014 from three teaching hospitals (i.e., Erasmus University Medical Center, Amphia Hospital and Franciscus Gasthuis hospital) and a regional hospital (i.e., Bravis hospital). Patients were enrolled if they met the following criteria: they were aged eighteen years or older, had a cytological or histological confirmed diagnosis of advanced or metastatic (i.e., stage IIIB and IV) NSCLC or unresectable malignant pleural mesothelioma and were treated with at least four cycles of pemetrexed in combination with a platinum compound as first-line therapy or with at least four cycles of pemetrexed monotherapy as second-line therapy. Patients were excluded if they were not able to read Dutch or could not complete the questionnaires due to a physical or mental condition. Informed consent was obtained from all individual participants included in the study**.** All procedures were in accordance with the ethical standards of the institutional review board of the Erasmus University Medical Center in Rotterdam, The Netherlands (approval number MEC-2012-232) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Procedures

The WHOQOL-BREF and EORTC QLQ-C30 were completed by patients before the first cycle of chemotherapy, after the second cycle (day 7 to 14) and after the fourth cycle (day 14 to 21). The CTSQ was completed by patients after the fourth cycle of chemotherapy simultaneously with the (HR)QoL questionnaires. In addition, we collected sociodemographic information (i.e., age, sex, educational level, ethnicity, employment, partner status (i.e., living or not living together with a partner)), and clinical information (i.e., Eastern Cooperative Oncology Group (ECOG) performance status and cancer stage, type of tumor, line of therapy and tumor response). In the four weeks before completion of the CTSQ, the severity and number of different chemotherapy-related clinical adverse events that patients experienced were assessed at a weekly basis according to Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. The information regarding these adverse events was collected directly form patients during patient interviews and from medical records in the hospital information system.

Study measures

The CTSQ contains three domains covering 16 items: Expectations of Therapy (five items), Feelings about Side Effects (four items) and Satisfaction with Therapy (seven items). [15,8] Each item is scored on a Likert-scale from 1 (worst response) to 5 (best response). Four items are reverse coded. Domain scores range from 0 to 100, with a higher score

representing a better outcome. All patients completed the Dutch translation of the original English CTSQ.[16] Previous studies have assessed the psychometric properties in patients with different forms of cancer, including advanced stage lung cancer, and demonstrated good results.[15,16]

The WHOQOL-BREF [17,18] is a short version of the original WHOQOL-100.[19,20] It contains a General Facet (two items) and four domains that represent Physical Health (seven items), Psychological Health (six items), Social Relationships (three items), and Environment (eight items). Each item is scored on a Likert-scale from 1 (worst response) to 5 (best response). Domains of the WHOQOL-BREF are scored on a 4-20 scale and the General Facet on a 2-10 scale with higher scores indicating a better quality of life.[17] Previous studies have demonstrated satisfactory psychometric properties of the WHOQOL-BREF in patients with lung cancer [21] and in patients with chronic diseases or different forms of cancer [18] except for the Social Relationships domain.[21,18]

The EORTC QLQ-C30 is a cancer specific HRQoL instrument with demonstrated psychometric properties [22] and was originally developed with lung cancer patients. [23] It consists of 30 items and incorporates a Global Health Status/ QoL scale, five functional scales and a number of items assessing additional symptoms or problems. The functional scales represent Physical Functioning (five items), Cognitive Functioning (two items), Emotional Functioning (four items), Role Functioning (two items), and Social Functioning (two items). Each of the EORTC QLQ-C30 domains is scored on a 0-100 scale, with higher scores on the functional scales being indicative of better HRQoL, whereas higher scores on the symptom scales are reflective of worse symptoms.[23]

Statistics

Patient characteristics were analyzed with descriptive statistics. The Fisher's exact test was used to compare patients that completed the CTSQ and (HR)QoL questionnaires with those that did not on a selection of categorical clinical and sociodemographic variables**.** For the variables 'age' and 'grade 1 or 2 chemotherapy related clinical adverse events' the independent samples *t*-test was used. The Mann-Whitney *U* test was used for the variable 'grade 3 or 4 chemotherapy-related clinical adverse events'.

Multiple linear regression analyses were performed to identify the relationship between Expectations of Therapy, Feelings about Side Effects and Satisfaction with Therapy with (HR)QoL without prior simple linear regression analyses given the low number of independent variables. As no specific data has been reported in lung cancer, we expected each potential factor to show a medium effect size. According to Cohen, a correlation of 0.3 (or $R^2 = 0.09$) constitutes a medium effect.[24] Thus, given an effect size of $R^2 = 0.09$, a power of 0.80 and an alpha of 0.05, 69 patients were needed for our main analyses.

Subsequently, simple linear regression analyses were performed to assess the relationship between sociodemographic (i.e., age, sex, ethnicity, education, employment, partner status) and clinical variables (i.e., type of tumor, ECOG performance status, cancer stage and treatment response) and Expectations of Therapy, Feelings about Side Effects or Satisfaction with Therapy. Regression analyses were performed only on the independent variable (i.e., Expectations of Therapy, Feelings about Side Effects or Satisfaction with Therapy) that was most often significantly associated with (HR)QoL in the previous multiple regression analyses.

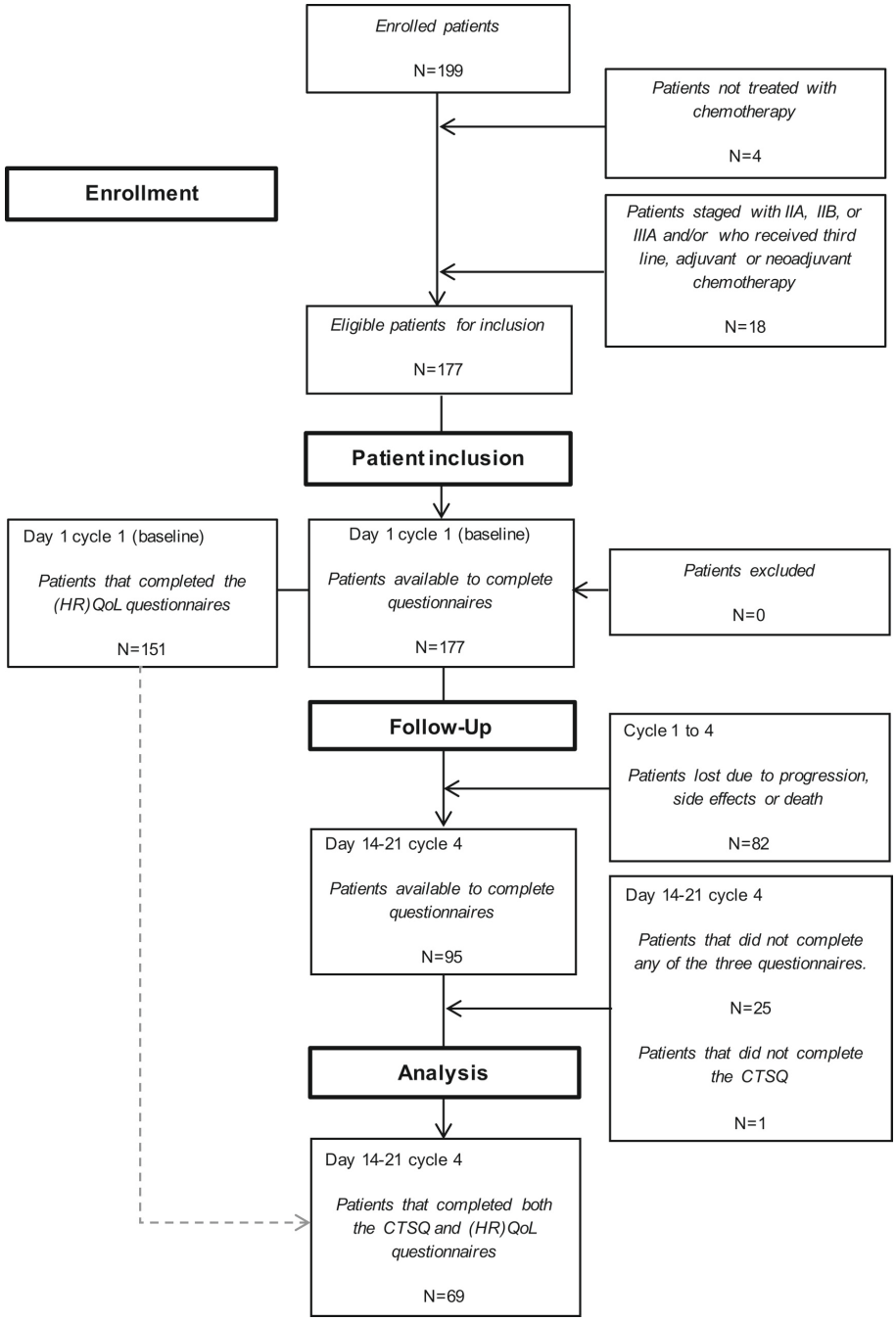
A *P*-value < 0.05 was considered to be statistically significant. All analyses were performed with IBM SPSS Statistics for Windows version 21.0.

RESULTS

Patient selection and characteristics

Of the 177 patients eligible for inclusion, 95 patients (54%) with stage IIIB or IV NSCLC or mesothelioma completed all four cycles of chemotherapy (figure 1). Twenty-six of these patients (27%) did not complete the (HR)QoL questionnaires and/or the CTSQ. These patients did not differ with the 69 patients (73%) who completed the questionnaires with regard to the different sociodemographic and clinical variables. Table 1 summarizes the characteristics of all 177 patients and the 69 patients used for the analyses.

