Understanding and Managing Side Effects of Frequently used Anticancer Drugs

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This thesis is based on the experience gained from the treatment of patients with solid tumors at the department of Medical Oncology, Erasmus MC Cancer Institute Rotterdam, The Netherlands.

Understanding and Managing Side Effects of Frequently used Anticancer Drugs

Het begrijpen en behandelen van bijwerkingen van vaak gebruikte antikanker medicijnen

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Voor Marian en Hanneke,
zij kennen de geschiedenis.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Cancer has a major impact on life expectancy around the world and is also a major cause of morbidity.[1] These days, the role of cytotoxic agents in the anticancer treatment of solid tumors is well established and is still increasing.[2] Although much cancer research is focused primarily on immunotherapy nowadays; classic cytotoxic agents and molecular targeted agents remain the most important systemic treatment modalities in the majority of cancer types, either as monotherapy or in combination with immunotherapy, surgery and/or radiotherapy.[3] Cancer treatment needs to be balanced between what is best to target the tumor and what is best for the patient. The potency of anticancer treatment is limited by factors such as drug resistance of tumor cells and the toxic effects on healthy normal tissue, leading to tissue damage (e.g., bone marrow, gastrointestinal tract mucosa, hair follicles) and to serious side effects, delay in cancer treatment and a serious decrease in quality of life.[4] Individual factors such as gender, age, genetic factors, illness related, organ function, body size measurements, co-medication and lifestyle [5] may all influence the systemic exposure to the anticancer drugs. Usually, a low exposure of systemic treatment can lead to ineffective treatment, while high exposure can lead to increased toxicity.

Side effects are a major concern for patients and the oncology team consisting of (amongst others) physicians, nurse practitioners and physician assistants, oncology nurses, pharmacists and dietitians.[6] Drug exposure, minimization of interactions and adequate management of side effects are key factors in the tolerance of the treatment and in reducing the negative consequences of the anticancer treatment for patients.[7] Therefore, it is important to gain more insight into the exposure, interactions and treatment of side effects of anticancer treatments.

This thesis describes studies on drug exposure, safety management and intervention methods in the chemotherapeutic drugs capecitabine and paclitaxel, and in tyrosine kinase inhibitors (in particular sorafenib), with the aim to improve control over these side effects.

PART I: CHEMOTHERAPY

Chemotherapeutic drugs are classified according to their mechanism of action and include DNA-interactive agents, antimetabolites, anti-tubulin agents amongst others. These drugs interfere with cell proliferation by targeting cellular DNA and their metabolism; antimetabolites as 5-fluorouracil target pyrimidine metabolic enzymes, whereas anti-tubulin agents such as paclitaxel interfere with microtubules.[4] Side effects of many cytotoxic agents include bone marrow suppression, an increased risk of infections, hair loss, nausea, mucositis, diarrhea, peripheral neuropathy, skin toxicities, allergic drug reactions and the development of clinical resistance. For capecitabine, especially hand-

foot syndrome is a characteristic side effect with a significant impact on daily live and consequently the dosage of this therapy.

CAPECITABINE

Capecitabine is an orally administered prodrug of 5-fluorouracil (5-FU). As it is administered orally, capecitabine has major advantages over other cytotoxic agents that need to be administered intravenously. In addition, it is widely used for the treatment of breast cancer, esophagus- and gastric cancer and colorectal cancer.[8]

Hand-foot syndrome (HFS), also known as palmar-plantar erythrodysesthesia syndrome or acral erythema, is a common cutaneous reaction to capecitabine treatment that can affect the palms and/or soles of the feet. HFS causes redness, swelling, pain, cracked or scaly skin, sometimes blisters appear. The skin can also look tight or thin.[9] It has anecdotally been reported that HFS is responsible for the loss of fingerprints during capecitabine treatment.[10] Loss of fingerprints can cause serious identification problems and adds stress to the patient's daily life. In **Chapter 2** a prospective cohort study is presented in which the association between HFS and fingerprint loss during capecitabine treatment is investigated.