Figure 1. Selection of patients



Abbreviations: N, number of patients; CTSQ, Cancer Therapy Satisfaction Questionnaire; (HR) QoL, (health related) quality of life

Table 1. Patient characteristics

Characteristic	All patients (N = 177)	Patients that completed all questionnaires (N = 69)	Patients that did not complete (all) questionnaires (N = 26)	*P*-value*
Age, years				
Mean (SD)	63.5 (9.0)	62.7 (8.0)	64.4 (9.8)	0.38
Min, max	37, 83	45, 79	37, 78	
Sex				
Male	94 (53.1)	38 (55.1)	13 (50.0)	0.82
Female	83 (46.9)	31 (44.9)	13 (50.0)	
Ethnicity				
Caucasian	167 (94.4)	64 (92.8)	24 (92.3)	1.00
Other	10 (5.6)	5 (7.2)	2 (7.7)	
Education[a]				
Low	113 (63.8)	51 (73.9)	18 (69.2)	0.75
High	32 (18.1)	13 (18.8)	3 (11.5)	
Unknown	32 (18.1)	5 (7.2)	5 (19.2)	
Employment				
Yes	39 (22.0)	20 (29.0)	4 (15.4)	0.41
No	112 (63.3)	48 (69.6)	17 (65.4)	
Unknown	26 (14.7)	1 (1.4)	5 (19.2)	
Partner status[b]				
Yes	123 (69.5)	59 (85.5)	15 (57.7)	0.18
No	28 (15.8)	9 (13.0)	6 (23.1)	
Unknown	26 (14.7)	1 (1.4)	5 (19.2)	
Cancer stage[c]				
Locally advanced (IIIB)	21 (11.9)	5 (7.2)	2 (7.7)	0.89
Metastatic (IV)	147 (83.1)	60 (87.0)	22 (84.6)	
Other	9 (5.1)	4 (5.8)	2 (7.7)	
Type of tumor[c]				
Adenocarcinoma	160 (90.4)	63 (91.3)	21 (80.8)	0.17
Large cell carcinoma, mesothelioma, other	17 (9.6)	6 (8.7)	5 (19.2)	
Line of therapy				
First line	161 (91.0)	64 (92.8)	24 (92.3)	1.00
Second line	16 (9.0)	5 (7.2)	2 (7.7)	
ECOG performance status				
Grade 0 or 1	155 (87.6)	66 (95.7)	26 (100.0)	0.20
Grade 2 or higher	21 (11.9)	1 (1.4)		
Unknown	1 (0.6)	2 (2.9)		

Table 1. Patient characteristics (continued)

Characteristic	All patients (N = 177)	Patients that completed all questionnaires (N = 69)	Patients that did not complete (all) questionnaires (N = 26)	*P*-value*
Grade 1 or 2 chemotherapy related clinical adverse events				
Mean		9.2 (3.2)	8.5 (4.0)	0.33
Min, max		3, 19	1, 18	
Unknown		1 (1.4)		
Grade 3 or 4 chemotherapy related clinical adverse events				
Median		0.0	0.0	0.93
Min, max		0, 4	0, 5	
Unknown		1 (1.4)		

Values are given in numbers (percentages) unless stated otherwise.
*P-values describe differences observed with Fisher's exact test for all categorical variables and with the independent *t*-test and Mann-Whitney U test for the variables 'age' and 'grade 1 or 2 chemotherapy related clinical adverse events' and the variable 'grade 3 or 4 chemotherapy related clinical adverse events', respectively.
[a]Low education: persons whose highest level of education is primary education, lower general education or lower vocational education. High education: persons whose highest level of education is higher general education, higher vocational education or university.
[b]Partner status: living or not living together with a partner
[c]Measured at baseline
Abbreviations: n, number of patients; SD, standard deviation; ECOG, Eastern Cooperative Oncology Group

CTSQ domain scores

The median score of the Expectations of Therapy domain was 55.0 (Inter Quartile Range (IQR) 38.8) and that of the Feelings about Side Effects domain was 56.3 (IQR 42.2). Satisfaction with Therapy had a median score of 82.1 (IQR 17.9).

(HR)QoL scale and domain scores

Table 2 demonstrates the scores of the different scales and domains of the EORTC QLQ-C30 and WHOQOL-BREF. For the WHOQOL-BREF, mean domain scores of the normally distributed domains were 13.6 (SD 3.1) for Physical Health and 16.1 (SD 2.1) for Environment. Median scores of the non-normally distributed domains were 13.7 (IQR 4.0) and 15.3 (IQR 2.7) for, respectively, Psychological Health and Social Relationships. The median score of the General Facet was 6.0 (IQR 3.0). Median scores for the different scales of the EORTC QLQ-C30, including the Global Health Status/QoL scale, ranged from 50.0 (IQR 50.0) to 83.3 (IQR 33.3).

Table 2. Results of the WHOQOL-BREF and EORTC QLQ-C30

Questionnaires	N	Min, Max	Mean	SD	Median	IQR
WHOQOL-BREF						
Overall QoL/General Health	69	3.0, 10.0	6.2	1.7	6.0	3.0
Physical Health	67	6.9, 20.0	13.6	3.1	13.7	4.1
Psychological Health	68	10.0, 18.7	14.1	2.2	13.7	4.0
Social Relationships	68	6.7, 20.0	15.5	2.4	15.3	2.7
Environment	67	11.0, 20.0	16.1	2.1	16.3	3.5
EORTC QLQ-C30						
Global Health Status/QoL	67	8.3, 100.0	57.3	24.6	66.7	41.7
Physical Functioning	69	13.3, 100.0	65.1	22.4	66.7	33.3
Role Functioning	69	0.0, 100.0	53.1	33.9	50.0	50.0
Emotional Functioning	68	16.7, 100.0	75.1	21.5	75.0	25.0
Cognitive Functioning	68	0.0, 100.0	77.0	24.4	83.3	33.3
Social Functioning	67	0.0, 100.0	74.6	26.8	83.3	33.3
CTSQ						
Expectations of Therapy	68	15.0, 100,0	58.1	23.8	55.0	38.8
Feelings about Side Effects	69	12.5, 100	53.7	25.3	56.3	42.2
Satisfaction with Therapy	69	42.9, 100	79.6	13.1	82.1	17.9

Abbreviations: WHOQOL-BREF, World Health Organization Quality of Life-BREF questionnaire; EORTC QLQ-C30, European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30; CTSQ, Cancer Therapy Satisfaction Questionnaire; n, number of patients; SD, standard deviation; IQR, interquartile range

Adverse events

Table 3 describes the occurrence of different chemotherapy-related clinical adverse events according to their grade. Fatigue was the most frequently experienced adverse event with 87.0% of patients reporting fatigue followed by nausea (71.0%) and anorexia (63.8%).

The association of the CTSQ with (HR)QoL

For all domains and scales of the (HR)QoL questionnaires, except for the WHOQOL-BREF domain Social Relationships, the Feelings about Side Effects domain was significantly associated with (HR)QoL (Table 4). Positive feelings about side effects were associated with higher (HR)QoL scores whereas negative feelings about side effects related with lower (HR)QoL scores. In contrast, high Expectations of Therapy were only significantly associated with increased Psychological Health and high Satisfaction with Therapy solely with increased Global Health Status/Quality of Life. No other associations between the (HR)QoL questionnaires and the Expectations of Therapy and Satisfaction with Therapy domain were found.

Table 3. 10 most frequently reported adverse events according to CTCAE 3.0

Adverse events	N	Grade 1 or 2	Grade 3 or 4
Total	69		
Fatigue	60	53 (76.8)	7 (10.1)
Nausea	49	46 (66,7)	3 (4.3)
Anorexia	44	42 (60.9)	2 (2.9)
Altered taste	38	38 (55.1)	0 (0.0)
Mucositis	34	33 (47.8)	1 (1.4)
Dry skin	30	30 (43.5)	0 (0.0)
Constipation	30	29 (42.0)	1 (1.4)
Neuropathy sensory	25	25 (36.2)	0 (0.0)
Dizziness	24	24 (34.8)	0 (0.0)
Rash	21	21 (30.4)	0 (0.0)

Values are given in numbers (percentages)
Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; N, number of patients

Table 4. Results of the multiple regression analyses for the WHOQOL-BREF and EORTC QLQ-C30 domains/scales with the CTSQ domains as variables

Variables	N	B	SE	β	*P*-value	95% CI for B	R^2
				WHOQOL-BREF			
				Overall QoL/General Health			
ET	68	0.010	0.008	0.143	0.199	-0.005, 0.026	0.258
FSE		0.031	0.008	0.472	<0.001*	0.016, 0.046	
SWT		0.003	0.015	0.026	0.824	-0.027, 0.033	
				Physical Health			
ET	66	0.017	0.014	0.135	0.217	-0.010, 0.045	0.309
FSE		0.063	0.014	0.527	<0.001*	0.036, 0.090	
SWT		0.005	0.027	0.022	0.851	-0.048, 0.059	
				Psychological Health			
ET	67	0.020	0.009	0.212	0.032*	0.002, 0.038	0.439
FSE		0.050	0.009	0.578	<0.001*	0.033, 0.068	
SWT		0.015	0.017	0.091	0.377	-0.019, 0.050	
				Social Relationships			
ET	67	0.015	0.013	0.144	0.256	-0.011, 0.041	0.044
FSE		0.014	0.013	0.141	0.286	-0.012, 0.039	
SWT		0.002	0.025	0.013	0.925	-0.048, 0.052	
				Environment			
ET	66	0.011	0.011	0.121	0.310	-0.010, 0.032	0.166
FSE		0.031	0.010	0.364	0.004*	0.010, 0.052	
SWT		0.008	0.021	0.052	0.682	-0.033, 0.050	

Table 4. Results of the multiple regression analyses for the WHOQOL-BREF and EORTC QLQ-C30 domains/scales with the CTSQ domains as variables (continued)

Variables	N	B	SE	β	*P*-value	95% CI for B	R^2
EORTC QLQ-C30							
Global Health Status/Quality of Life							
ET	66	-0.018	0.109	-0.017	0.869	-0.237, 0.200	0.339
FSE		0.425	0.106	0.442	<0.001*	0.212, 0.637	
SWT		0.478	0.210	0.257	0.026*	0.059, 0.898	
Physical Functioning							
ET	68	0.154	0.103	0.162	0.142	-0.053, 0.360	0.275
FSE		0.376	0.101	0.421	<0.001*	0.174, 0.577	
SWT		0.237	0.200	0.137	0.240	-0.162, 0.635	
Role Functioning							
ET	68	0.179	0.147	0.125	0.227	-0.114, 0.472	0.360
FSE		0.817	0.143	0.607	<0.001*	0.531, 1.102	
SWT		-0.192	0.283	-0.074	0.499	-0.758, 0.373	
Emotional Functioning							
ET	67	0.027	0.105	0.030	0.795	-0.182, 0.237	0.190
FSE		0.347	0.102	0.412	<0.001*	0.144, 0.550	
SWT		0.085	0.201	0.052	0.672	-0.316, 0.487	
Cognitive Functioning							
ET	67	-0.043	0.126	-0.041	0.737	-0.295, 0.209	0.099
FSE		0.315	0.122	0.329	0.012*	0.071, 0.559	
SWT		-0.222	0.242	-0.120	0.361	-0.705, 0.260	
Social Functioning							
ET	66	0.019	0.135	0.017	0.887	-0.251, 0.290	0.149
FSE		0.414	0.131	0.395	0.003*	0.151, 0.677	
SWT		-0.061	0.260	-0.030	0.815	-0.581, 0.459	

*_P_-values of < 0.05

Abbreviations: CTSQ, Cancer Therapy Satisfaction Questionnaire; WHOQOL-BREF, World Health Organization Quality of Life-BREF questionnaire; EORTC QLQ-C30, European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30; ET, Expectations of Therapy; FSE, Feelings about Side Effects; SWT, Satisfaction with Therapy

Factors associated with Feelings about Side Effects

In the simple regression analyses, only low-grade chemotherapy-related clinical adverse events (i.e., grade 1 or 2 adverse events) were significantly associated with Feelings about Side Effects ($P < 0.01$) (Table 5). No other relationship was observed.

Table 5. Results of the simple regression analyses for the CTSQ FSE domain score

	FSE						
	N	B	SE	β	*P*-value	95% CI for B	R^2
Age	69	-0.134	0.383	-0.043	0.728	-0.899, 0.631	0.002
Sex	69	-4.968	6.132	-0.099	0.421	-17.206, 7.271	0.010
Ethnicity: Caucasian/other	69	-8.092	11.780	-0.084	0.494	-31.606, 15.421	0.007
Type of tumor: adenocarcinoma/other	69	14.368	10.734	0.161	0.185	-7.058, 35.795	0.026
ECOG performance score: 0 or 1/higher	69	-23.878	12.787	-0.222	0.066	-49.400, 1.644	0.049
Cancer stage: IIIB/IV	69	9.896	9.020	0.133	0.277	-8.108, 27.899	0.018
Education: low/high	64	0.129	7.730	0.002	0.987	-15.323, 15.581	0.000
Employment: yes/no	68	8.238	6.659	0.151	0.220	-5.056, 21.532	0.023
Partner status: yes/no	68	-6.128	9.025	-0.083	0.499	-24.147, 11.890	0.007
Tumor response: complete and partial response/stable or progressive disease	69	5.525	6.466	0.104	0.396	-7.382, 18.432	0.011
Grade 1 or 2 chemotherapy clinical AE's	68	-2.543	0.907	-0.326	0.007*	-4.354, -0.733	0.107
Grade 3 or 4 chemotherapy clinical AE's	68	1.527	2.984	0.063	0.610	-4.430, 7.484	0.004

*P-values <0.05
Abbreviations: CTSQ, Cancer Therapy Satisfaction Questionnaire; FSE, Feelings about Side Effects; ECOG, Eastern Cooperative Oncology Group; AE, adverse event

DISCUSSION

Preservation of (HR)QoL is an important goal during chemotherapy considering that patients with advanced stage lung cancer have a limited prognosis.[1,2] Therefore, identification of patients at risk for decreases in (HR)QoL due to treatment may offer opportunities for improvement. We observed, using a validated scoring system to determine patients' judgement about therapy in different domains, that negative feelings about side effects were associated with decreased (HR)QoL. Especially for patients experiencing low grade adverse events at a regularly basis, this seems important.

Of the three CTSQ domains, Expectations of Therapy, Satisfaction with Therapy and Feelings about Side Effects, the last one was associated with (HR)QoL. In contrast, Satisfaction with Therapy was only related with the Global Health State/QoL scale of

the EORTC QLQ-C30. A reason for this may be that none of the seven items of the Satisfaction with Therapy domain except one (i.e., chemotherapy was worth taking even with side effects), refer to adverse events or (HR)QoL. Moreover, patients may associate Satisfaction with Therapy with treatment response and survival and not with particular aspects of (HR)QoL. Since the Feelings about Side Effects domain was most often related to (HR)QoL, we studied the underlying factors of this domain. It appeared that the number of different grade 1 or 2 chemotherapy-related clinical adverse events were significantly associated. As these were often experienced on a regularly basis over longer periods of time, vigorous management of them is warranted. Therefore, it is recommended that health care providers have high awareness and consequently check the occurrence and impact of low-grade adverse events as our results clearly demonstrate that patients are bothered by them. In contrast, no relation with chemotherapy related clinical grade 3 or 4 adverse events was found. This may be because high-grade toxicities were much less experienced in this patient cohort and that the study lacked power. In addition, patients completed the CTSQ after four cycles of chemotherapy. Patients that experienced severe complications may have interrupted chemotherapy and were therefore not included.

Earlier, it was found that HRQoL issues were more often discussed between doctors and patients when the EORTC QLQ-C30 was completed by patients than when this was not the case.[13] All participating physicians and 87% of patients were interested in the persistent use of the questionnaire. These results demonstrated the value of questionnaires in oncological practice. However, application of such an instrument does not provide information about what people think and feel about their treatment. Moreover, (HR)QoL instruments are often more extended than the sixteen items of the CTSQ and require more time to be completed which hampers their application during clinical practice. Also, simply the registration of adverse events does not provide information about the extent to which patients are bothered by them. Therefore, considering the results of this study, we advocate the use of the four items of the Feelings about Side Effects domain of the CTSQ as this seems more time efficient and patient friendly.

In the present study, feelings about side effects were more often significantly associated with (HR)QoL than satisfaction with therapy. This is an important observation that may be used by physicians and patients when making treatment decisions. Although several reports revealed that patients may accept a decrease in QoL or treatment related toxicity given a possible survival benefit,[11,12] a systematic review demonstrated that most cancer patients (>50%) in the included studies required moderate survival benefits to make chemotherapy and its risk for toxicity acceptable.[25] Given that, according to our results, patients with negative feelings about side effects could have low (HR)QoL and that prognosis is limited in advanced stage lung cancer, we propose that the CTSQ results of previously treated patients may be used to help newly diagnosed patients at risk for adverse events (i.e., decreased performance score, significant comorbidity) in making treatment decisions. For instance, if a considerable proportion of patients who received

chemotherapy were often hampered by adverse events according to their CTSQ results, newly diagnosed patients with a limited prognosis could take knowledge of these results and make a more considered treatment decision. In such a way, CTSQ results are handled in a similar manner during decision-making as treatment response and survival rates.

Satisfaction with therapy was significantly associated with the Global Health Status/QoL scale of the EORTC QLQ-C30 whereas this was not observed for the General Facet of the WHOQOL-BREF. It is possible this observation is merely due to the idiosyncrasies of the data at hand or simply chance. Also, the relatively small number of patients or selection bias may be responsible for this. In addition, patients may consider occurrence and management of adverse events when they evaluate satisfaction (although this is not directly described by the items that form the Satisfaction with Therapy domain). Given that adverse events can directly affect a patient's HRQoL, the interest of health care professionals for adverse events could influence the relation of Satisfaction with Therapy score with the Global Health Status/QoL scale. For instance, adequate management of adverse events may lead to high patient satisfaction with their care. This may result in increased Satisfaction with Therapy scores. Given that treatment of adverse events could also enhance HRQoL, increased patient satisfaction with care may result in the observation of an association between Satisfaction with Therapy and Global Health Status/QoL. Expectations of Therapy were significantly associated with Psychological Health. Besides the possibility of related constructs, reasons for this may be related to coping. For instance, the coping capacity three months after baseline in patients with advanced stage lung cancer was a predictor for HRQoL.[26] Patients with good coping capacity may have high expectations and may value (HR)QoL more positively than those with few coping capabilities. In addition, coping style may also be of influence as patients that demonstrate 'a fighting spirit' may report higher expectations than those that have no hope of a good outcome. Moreover, non-acceptance of the diagnosis and/or prognosis could result in a paradoxical expression of high expectations.

Some limitations of this study have to be addressed. First, the included patients were not asked for their motivation to receive chemotherapy, nor was determined which factors could influence patients' treatment preferences and opinions. This limited us, together with the observational design of this study and the calculation of associations, to investigate causal relationships between the CTSQ and the (HR)QoL questionnaires. As the present study is part of a larger project in which patients' motivations were not routinely assessed, we could not provide this information. However, a review that evaluated cancer patients' preferences for adjuvant therapy reported that in addition to treatment benefit and toxicity, personal experience of the treatment and having dependents (e.g., children) were important determinants of patients' preferences.[27] Acquiring such information is of importance as it may help physicians to plan their communication strategy towards patients and provides opportunities for personalized treatment.

Second, patients treated with less than four cycles of chemotherapy were not included in this study. These patients dropped out due to progression or adverse events. Given that they had to discontinue treatment with chemotherapy earlier than expected, it is possible they could have valued satisfaction with therapy more often as important. This could have confounded our results and may explain why Satisfaction with Therapy in our study was not associated with (HR)QoL. However, other observational studies in patients with advanced stage lung cancer have experienced similar difficulties with patients dropping out during treatment. In addition, we observed consistent findings regarding the associations of the CTSQ domains with (HR)QoL. Therefore, the findings of the present study contribute to the results of the limited number of reports that discussed the relation of patients' disease and treatment related opinions with (HR)QoL.

Third, the observed *R* squares of the simple regression analyses for the Feelings about Side Effects domain in Table 5 were relatively small. To demonstrate with reasonable power that the other predictors were truly not a determinant of Feelings about Side Effects domain score would require the inclusion of many more patients. Given that the *R* square of the analysis in which low grade adverse events were associated with Feelings about Side Effects score was relatively high, suggesting an acceptable power, the result of this analysis remains of importance.

In conclusion, we demonstrated that patients with advanced stage lung cancer who experience strong negative feelings about side effects have a decreased (HR)QoL. Our findings demonstrate that low grade adverse events are of importance for patients' feelings about side effects. Therefore, it is recommended that in clinical practice, physicians facilitate vigorous management of low-grade adverse events to enhance the (HR)QoL of patients. In addition, the observed results may aid physicians and patients in making treatment decisions.