A potential problem with oral antineoplastic drugs such as capecitabine is the variability in absorption due to various factors such as co-medication. Earlier, Chu et al. and Sun et al. have demonstrated a significant decrease in progressive free survival and overall survival in patients using capecitabine concomitantly with gastric acid suppressive therapy such as proton pump inhibitors (PPIs). They proposed that this might be explained by a drug-drug interaction with PPIs leading to a decrease in capecitabine exposure.[11,12] Gastric acid suppressive therapy is concomitantly used in one third of all cancer patients as prophylaxis for gastro-intestinal bleedings or treatment for gastroesophageal reflux disease.[13] Self managing of reflux-related symptoms is easier with the availability of over-the-counter PPIs.[14] Given the impact of the potential drug-drug interaction between capecitabine and PPIs, a randomized crossover study on the potential pharmacokinetic interaction between the proton pump inhibitor esomeprazole and capecitabine is described in **Chapter 3**.

PACLITAXEL

Paclitaxel –another important chemotherapeutic drug –is a widely used intravenously administered agent and was found in 1963 in the needles and bark of the Pacific yew tree, *Taxus brevifoli*. It was approved for clinical practice in 1993,[15] and in combination with carboplatin, it is now the cornerstone treatment for ovarian, breast, esophageal and lung cancer amongst others.[16-18]

Hair loss (or alopecia) is a common side effect of paclitaxel. Particularly for women this is one of the most distressing adverse events and may affect a patient's quality of life

dramatically.[19] Scalp cooling in patients treated with taxane-based chemotherapy such as paclitaxel, led to hair conservation in more than 50% of patients, depending on the dose, compared with those who received no scalp cooling. Using scalp cooling, liquid refrigerant is pumped as coolant through a cooling cap which is placed on the head of the patient, usually 20-45 minutes before the infusion of chemotherapy and continues during and for 20-150 minutes after infusion of chemotherapeutic drugs.[20] The hypothesis for the hair preservation effect of scalp cooling is that cooling of the scalp reduces the blood flow and causes locally a subcutaneous vasoconstriction, which can lead to less availability for uptake of the paclitaxel by the scalp. It also reduces biochemical activity, which may make hair follicles less susceptible to the damage by chemotherapy.[20] As scalp cooling can potentially reduce the body temperature and as a result, systemic metabolic processes, we investigated in a prospective pharmacokinetic study the impact of scalp cooling on paclitaxel pharmacokinetics as described in **Chapter 4**.

Another frequent side effect during paclitaxel infusion is a hypersensitivity reaction (HSR), which usually occurs during the first or second dose of paclitaxel within the first minutes of starting the infusion. HSRs to paclitaxel are primarily due to Cremophor-EL; the pharmaceutical vehicle for paclitaxel.[15] Symptoms include flushing, chest and/or back pain, dyspnea and cardiovascular involvement ranging from hypertension to hypotension.[21] A HSR usually quickly resolves after discontinuation of the infusion. To prevent HSR, premedication regimens were introduced as standard of care during paclitaxel treatment and generally consist of the corticosteroid dexamethasone combined with a histamine 1 (H_1)-receptor antagonist (e.g., clemastine or diphenhydramine) and the histamine 2 (H_2)-receptor antagonist ranitidine.[22] Interestingly, during the years doubt arose if ranitidine is effective in the prevention of HSRs.[23-25] Despite these findings, the use of an H_2-antagonist during paclitaxel infusion is still recommended as standard premedication to prevent paclitaxel induced HSRs. In **Chapter 5,** a non-inferiority study is described in which the incidence of HSR during paclitaxel is compared between patients treated with premedication regimens with and without ranitidine.

In daily practice, paclitaxel administration is based on a patient's body surface area (BSA), which is calculated from height and weight and is a surrogate for body size. Despite this '*individualization*', the interindividual variability in paclitaxel pharmacokinetic remains high. That is unfortunate, as a low paclitaxel clearance may put patients at risk for drug-related toxicities, while patients with a high clearance are at risk of suboptimal systemic drug levels with potentially a reduced therapeutic effect.