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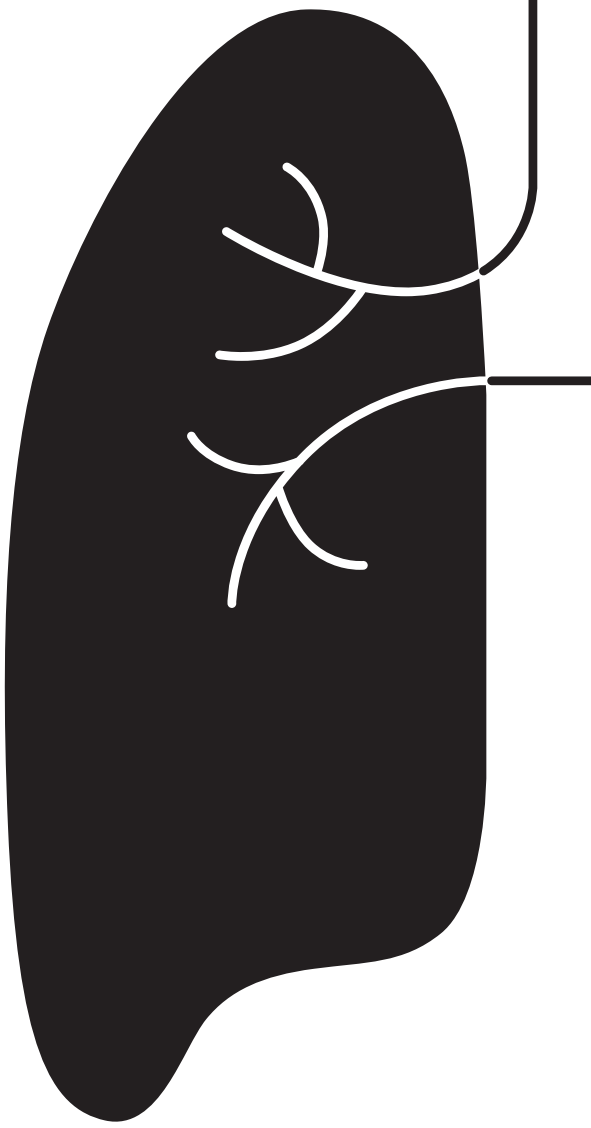


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CHAPTER 12

General discussion

In personalized medicine, the aim is to provide specific treatments tailored to individual patients based on the presence of predictive biomarkers or other determinants which indicate sensitivity to corresponding therapies and/or lower toxicity risk. In this way, patients have the highest chance of deriving benefits from the treatment. In patients with non-small-cell lung cancer (NSCLC) this tailored treatment was introduced more than 10 years ago with the identification of a genetic abnormality called EGFR, which was present in about 8% of the western population and for which an EGFR targeted therapy was available. The increasing identification of new genomic drivers in lung cancer, and the emergence of selective molecular targeted agents and more recently immunotherapy have changed the treatment of NSCLC dramatically to a more personal approach. However, in a significant proportion of patients no specific predictive marker is found and those patients are treated with a so-called untargeted treatment: chemotherapy.

In NSCLC, pemetrexed is a frequently used and active chemotherapeutic agent. Pemetrexed-based chemotherapy was introduced as a treatment against lung cancer since the early 2000s. pemetrexed is still – and presumably will be for the next years – the backbone of anticancer treatment in the majority of patients with nonsquamous NSCLC who lack targetable molecular abnormalities and marked sensitivity to immunotherapy as single agent. As discussed in **Chapter 1**, although substantial progression has been made in the last decade to tailor anticancer treatment in advanced NSCLC by using targeted therapy and immunotherapy based on the presence of targetable genetic aberrations or a high PD-L1 expression respectively, no such predictive markers are known for patients treated with chemotherapy.

Identification of patients who are prone to respond to pemetrexed and who are at risk of (severe) treatment-related toxicities is mandatory to make pemetrexed-based therapy more targeted and more personalized. Of course, the ultimate goal would be to find a predictive biomarker for the selection of the ideal patient population benefitting from pemetrexed treatment. In this thesis, we focused on determining mechanisms of treatment resistance and toxicity, which is a necessary puzzle to solve before a potential biomarker can be determined.

Another aspect of personalised treatment is incorporating shared decision-making in clinical practice. The aim here is to really have the patient deciding her or his preference in treatment options. However, there is a lack of understanding what makes patients (un) satisfied with their treatment. We determined patients' treatment satisfaction and their feelings about adverse effects during pemetrexed-based chemotherapy.

PREDICTION OF TREATMENT EFFECTIVENESS AND TOXICITY

Since the introduction of pemetrexed treatment in lung cancer, much effort has been put in the exploration of the underlying mechanisms determining tumor response to pemetrexed. Overexpression of thymidylate synthase (TS), the main target enzyme of pemetrexed, is the most extensively studied drug-resistance mechanism involved in pemetrexed chemo-resistance. Both gene and protein expression of TS have been explored as potential predictive biomarkers of response to pemetrexed therapy.[1–6] Although preclinical and retrospective data were promising, this marker never found its way into practice. A randomized phase II trial was performed in which patients were stratified according to TS-negative and TS-positive tumors and received pemetrexed/cisplatin or gemcitabine/cisplatin, to provide clear answers about the prognostic or predictive role of thymidylate synthase.[7] Regardless of the received treatment, patients with TS-negative tumors survived longer than patients with TS-positive tumors, suggesting a prognostic role of TS. Although the interaction between treatment allocation and TS expression status was significant for the objective response rate, it was not for progression-free survival. Moreover, there was no significant difference in response rate of pemetrexed/cisplatin between TS-negative and TS-positive groups. Thus, no indisputable evidence was found for the predictive abilities of TS protein expression.

Although TS has been considered as the main target of pemetrexed, it is known that pemetrexed also hinders DNA synthesis by binding to other intracellular targets involved in the purine synthesis.[8,9] Based on the expression of the genes encoding different target enzymes of pemetrexed, including but not limited to TS, a gene expression signature with correlated genes was used to classify primary tumor samples of patients with NSCLC who underwent surgery with curative intent as predicted responders and non-responders to pemetrexed.[10] In **Chapter 2**, protein expression of these genes was measured using commercially available immunohistochemical (IHC) staining antibodies, and ultimately a prediction model for pemetrexed response based on IHC staining scores of Enhancer of zeste homolog 2 (EZH2) and Microtubule Nucleation Factor (TPX2) was built. However, we were not able to validate the results in an independent retrospective cohort of patients with advanced NSCLC who were actually treated with pemetrexed. In these patients, the sensitivity and positive predictive value of the prediction model were disappointing. Thus, patients actually responding to pemetrexed were not identified by the model and predicted responders were actually non-responders in practice. Tumor heterogeneity may have contributed to the failure of this model, as the model was built using protein expressions in primary tumors of patients with stage I/II disease and subsequently tested in primary tumors or metastases of patients with an advanced stage. Comparable to studies examining the role of TS protein expression, possibly the used IHC assays and the semi-quantitative scoring mechanism lacked sensitivity to detect clinically relevant differences in protein expression. Currently, we also recognize this issue with the use

of IHC stainings to determine the PD-L1 tumor protein score. Although it is considered the best biomarker to date and used to guide treatment decisions whether to treat NSCLC with immunotherapy monotherapy (PD-L1 high) or combined with chemotherapy (PD-L1 low), it is clear that PD-L1 immunochemistry score cut-offs are arbitrary and nondefinitive.[11,12] Furthermore, recent data extracted from the nationwide Dutch cyto- and histopathology registry, showed substantial interlaboratory variation in reported PD-L1 positivity.[13]

Compared to the measurement of protein expression, genotyping procedures are more robust and are less prone to variation by technical aspects and human interference. Additionally, besides target enzymes other processes might play a role in the effectiveness and toxicity of pemetrexed treatment. Therefore, another approach to explore driving mechanisms of innate resistance of tumors and toxicity to pemetrexed is examining germline alterations of genes involved in the biological mechanism of pemetrexed including target enzymes, but also metabolic and cell transport enzymes. Earlier research primarily focused on genotyping the main target TS and was often performed in small cohort studies and in patients who already received prior treatment. In **Chapter 3** we observed that a single nucleotide polymorphism (SNP) in *ATIC*, one of the target enzymes of pemetrexed, was both associated with the occurrence of (severe) toxicities and worse overall survival (OS) in patients who received pemetrexed-based first-line treatment. The absent association between the *ATIC* SNP and treatment response and progression-free survival (PFS) but the present association with dose reductions and less maintenance treatment, generates the hypothesis that severe toxicity might be the cause of worse overall survival. As an example in the field of clinical oncology, reduced doses of fluoropyrimidine are administered in patients with certain genotypes of *DPYD* associated with severe toxicity.[14] However, such clinical implementation of structural *ATIC* genotyping before start of treatment with pemetrexed is unlikely in the near future. Firstly, our findings should be validated in an external cohort. Preferably, a comparator arm should be used without pemetrexed treatment to exclude a prognostic role of certain *ATIC* variants. If a comparator arm is chosen with the same backbone as the pemetrexed treatment arm it is possible to discriminate the role of ATIC in overall toxicity and pemetrexed-related toxicity. Secondly, it is questionable whether upfront dose reductions of pemetrexed in patients with an unfavorable genotype of *ATIC* will result in less toxicity while maintaining treatment effectiveness.

One of the major difficulties that complicates biomarker detection based on the presence of an alteration in a specific gene or the protein it encodes for, is the so called spatial and temporal tumor heterogeneity: due to ongoing mutational changes in a tumor and selective pressure of a treatment, molecular characteristics of a tumor can change over time. Additionally, the primary tumor may differ from the metastastic lesions, metastatic lesions may differ from each other and in a single lesion heterogeneous areas are present.[15–17] Analysing cell free DNA (cfDNA) in blood plasma, the so called “liquid biopsy”,

has been proposed to cover the issue of tumor heterogeneity and has the additional advantage to be less invasive than (sequential) tumor biopsies.[18] Developments in genotyping techniques now offer the possibility to perform high throughput analyses of the whole exome or genome using next-generation sequencing.[19,20] Our work in **Chapter 4** contributed to the current knowledge of acquired resistance to pemetrexed in NSCLC and showed new insights in *de novo* gene mutations and variant frequency alterations due to selective pressure of pemetrexed. Whole exome sequencing (WES) analysis performed on cfDNA retained from blood plasma, showed significant increases at progressive disease compared to baseline of variant allele frequencies of two variants in genes (*GGH* and *MTR*) involved in the biological process of the folic acid cycle, in which pemetrexed interferes. We also found *de novo* variants and recurrently significantly increased variants of *MUC* genes at progressive disease. Increased expression of *MUC* genes may lead to an immunosuppressive environment, which poses an interesting question whether the observed *MUC* variants contribute to pemetrexed resistance in this manner. However, the results of our study in a small sample size should be interpreted with caution and should only be considered as hypothesis generating. *MUC* genes are large and thus the chance of false discovery due to missed germline variants, passenger variants and sequencing errors should not be underestimated. Our study also underlined another important problem with the identification of cfDNA derived from tumors, in other words circulating tumor DNA (ctDNA): although all patients had KRAS positive tumors and they had a large tumor burden, there was a significant lack of sensitivity to detect the *KRAS* mutation in plasma both by digital PCR and NGS. Levels of detectable circulating tumor DNA are not only impacted by tumor burden, but also by anatomic location and genomic genotype.[21] However, it means that other important *de novo* variants or increase in variant allele frequencies might not be picked up as well by these techniques.