Other biometric parameters, such as skeletal muscle mass (i.e., skeletal muscle index, SMI), adipose tissue, and skeletal muscle density (SMD; i.e., a measure for skeletal muscle quality and intramuscular fat infiltration), could potentially serve as predictive covariates, as they are associated with altered volumes of distribution, metabolism, and clearance of cytotoxic drugs.[26] Previous studies demonstrated a wide variation in muscle

mass and visceral adipose tissue (VAT) in patients with identical BSA and/or body mass index (BMI), leading to heterogeneity in chemotherapy tolerance and treatment-related toxicity such as neutropenia.[27-29] Taking into account the parameters SMI, SMD and VAT could in theory help to optimize paclitaxel dosing strategies. In **Chapter 6** we assessed whether paclitaxel dosing could be optimized by correcting for SMI, VAT and SMD in a retrospective cohort of patients with esophageal cancer.

In combination with carboplatin and radiotherapy paclitaxel is highly effective in the curative setting of esophageal cancer, and in combination with carboplatin alone it has shown efficacy both during induction settings and in the palliative setting of this tumor type.[17,30,31] A substantial part of patients with esophageal cancer however do not benefit from paclitaxel treatment.[30,32] In a previous study from our group, no correlation between systemic paclitaxel clearance and esophageal cancer was shown.[33] However, knowledge about intra-tumoral concentrations of paclitaxel and its influence on the effectiveness of paclitaxel treatment is lacking. Therefore, in **Chapter 7,** a prospective explorative analysis is presented to identify differences between patients in systemic paclitaxel pharmacokinetics and intra-tumoral paclitaxel exposure in esophageal cancer patients, aiming to find correlations which could guide treatment decisions.

PART II: TARGETED ORAL ANTICANCER THERAPY

Targeted anticancer drugs block the growth and spread of cancer by interfering with specific kinase molecules (molecular targets, tyrosine kinase inhibitors (TKIs)) involved in the growth, progression and spread of cancer. They are approved for the treatment of many malignancies such as colorectal, thyroid, hepatocellular, renal cell, breast and lung cancers.[34] There are different types of TKIs; e.g., vascular endothelial growth factor (VEGF) receptors, inhibitors selective for BRAF and c-KIT. Depending on their target, vascular, cutaneous, endocrine, coagulation and pulmonary side effects can occur, such as hypertension, proteinuria, skin rash, hand-foot skin reaction and hypothyroidism.[35] To maximize clinical effectiveness, it is important to prevent and/or treat side effects that patients put at risk (or greatly reduce their quality of life).

SORAFENIB

Sorafenib is an orally administered small molecule kinase inhibitor that inhibits tumor-cell proliferation and tumor angiogenesis (growth of new blood vessels).[36] Sorafenib is registered for the treatment of hepatocellular carcinoma (HCC),[37] thyroid cancer [38] and renal cell carcinoma (RCC).[39] Treatment is continued as long as clinical benefit is observed or until unacceptable toxicity occurs.[40]

Hand-foot skin reaction (HFSR) is one of the most observed side effects (20% ≥ grade 3) of sorafenib in which redness, painful hyperkeratotic plaques and blistering develop especially on fingertips and toes.[41] There is no effective treatment option besides dose reduction or discontinuation of treatment. A preclinical study by Zimmerman et al.[42] showed that the drug transporter OAT6 in keratinocytes is responsible for the uptake of sorafenib in the skin and that inhibiting OAT6 with probenecid, a drug used to prevent gout,[43] prevented HFSR in mice. In **Chapter 8**, we performed a prospective cohort study in patients using sorafenib in order to characterize the effects of probenecid on sorafenib distribution into keratinocytes and on its systemic exposure.

Another frequent side effect of sorafenib is hypothyroidism, which occurs in 18%-50% of patients.[44] Back in 2011, we reported on two patients who suffered from thyroiditis during treatment with sorafenib.[45] The pathogenesis of thyroid disease due to sorafenib had not been fully elucidated up till then,[46] and these cases led us to set up a prospective cohort study, designed to better describe the clinical presentation of thyroid dysfunction related to sorafenib treatment in **Chapter 9**.