Despite the preliminary results of our work in **Chapter 4**, we believe that plasma mutation analysis holds promise for the future to improve care of patients treated with pemetrexed-based therapy. A liquid biopsy might be more sensitive than tumor genotyping in discovering mutation diversity.[22,23] Moreover, its non-invasive nature and the possibility to monitor treatment response and detect tumor progression earlier than by imaging are other important advantages.[24] Therefore, plasma tumor genotyping could be a promising future tool in clinical practice if high sensitivity and specificity are warranted. In fact, for patients with oncogene-driven advanced NSCLC the use of a liquid biopsy is sometimes already preferred above tissue analysis for evaluation of mechanisms of resistance ("plasma-first" instead of "tissue-first").[25] Recent research also showed promising utility of (the dynamics of) circulating tumor DNA to predict the treatment response to immune checkpoint inhibitors.[26,27] In the era of combined treatment of pemetrexed-based chemotherapy with immunotherapy, the finding of *MUC* genes possibly playing a role in pemetrexed resistance through changes into a more immunosuppressive microenvironment is worthy of further investigation. Whether

these variants in *MUC* genes play a role in the synergic pathway of pemetrexed and immune checkpoint inhibitors, could be further evaluated in patients receiving the combination treatment. In future studies, matched normal DNA should be used to guarantee specificity.

RENAL TOXICITY AND PEMETREXED

In the last decade more and more treatment modalities have become available for advanced NSCLC, resulting in different possible combinations and sequential lines of treatment with chemotherapy, targeted agents and immune checkpoint inhibitors. These recent therapeutic advances have led to a significant reduction of mortality from stage IV NSCLC.[28] But, to take optimal advantage of all these treatment possibilities in terms of survival benefit, patients need to be able to undergo multiple lines of treatment.[29] An important condition to start and maintain most of these treatments is an adequate renal function. The pivotal PARAMOUNT trial leading to approval of pemetrexed maintenance treatment, only reported treatment-related renal impairment in <10% of the patients and in <5% this led to discontinuation of maintenance treatment.[30] However, it is known that the incidence of treatment-related toxicity is often underestimated in clinical trials compared to real-world data.[31] In **Chapter 5**, this was also acknowledged for renal impairment due to pemetrexed-based chemotherapy. We found that 30% of the patients developed acute kidney disease during pemetrexed maintenance treatment. More disturbing, this had important consequences in half of these patients as they developed chronic kidney disease and/or were forced to discontinue pemetrexed maintenance. Without a pemetrexed monotherapy comparator arm, we could not exclude that the platinum, used during four cycles induction treatment, contributed to the renal impairment found during maintenance. At the time of this study, immune checkpoint inhibitors made their entrance as a new treatment modality of NSCLC after showing superiority to docetaxel as a second-line therapy.[32,33] In the light of this development, the findings of the frequent occurrence of renal toxicity with clinical consequences during pemetrexed treatment in our study gained greater importance. The margin to maintain an acceptable renal function is smaller if renal injury is already suboptimal before start of this line of treatment, thus a further decrease of renal function due to the potential nephrotoxic immune checkpoint inhibitors (or due to whatever other reason) could jeopardize treatment with these agents.

Renal impairment can be a precursor and modifier of other toxicities. For drugs that are eliminated renally, like pemetrexed, one could hypothesize that the systemic exposure to the drug will increase with renal impairment. The current dosing strategy of pemetrexed is based on the body surface area (BSA), following the idea that small patients need a lower dose than large patients to maintain the same systemic exposure. However, this mechanism has been shown to be outdated for many anticancer drugs.[34] In **Chapter 6**, we have reported that – in contrast to BSA - a patient's renal function

expressed as the estimated glomerular filtration rate (eGFR) significantly contributes to the reduction of the interindividual variation of renal clearance of pemetrexed. Severe haematological and clinical chemotherapy-related adverse events were more common in patients with a higher systemic exposure to pemetrexed. With the current BSA-based dosing strategy, large patients and patients with renal impairment are more prone to treatment-related severe toxicity. Compared to the BSA-based dosing strategy, we were able to demonstrate a reduction of the interindividual variation in systemic exposure if more rational dosing strategies would be used, like eGFR-based or flat-fixed with a dose reduction of pemetrexed in renally impaired patients (eGFR < 60). These results point out that the current dosing strategy of pemetrexed is far from ideal with regard to treatment-related toxicity. The question remains whether effectiveness endpoints will not be negatively influenced, when the dose of pemetrexed – and thus Cmax – is lower (what definitely occurs in a substantial number of patients with the other two treatment strategies). Therefore, a head-to-head comparison of the current BSA-based dosing strategy against an alternative dosing strategy would be needed. In **Chapter 7**, we investigated what would be the minimum number of blood withdrawals needed to accurately assess the pharmacokinetics of pemetrexed. We discovered that a limited sampling schedule with four sampling times at 0.5-2-4-8 hours resulted in an acceptable estimation of pemetrexed clearance (a proxy of total systemic exposure). Compared to the seven sampling times used in **Chapter 6**, this is - most importantly - less burdensome for the patients but also less demanding and costly for investigators. Currently, the limited sampling schedule is used in the IMPROVE-II randomized trial (ClinicalTrials.gov Identifier NCT03655821) investigating the renal-dosing strategy compared to the BSA-based strategy. Unfortunately, the main endpoints are pharmacokinetic parameters and treatment effectiveness is not taken into account. Thus, despite the outcome of this trial, we believe at this point there will not be substantial evidence to change the dosing strategy.

In the meantime, we should handle renal toxicity during pemetrexed treatment to the best of our abilities. The results of **Chapter 5** gave us more insight into which patients are at higher risk to develop renal impairment during pemetrexed maintenance treatment. Patients with an already decreased renal function before the start of induction treatment combined with platinum and patients with a decline in renal function during this induction treatment should be monitored closely. Although the association between the number of maintenance cycles and the development of renal injury was not statistically significant, others demonstrated that cumulative pemetrexed dose increased the risk of nephrotoxicity.[35,36] Probably better than only monitoring closely would be to proactively act upon the knowledge that these patients are more prone to this toxicity. Besides preventing other risk factors for nephrotoxicity (like the use of nephrotoxic agents, hypovolemia) one should consider hyperhydration regimens, already a standard of care procedure with the nephrotoxic agent cisplatin.[37] The physiologic rationale is to lower pemetrexed half-life, urinary pemetrexed concentrations and the proximal

tubule transit time. Whether it actually leads to a decline in renal impairment and its clinical consequences should be investigated in a clinical trial, randomising patients to pemetrexed maintenance with or without hydration stratified to prior platinum-combination during induction. In the near future, this trial is going to start in our institution. Handling renal toxicities of pemetrexed in the era of combination with immune checkpoint inhibitors has become even more challenging, for the latter are known to cause renal damage as well. In **Chapter 8**, we provided a clinically useful algorithmic tool both to diagnose and to manage renal injury during combination treatment with chemo- and immunotherapy. When reviewing the literature, again we noticed an important disparity between the incidence of renal injury reported in clinical trials (0.5% - 5%) and in real-world observational studies (14.2% - 29%).[38–40] Of course, patient selection plays an important role as relatively "good/healthy" patient are selected for trials (no renal impairment at baseline, no concomitant nephrotoxic medication). However, the use of different definitions (CTCAE vs KDIGO) of renal impairment also likely contributes to this difference (also demonstrated in **Chapter 5**). We advocate the use of the more sensitive definition of acute kidney damage according to KDIGO, as earlier detection of nephrotoxicity hopefully leads to more timely intervention. It is key to prevent (long) interruption of chemo- and/or immunotherapy treatment and the development of chronic kidney injury which may jeopardize further lines of treatment. Future research should focus on finding (non-invasive) markers to help to discriminate between different causes of kidney injury and to inform about the risk of recurrent kidney injury after rechallenge with chemotherapy/immunotherapy.

PATIENTS' VIEW ON PEMETREXED TREATMENT

Quality of life (QoL) outcome measures are often secondary endpoints in clinical trials. However, QoL is a primary goal to maintain during palliative treatment of NSCLC. Patients want to be involved in the process of making important treatment decisions and they actually value the experienced quality of care higher if the principle of shared decision-making is used.[41,42] Helpful in shared decision-making are patient-reported outcome measures (PROMs), which are supportive in the assessment of and communication about symptoms and (health-related) QoL.[43,44] But despite receiving direct input from patients about their perceived QoL and symptoms during treatment via PROMs, there is no value judgment from a patient's perspective attached to these items. It is known that caregivers often do not correctly interpret the impact of adverse events.[45] Thus, there is a lack of knowledge about how patients value their treatment overall, whether they believe it was worth it. The Cancer Therapy Satisfaction Questionnaire (CTSQ) is a tool to assess patients' feelings about treatment, but has not been extensively evaluated in patients with chemotherapy. Therefore, in **Chapter 9**, we validated the questionnaire in patients with advanced NSCLC receiving pemetrexed-based chemotherapy. The CTSQ is a valid and reliable instrument to examine its three domains: patients' feelings about side effects (FSE) and their perceived expectations of treatment (ET) and satisfaction

with therapy (SWT). We were able to define minimally clinically important differences (MCID) for these three domains of the CTSQ, which enables a better interpretability in clinical practice. After validating the questionnaire, we evaluated the satisfaction with pemetrexed-based therapy of patients with advanced NSCLC according to the CTSQ in **Chapter 10**. The explained variances of different (health-related) QoL scales of the EORTC QLQ C-30 and QoL domains of the WHOQoL-BREF by SWT were small (maximum 17% for global health status/QoL Scale of the EORTC QLQ C-30). Moreover, patients were satisfied with pemetrexed treatment regardless of changes in QoL or experience of (severe) adverse effects. Thus, SWT brings additional information about treatment into the consulting room, supplementary to patient-reported QoL and adverse events. More than 80% of the patients considered that the chemotherapy was worth receiving even with adverse effects, and even 86% of the patients would undergo the same treatment again. Not surprisingly, a higher SWT score was associated with a better tumor response. We should be aware that this also touches upon a main pitfall of our study. One third of the patients did not finish four cycles of treatment due to progressive disease or treatment-related toxicity and therefore did not complete the CTSQ, which may have resulted in an overestimation of SWT. Patients with better feelings about their adverse effects were also more satisfied with their treatment. Clinicians often underestimate the impact of adverse effects compared to patients and they frequently rate different symptoms as more problematic than patients do. [45] The reported feelings about side effects were further explored in **Chapter 11**. More negative feelings about adverse effects were associated with a lower reported (health-related) QoL. We identified the experience of grade 1 or 2 treatment-related clinical adverse events as a determinant of lower FSE domain score. Notably, the fact that we did not find an association between the experience of severe adverse effects and FSE should be interpreted with caution. The aforementioned dropout of patients with severe toxicity before completion of the CTSQ is an important limitation. We tend to pay more attention to severe adverse events, but these results certainly justify adequate counselling about low grade adverse effects. It would be valuable to further look into which adverse events are most burdensome for patients, to target these events more specifically.

CONCLUDING REMARKS AND PROSPECTS FOR FUTURE RESEARCH

In advanced NSCLC, discoveries in molecular cancer biology and immunology continue to proceed and new treatments find their way into clinical practice. In the near future, an important challenge will be to rationally combine different treatment regimens in the best sequential order, while maintaining acceptable toxicity and QoL. In order to do so, we need to understand what drives tumor resistance against cancer treatments. We made efforts to unravel mechanisms of innate and acquired resistance to pemetrexed treatment. Although we found some interesting leads, a predictive biomarker for pemetrexed treatment is still not available. Other (large) prospective cohorts are needed

to validate the association between the *ATIC* SNP and overall survival and toxicity. It is key to adequately select patients with regard to comparative treatment arms in order to distinguish between predictive and prospective characteristics of *ATIC***.** But maybe, now treatment regimens like chemotherapy and immunotherapy are combined and are synergistically active, we should ideally look for a combination of predicting markers or for a marker involved in a common or synergistic pathway, instead of a single biomarker predicting the effectiveness of a single agent. Preferably, in our search for the most effective treatment combinations and sequences against NSCLC we should include well thought-out biomarker research in the clinical trials. Both pre- and on-treatment biomarker analysis is required, in order to better understand the mechanisms behind succeeded or failed investigated treatment options, to optimally stratify patients for individual treatment combinations and to adjust treatment when secondary or acquired resistance occurs. As another advantage from a scientific point of view, this might also increase the efficiency and pace of oncological research. A liquid biopsy is a promising non-invasive tool with the opportunity to perform a variety of (high-throughput) analysing techniques to investigate circulating tumor DNA in a quantitative and qualitative manner in order to acquire more insight into these resistance mechanisms. A challenge will be to restrict the number of false positives and false negatives to an acceptable level.

An eye-opener is the high incidence of renal toxicity in a real-life population, which is especially alarming since nowadays pemetrexed-based chemotherapy is combined with potential nephrotoxic immune checkpoint inhibitors. It also confirms the persistent need for real-world cohort studies alongside clinical trials, because they aim to answer different but both important questions: clinical trials will provide the knowledge about the efficacy and toxicity of treatment options compared to others. Although more prone to information and selection bias in general, well designed cohort studies in real-world populations will give more insight into the actual survival times and number of (treatment-related) adverse events. Further research aimed at early (non-invasive) detection of (severe) renal toxicity and assigning the toxicity to the right drug is key to prevent (definitive) treatment discontinuation and to keep the ability to undergo further lines of treatment. At the same time, we should concentrate studies on ways to prevent and manage these renal toxicities based on the pharmacokinetic characteristics of pemetrexed.

Besides renal toxicity, the incidence of other toxicities also often increases with combination treatments. The question is whether this is always troublesome. Patients may actually be willing to accept loss in QoL and more toxicity, maybe more than we think as caregivers, and still value the treatment as worthwhile. This trade-off between treatment benefit and treatment harm, might shift towards the willingness to accept even more treatment harm if life expectancy further increases. But, we should keep an eye on low-grade adverse events, which are often below the radar as well in research

as in clinical practice. It would be interesting to investigate which adverse events are specifically troublesome for patients and of course whether counselling to patients about the nature and management of these adverse events beforehand would lead to a better satisfaction with treatment.

Altogether, the findings of our research present small pieces to the puzzle to optimize personalized treatment and care with pemetrexed-based chemotherapy. This thesis provides potential leads to pursue further finetuning of individualized treatment with pemetrexed by exposing points of concern with regard to the management of renal toxicity and low-grade adverse effects. But most importantly, to solve this puzzle we should strive to continue our search for a clinically useful biomarker predicting treatment response and toxicity. With the expanding number of therapies available, treatment choices will only be more complex. Rational determination of treatment combination and sequences should be biomarker-driven, representing underlying resistance mechanisms, which is already becoming successfully embedded in the use of molecular targeted agents but is now still lacking for chemo- and immunotherapy. Our results can be used as a starting point to further unravel the innate and acquired resistance to pemetrexed, preferably using patient-friendly techniques like liquid biopsies. But, we also ran into the limitations of a single-arm cohort study in our research. Ideally, biomarker research should be implemented and validated in randomized controlled trials, based on findings from preclinical data, early-phase clinical trials or cohort studies. Those biomarker-driven randomized trials can be designed more efficiently by making use of so-called adaptive trial designs like the basket and umbrella trials. However, in our search for a predictive biomarker we also should be more practical and innovative sometimes by using other study designs and exploiting already available research data. History has shown more than once that other research designs are also able to identify biomarkers, despite the theoretical inferiority to randomized controlled trials. For example, the discovery of the predictive abilities of the important genetic mutations in *KRAS* in colorectal carcinoma and *EGFR* in NSCLC was done in retrospective cohorts.[46–49] A possible strategy would be to collect samples prospectively in standard of care and (re)use already prospectively collected samples from clinical trials or cohorts, and study them retrospectively by comparing objective response rates to different treatment strategies in unselected patients with various biomarker outcomes. Another option would be to use selected groups of patients representing marked responders and non-responders to the studied treatment, identifying the extreme phenotypes in this manner. Because treatment effectiveness differs greatly between the phenotypes, a smaller sample size is needed to detect potential biomarkers. Thus, we should keep our eyes open to the opportunities less costly and time-consuming than randomized controlled trials to hopefully speed-up biomarker driven oncology.

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CHAPTER 13

Summary

In the Netherlands, 14.000 new cases of lung cancer were detected in 2020 with only breast and prostate cancer having a higher incidence. Lung cancer has the highest mortality rate of all malignancies, responsible for 22% of all cancer deaths in 2020. Despite the rapid developments in the treatment landscape over the last decade with the introduction of molecular targeted agents and immunotherapy after the discovery of molecular targetable drivers, its resistance mechanisms and the possibility to influence the immune response to the tumor to our advantage, pemetrexed-based chemotherapy is still widely used as a backbone of treatment in patients with advanced non-small-cell lung cancer (NSCLC). This antimetabolite, interfering in the folic acid cycle, accomplishes overall survival benefits at the cost of low-grade and severe therapy-related toxicities, like other anticancer treatment. Although precision medicine has become a primary goal in cancer care, this is still largely uncharted territory for this chemotherapeutic treatment. In this thesis, we aimed to define mechanisms of resistance and toxicity more precisely and to investigate patient's value of and feelings about their own treatment in order to personalize treatment decisions.

In **Chapter 1** we have provided an overview of the use of pemetrexed in the treatment of advanced NSCLC since its introduction in clinical practice and we explained the current position of pemetrexed treatment next to the more recently introduced immune checkpoint inhibitors and targeted therapy for oncogenic driven NSCLC. For patients with advanced nonsquamous NSCLC without targetable driver mutations and with a low PD-L1 expression, combined treatment of pemetrexed-based chemotherapy with immune-checkpoint inhibitors is the first choice at this moment. Despite the improvements in tailoring lung cancer care in general, we exposed multiple knowledge gaps which hinder accomplishment of treatment personalization with regard to pemetrexed-based therapy: There are no biomarkers available to predict treatment response and toxicity to pemetrexed, mechanisms of innate and acquired resistance are still to be explored and despite the increasing use of patient-reported outcome measurement we do not know how patients value their own treatment. In order to seek answers to these questions we performed a real-world prospective multi-center cohort study (PEmetrexed and biomaRkerS: an observatiONAL study; PERSONAL), in which patients who received pemetrexed-based chemotherapy as first- or second-line treatment for advanced NSCLC were included between 2012 and 2014. Except for **Chapters 2** and **8**, the analyses in this thesis were performed using the data collected in the PERSONAL study.

In a retrospective analysis in **Chapter 2**, we aimed to develop a model to predict treatment response to pemetrexed based on immunohistochemical staining scores of genes which expression was correlated to the gene expression of the main target enzymes of pemetrexed (TS, GARFT, DHFR). In the training cohort including patients with early stage NSCLC who underwent surgical treatment, patients were classified as predicted responders and non-responders based on an earlier published optimized gene expression signature including but not limited to target enzymes of pemetrexed.

The prediction model showed that high protein expression of EZH2 and TPX2 were predictors of poor treatment response. These results could not be validated in an independent validation cohort with patients who were actually treated with first-line pemetrexed. In **Chapter 3**, mechanisms of innate resistance and toxicity to pemetrexed-based treatment were assessed by performing pharmacogenetic analyses of 10 single nucleotide polymorphisms (SNPs) of genes encoding for enzymes involved in the transport, metabolism and target function of pemetrexed. The polymorphism of the *ATIC* target gene was significantly associated with decreased overall survival and severe laboratory and clinical chemotherapy-related adverse events, but no association was observed between this SNP and treatment response and progression-free survival. The suggestion that decreased survival in patients with the *ATIC* SNP might be a result of increased toxicity is unnerving, but needs validation in another prospective cohort study. Mechanisms of acquired resistance to pemetrexed were explored in **Chapter 4**. Blood plasma samples of good responders to pemetrexed treatment were used as liquid biopsies and we compared the cell free DNA in their blood both at baseline and at disease progression. Increased variant allele frequencies of *GGH* and *MTR,* genes involved in the folic acid cycle, were observed at disease progression compared to baseline. We also detected *de novo* (not present at baseline) variant allele frequencies of genes at disease progression and recurrently increased variant allele frequencies in *MUC* genes in multiple patients. Prior research suggested that increased *MUC* expression leads to an immunosuppressive environment. Although the results of this analysis are preliminary due to the nature of this exploratory analysis, we believe these findings are worthy of further investigation in a cohort of patients treated with combination chemotherapy and immunotherapy.

Pemetrexed is known to be a chemotherapeutic agent with nephrotoxic potential. Although clinical trial data were already available, it was largely unknown how often patients from a real-world population are affected by renal toxicity during pemetrexed treatment. In **Chapter 5**, we found that approximately 30% of the patients developed acute kidney disease in a real-world population, frequently leading tot chronic kidney disease and treatment discontinuation. Both a patient's renal function before the start of induction treatment and its decrease during induction treatment were found to be risk factors for the development of acute kidney disease. These results were validated in an independent retrospective cohort of patients. Hyperhydration as a regimen to prevent renal toxicity, comparable to the common practice during cisplatin administration, will be further investigated in a clinical trial. Because pemetrexed is cleared renally to a large extent and the dose is based on body surface area (BSA) and not renal function, renal toxicity could lead to a higher systemic exposure. We tested this hypothesis in **Chapter 6**, by performing a pharmacokinetic-pharmacodynamic analysis. Total clearance of pemetrexed (and thus systemic exposure) was significantly affected by its renal clearance and not BSA. Using the current BSA-based dosing strategy, large patients and patients with a decreased renal function had a higher systemic exposure and were more prone to severe treatment-related toxicity. Simulations of renal function based and fixed single

dosing schedules showed less interindividual variation of systemic exposure. In order to investigate these alternative dosing schedules in the future, we aimed to develop more patient-friendly limited blood sampling designs for the estimation of pemetrexed pharmacokinetics in **Chapter 7**. A validated pharmacokinetic model was used to obtain estimates of pemetrexed clearance of pemetrexed using two separate datasets, both including rich pharmacokinetic data of seven and nine blood withdrawals within 24 hours respectively. Pemetrexed clearance, serving as a proxy of systemic exposure, could be adequately estimated with an acceptable precision and accuracy using 4 blood samples within a timeframe of eight hours after administration or with a single sample 24 hours after administration. In **Chapter 8**, we performed a review explaining the importance of using different definitions of renal toxicity and the mechanisms of renal toxicity due to pemetrexed-based chemotherapy and immune-checkpoint inhibitors. We provided an algorithmic tool based on clinical, laboratory, imaging and histological data to guide clinicians in diagnosing and treating renal toxicity in the era of combined chemotherapy and immunotherapy.

In personalized medicine, the shared decision-making process between the caregiver and the patient has an important role. Although treatment response, adverse events and quality of life are regularly measured during treatment, there is a lack of a single parameter balancing all these data from a patient's perspective. In **Chapter 10**, we evaluated patient's satisfaction with pemetrexed-based treatment using the Cancer Therapy Satisfaction Questionnaire (CTSQ), which we had previously validated in **Chapter 9**. The majority of patients were satisfied with the treatment regardless of deterioration of quality of life or experiencing (severe) adverse effects. We know that clinicians often do not correctly interpret the impact of adverse events on patients. In **Chapter 11**, an association between negative feelings about adverse effects from a patient's perspective and worse reported quality of life is noted. We identified low grade adverse effects as determinants of worse feelings about adverse effects.

This thesis concludes with a discussion in **Chapter 12,** where the findings of the previous chapters are put into context. In the last years, tremendous progress has been realized in the treatment of NSCLC by making the treatment more precise and more effective with the entrance of molecular targeted therapy and immunotherapy. However, there is still plenty of room for improvement. Pemetrexed-based chemotherapy is still a frequently used backbone in the treatment of advanced NSCLC. In this thesis, we contributed to personalization of the use of this therapy by providing insights into innate and acquired resistance mechanisms, determinants and incidence of (renal) toxicity, optimization of drug exposure and value judgment of treatment from a patient's perspective. Future research should focus on finding determinants to further optimize and personalize treatment choices, which will only become more complex with the increasing number of (classes of) anticancer agents available.

CHAPTER 14

Nederlandse samenvatting

In het jaar 2020 werden in Nederland ongeveer 14.000 nieuwe gevallen van longkanker vastgesteld en enkel van borst- en prostaatkanker werd een hoger aantal nieuwe gevallen gedetecteerd. Van alle verschillende soorten kanker, overlijden het meeste aantal mensen aan longkanker: in 2020 was deze ziekte verantwoordelijk voor 22% van alle overlijdens ten gevolge van kanker. Na de ontdekkingen van zogenaamde "driver-mutaties" in de tumor waar gerichte therapie op gegeven kan worden, de resistentiemechanismen die met deze gerichte behandeling gepaard gaan en de mogelijkheid om de immuunrespons van het lichaam in ons voordeel te beïnvloeden, heeft er in het laatste decennium een stormachtige ontwikkeling plaatsgevonden in het behandelingsveld van longkanker met de introductie van moleculair gerichte therapie en immuuntherapie. Desondanks is pemetrexed-gebaseerde chemotherapie nog steeds een veelgebruikte hoeksteen in de behandeling van patiënten met gevorderd niet-kleincellig longkanker. Pemetrexed is een antikanker medicijn in de categorie chemotherapie en grijpt als anti-metaboliet aan in het foliumzuur metabolisme. De behandeling gaat gepaard met een overlevingsvoordeel ten koste van milde en ernstige therapie-gerelateerde bijwerkingen, net zoals dit het geval is bij andere antikankerbehandelingen. Hoewel er tegenwoordig in de behandeling van kanker steeds meer wordt gestreefd naar precisiegeneeskunde, ofwel "behandeling op maat", is dit grotendeels nog onontgonnen terrein voor de behandeling met pemetrexed (en chemotherapie in het algemeen). In dit proefschrift hebben we de mechanismen van tumorresistentie en het ontwikkelen van toxiciteit nauwkeuriger in kaart gebracht en dieper inzicht verkregen in de waarden en gevoelens van de patiënt met betrekking tot hun eigen behandeling, met het doel om behandeling met pemetrexed beter op de individuele patiënt af te kunnen stemmen.

In **Hoofdstuk 1** hebben we een overzicht gegeven van het gebruik van pemetrexed voor de behandeling van gevorderd niet-kleincellig longkanker sinds dat het middel voor het eerst is ingezet in de klinische praktijk en hebben we de huidige plaats van het medicijn in de behandeling van longkanker besproken, te midden van de recenter geïntroduceerde immuun-checkpoint remmers en moleculair gerichte behandelingen. Op dit moment is een combinatie behandeling bestaande uit pemetrexed-gebaseerde chemotherapie en immuun-checkpoint remmers de therapie van eerste keuze voor patiënten met gevorderde niet-plaveiselcel niet-kleincellig longcarcinoom zonder driver mutaties waar gerichte therapie op gegeven kan worden en met een lage PD-L1 expressie (een eiwit dat tot expressie wordt gebracht door de tumorcel en waarvan een hoge expressie voorspelt voor een betere respons op bepaalde immuun-checkpoint remmers). Grofweg 60% van de patiënten bij wie dit type longkanker wordt vastgesteld, komt in aanmerking voor deze therapie. Ondanks dat de behandeling van longkanker in het algemeen steeds beter op maat van de specifieke patiënt kan worden gemaakt, hebben we diverse kennishiaten blootgelegd die verdere individualisering van de behandeling met pemetrexed vooralsnog in de weg staan. Zo zijn er geen biomarkers beschikbaar die kunnen voorspellen of de tumor respondeert op therapie of dat er toxiciteit optreedt ten gevolge van pemetrexed. Mechanismen van op voorhand aanwezige en tijdens

behandeling verworven resistentie moeten nog uitgezocht worden. En ondanks dat we steeds vaker patiënt-gerapporteerde uitkomsten gebruiken, weten we niet hoe patiënten hun eigen behandeling waarderen. Om deze vragen te kunnen beantwoorden hebben we een "real-world" prospectieve multicenter cohortonderzoek uitgevoerd (PEmetrexed and biomaRkerS: an observatiONAL studie; PERSONAL), waarin tussen 2012 en 2014 patiënten geïncludeerd hebben die behandeld werden met pemetrexed-gebaseerde chemotherapie als eerstelijns of als tweedelijns behandeling voor gevorderd niet-kleincelling longcarcinoom. Behalve de analyses in **Hoofdstuk 2** en **8**, zijn alle overige analyses in dit proefschrift verricht met de data die verzameld zijn in de PERSONAL studie.

In **Hoofdstuk 2** hebben we een retrospectieve analyse uitgevoerd met het doel om een model te ontwikkelen waarmee we de behandelrespons (ofwel aanslaan van therapie/krimpen van de tumor) op pemetrexed konden voorspellen op basis van scores van immunohistochemische kleuringen van genen, waarvan de expressie gecorreleerd was met de genexpressie van de belangrijkste zogenaamde target enzymen waarop pemetrexed aangrijpt in de tumorcel (TS, GARFT en DHFR). Met immunohistochemische kleuringen worden in het pathologisch laboratorium tumorcellen gekleurd, waarbij de mate van aankleuring (aantal cellen en intensiteit) correleert met de expressie van het eiwit waarvoor het gen codeert. In het trainingscohort, bestaande uit patiënten met een vroeg stadium niet-kleincellig longcarcinoom die hiervoor een operatie hadden ondergaan, werden de patiënten geclassificeerd als *voorspelde* responders en non-responders (deze patiënten zijn niet daadwerkelijk behandeld met pemetrexed). Deze classificatie werd gebaseerd op de expressie van een geoptimaliseerd samengesteld genprofiel vastgesteld in eerder gepubliceerde literatuur, waarin gekeken werd naar onder meer (maar niet uitsluitend) de genexpressie van targetenzymen (aangrijpingspunten) van pemetrexed. Het predictie model toont aan dat hoge eiwitexpressie van EZH2 en TPX2 voorspellers zijn van een slechte behandelrespons. Deze resultaten konden echter niet gevalideerd worden in een onafhankelijk cohort met patiënten die daadwerkelijk behandeling met eerstelijns therapie hadden ondergaan. In **Hoofdstuk 3** hebben we geprobeerd om meer inzicht te krijgen in resistentiemechanismen van de tumor en in het ontstaan van toxiciteit, met behulp van onderzoek naar afwijkingen van 10 genen die betrokken zijn in het werkingsmechanisme van pemetrexed. Zo hebben we genen onderzocht zie een rol spelen in het celtransport, het metabolisme en aangrijpingspunten van pemetrexed. Een variant van het gen *ATIC* ging gepaard met zowel een verminderde overleving als het optreden van meer ernstige bijwerkingen. Er werd geen relatie gezien tussen deze variant en snellere groei van de tumor of eerdere progressie van ziekte. Vanuit deze bevindingen zou gedacht kunnen worden dat de slechtere overleving van patiënten met de *ATIC* variant samenhangt met ernstige bijwerkingen. Dit is uiteraard alarmerend, echter kan deze conclusie niet zomaar getrokken worden. Hiervoor is het nodig om de bevindingen te bevestigen in een aanvullend prospectief onderzoek. Mechanismen van verworven resistentie van de tumor tijdens de behandeling met pemetrexed werden onderzocht

in **Hoofdstuk 4**. Er werd op 2 tijdspunten, namelijk vóór start van therapie en na het optreden van tumorgroei, bloed gebruikt van een klein aantal patiënten om daarin te zoeken naar nieuwe danwel toegenomen afwijkingen van genen tijdens de behandeling. Deze zogenaamde "vloeibare biopten" leverden een aantal interessante genen op, waaronder 2 genen die een rol spelen in het werkingsmechanisme van pemetrexed alswel van zogenaamde *MUC* genen. *MUC* genen hebben mogelijk een onderdrukkende rol op het immuunsysteem en daarom zou het interessant zijn om deze resultaten verder te onderzoeken in patiënten die zowel chemotherapie als immuuntherapie krijgen.

Pemetrexed is een soort chemotherapie die als bijwerking nierfunctiestoornissen kan geven. Ook al zijn hierover genoeg data beschikbaar vanuit klinische trials, data uit de dagelijkse praktijk ontbreken nagenoeg. In **Hoofdstuk 5** hebben we aangetoond dat ongeveer 30% van de patiënten die een onderhoudsbehandeling krijgen met pemetrexed acute nierfunctiestoornissen ontwikkelen en dat deze vaak leiden tot chronische nierschade en zelfs tot moeten stoppen met de behandeling. Patiënten hebben hierop een hoger risico als ze op voorhand al een slechtere nierfunctie hebben, maar ook als de nierfunctie tijdens de behandeling achteruit gaat. We hebben deze resultaten kunnen bevestigen in een andere groep patiënten. Pemetrexed wordt in de huidige praktijk gedoseerd op basis van het lichaamsoppervlaktie ("body surface area"; kortweg BSA), afhankelijk van de lengte en gewicht van een patiënt, en hiermee wordt dus geen rekening gehouden met de nierfunctie. Omdat pemetrexed grotendeels via de nieren wordt uitgescheiden, zou een achteruitgang van de nierfunctie invloed kunnen leiden tot stapeling van pemetrexed in het lichaam en hiermee tot meer bijwerkingen. Deze hypothese hebben we getoetst in **Hoofdstuk 6**, waarbij we inderdaad konden bevestigen dat de uitscheiding van pemetrexed uit het lichaam (en hiermee gepaard gaande dus de totale blootstelling van het lichaam aan het medicijn) sterk afhankelijk is van de nierfunctie en niet van het lichaamsoppervlak. Met de huidige doseringsstrategie op basis van het lichaamsoppervlak zagen we dat zowel grote patiënten en patiënten met een verminderde nierfunctie een hogere totale blootstelling aan het medicijn hadden en ook meer ernstige bijwerkingen ervoeren. Op basis van simulaties menen wij dat een gelijke startdosis bij elke patiënt mogelijk beter kan zijn dan doseren op basis van BSA. Om andere doseringsstrategieën te onderzoeken, is het nodig om concentraties van pemetrexed op verschillende tijdpunten te kunnen meten nadat het medicijn is toegediend. Het vervolgen van de geneesmiddelenconcentraties over de tijd zou een stuk patiëntvriendelijker kunnen verlopen. In **Hoofdstuk 7** hebben we laten zien, dat we met veel minder bloedafname momenten bij de patiënt toch voldoende betrouwbare gegevens van de concentraties van pemetrexed kunnen verkrijgen. Op dit moment wordt pemetrexed-gebaseerde chemotherapie frequent gecombineerd met immuuntherapie. Ook immuuntherapie kan leiden tot nierfunctiestoornissen. In **Hoofdstuk 8** staat beschreven wat de verschillende achterliggende mechanismen zijn voor het optreden van nierfunctiestoornissen door chemotherapie en immuuntherapie. Ook hebben we een algoritme ontwikkeld met het doel om de clinicus handvatten te geven wat betreft

de diagnostiek en behandeling van nierfunctiestoornissen die optreden tijdens de combinatie van chemotherapie en immuuntherapie.

In de precisiegeneeskunde heeft de gezamenlijke besluitvorming tussen de patiënt en de behandelaar een belangrijke rol. Ook al worden respons op behandeling, bijwerkingen en kwaliteit van leven regelmatig gemeten tijdens de behandeling, er is geen alomvattende parameter die al deze verschillende data tegen elkaar afweegt vanuit het perspectief van de patiënt. In **Hoofdstuk 10** hebben we tevredenheid met pemetrexed-gebaseerde chemotherapie geëvalueerd bij patiënten met behulp van een vragenlijst (Cancer Therapy Satisfaction Questionnaire; CTSQ) welke door de patiënt zelf moest worden ingevuld. De resultaten wezen uit dat tevredenheid met therapie niet per se gerelateerd is met kwaliteit van leven en het ondervinden van bijwerkingen. Daarom is deze meting mogelijk van meerwaarde voor in de spreekkamer. Overigens hebben we voorafgaand aan de analyses in **Hoofdstuk 10** eerst in **Hoofdstuk 9** aangetoond dat de vertaalde versie van de CTSQ naar het Nederlands in onze patiëntpopulatie een betrouwbaar en valide meetinstrument is. De impact van bijwerkingen op patiënten wordt vaak niet goed ingeschat door behandelaars. In **Hoofdstuk 11** hebben we een associatie beschreven tussen negatieve gevoelens over bijwerkingen en slechtere kwaliteit van leven van patiënten. Juist milde bijwerkingen (in plaats van ernstige bijwerkingen) bleken gerelateerd aan het ervaren van negatieve gevoelens over de bijwerkingen. Mogelijk kan verhoogde aandacht vanuit de behandelaar voor deze milde bijwerkingen (bijvoorbeeld door betere voorlichting of snellere interventie) dus leiden tot een betere kwaliteit van leven.

Dit proefschrift sluit af met een discussie in **Hoofstuk 12**, waarin alle bevindingen van de eerdere hoofdstukken in de context van de huidige literatuur worden geplaatst. In de laatste jaren, hebben de ontwikkelingen binnen de behandeling van niet-kleincellig longkanker een vlucht genomen met de introductie van moleculair gerichte therapie en immuuntherapie, waarmee de behandeling meer op maat en effectiever is geworden. Toch blijft er nog veel ruimte over voor verbetering. Pemetrexed-gebaseerde chemotherapie is nog steeds een hoeksteen in de behandeling van gevorderd niet-kleincellig longkanker. In dit proefschrift hebben we bijgedragen aan het preciezer maken van de behandeling met pemetrexed door op zoek te gaan naar (genetische) determinanten die een rol spelen in het optreden van resistentie in de tumor, door de incidentie en impact van nierfunctiestoornissen beter in kaart te brengen, door de blootstelling aan het geneesmiddel te optimaliseren en door een waardeoordeel over de behandeling vanuit het perspectief van de patiënt te meten. Toekomstig onderzoek zal zich moeten blijven richten op het optimaliseren van behandelbeslissingen en op het leveren van beter maatwerk naar de individuele patiënt, al zal dit enkel complexer worden met het toenemende aantal verschillende soorten en combinaties van antikankerbehandelingen.

APPENDICES **A**

List op publications
Co-author affiliations
Portfolio
Dankwoord
Curriculum Vitae

LIST OF PUBLICATIONS

Cheung K*, de Mol M*, **Visser S**, Den Oudsten BL, Stricker BH, Aerts JG. Reliability and validity of the Cancer Therapy Satisfaction Questionnaire in lung cancer. Qual Life Res. 2016 Jan;25(1):71-80
** Authors contributed equally*

Stoop MP*, **Visser S***, van Dijk E, Aerts JGJV, Stricker BH, Luider TM. A new quantification method for assessing plasma concentrations of pemetrexed and its polyglutamate metabolites. J Pharm Biomed Anal. 2016 Sep 5;128:1-8
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PORTFOLIO

Phd Training and teaching	Year:	ECTS
General courses		
GCP (Good clinical practice)	2012	0.5
BROK	2013	1.5
Scientific writing course	2016	1
GCP re-training and exam	2016	0.25
Specific courses		
NIHES postgraduate program clinical epidemiology	2014-2016	70
Oncologiecursus New York	2015	1
Presentations and conferences		
College Tour Longcarcinoom (Oral presentation)	2012	1
NRS 5th Young Investigator Symposium (Oral presentation)	2013	0.5
Amphia Wetenschapsdag (Oral presentations)	2015	1
PhD Day EMC	2015	0.5
WCLC Denver (Oral and poster presentations)	2015	3.5
WCLC Vienna (Oral and poster presentations)	2016	3.5
PhD Day EMC	2016	0.5
ELCC Geneva (Oral and poster presentations)	2018	3.5
Longkankerregiobijeenkomst (Oral presentation)	2019	0.5
Refereeravonden/ OIO meetings (Oral presentations)	2012-2018	4
Teaching master thesis		
Jermo van Toor	2015	2.5

DANKWOORD

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Sabine Visser,
Breda, augustus 2022

CURRICULUM VITAE

Sabine Visser was born on the 5th of October 1987 in Rijen, the Netherlands. She attended high school at the Stedelijk Gymnasium in Breda and graduaded (cum laude) in 2005. She then started medical school at Utrecht University and obtained her MD degree (cum laude) in 2011. After her studies, she continued as a resident not in training in 2012 at the department of Pulmonary Medicine of the Amphia hospital in Breda, supervised by dr. Remco Djamin and dr. Marco Grootenboers. In November 2012 the PhD project on personalizing pemetrexed-based chemotherapy under the supervision of prof. dr. Joachim Aerts and prof. dr. Bruno Stricker initiated, resulting in this thesis. During her PhD trajectory she combined the research with the performance of clinical activities at the Pulmonary department of the Amphia hospital and she obtained a master's degree in Health Sciences (Master of Science in Clinical Epidemiology, Netherlands Institute for Health Sciences). In 2017 she started her residency in Pulmonary Medicine with 20 months of Internal Medicine at the supervision of dr. Joost van Esser at the Amphia hospital. Subsequently, she continued her residency at the department of Pulmonary Medicine of the Erasmus Medical Center supervised by dr. Leon van den Toorn. Presently, she lives together in Breda with her fiancé Roland and their daughter Lotte.