As described above, sorafenib treatment is often limited by the occurrence of side effects. Concomitant medication might induce or aggravate these side effects. For example, immunosuppressive drugs are used by patients with HCC that progress after liver transplantation (approximately 20% of cases),[47] and are also known to inhibit CYP3A4, for which sorafenib is a substrate. Moreover, patients who has had a liver transplantation are known to experience more sorafenib-induced side effects. As this might be caused by sorafenib accumulation due to CYP3A4 inhibition, we assessed the pharmacokinetics of sorafenib in a case-series of patients who were also treated with immunosuppressive drugs in **Chapter 10**.

During sorafenib treatment, but also with other TKIs that inhibit the vascular endothelial growth factor (VEGF) signaling pathway, serious cardiovascular adverse events can occur such as hypertension, venous thromboembolism, left ventricular dysfunction and QTc interval prolongation.[48] The cardiovascular risk of VEGF inhibitors, either as monotherapy or in combination with immune checkpoint inhibitors, is reviewed in **Chapter 11**. Hypertension, a frequent cardiovascular adverse event, is seen in 25-87% of patients using VEGF inhibiting TKIs and has been proven to limit optimal treatment.[49,50] Novel strategies to prevent these unwanted side effects are needed to improve quality of life and survival in patients with cancer. Although it is theoretically logical to reduce sodium consumption in these patients, as has been shown in sunitinib treated rats fed with a sodium-rich diet,[51] sodium restriction has never been tested in hypertensive patients with cancer using VEGF inhibitors. Therefore, in **Chapter 12** we prospectively studied the effect of dietary sodium restriction in patients treated with VEGF inhibiting TKIs.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin.* 2021;71(3):209-249.
2. Wilson BE, Jacob S, Yap ML, Ferlay J, Bray F, Barton MB. Estimates of global chemotherapy demands and corresponding physician workforce requirements for 2018 and 2040: a population-based study. *Lancet Oncol.* 2019;20(6):769-780.
3. Falzone L, Salomone S, Libra M. Evolution of Cancer Pharmacological Treatments at the Turn of the Third Millennium. *Front Pharmacol.* 2018;9:1300.
4. Nussbaumer S, Bonnabry P, Veuthey JL, Fleury-Souverain S. Analysis of anticancer drugs: a review. *Talanta.* 2011;85(5):2265-2289.
5. Mathijssen RH, Sparreboom A, Verweij J. Determining the optimal dose in the development of anticancer agents. *Nat Rev Clin Oncol.* 2014;11(5):272-281.
6. Nurgali K, Jagoe RT, Abalo R. Editorial: Adverse Effects of Cancer Chemotherapy: Anything New to Improve Tolerance and Reduce Sequelae? *Front Pharmacol.* 2018;9:245.
7. Scripture CD, Figg WD. Drug interactions in cancer therapy. *Nat Rev Cancer.* 2006;6(7):546-558.
8. Walko CM, Lindley C. Capecitabine: a review. *Clin Ther.* 2005;27(1):23-44.
9. Kwakman JJM, Elshot YS, Punt CJA, Koopman M. Management of cytotoxic chemotherapy-induced hand-foot syndrome. *Oncol Rev.* 2020;14(1):442.
10. Chavarri-Guerra Y, Soto-Perez-de-Celis E. Images in clinical medicine. Loss of fingerprints. *N Engl J Med.* 2015;372(16):e22.
11. Chu MP, Hecht JR, Slamon D, et al. Association of Proton Pump Inhibitors and Capecitabine Efficacy in Advanced Gastroesophageal Cancer: Secondary Analysis of the TRIO-013/LOGiC Randomized Clinical Trial. *JAMA Oncol.* 2017;3(6):767-773.
12. Sun J, Ilich AI, Kim CA, et al. Concomitant Administration of Proton Pump Inhibitors and Capecitabine is Associated With Increased Recurrence Risk in Early Stage Colorectal Cancer Patients. *Clin Colorectal Cancer.* 2016;15(3):257-263.
13. Smelick GS, Heffron TP, Chu L, et al. Prevalence of acid-reducing agents (ARA) in cancer populations and ARA drug-drug interaction potential for molecular targeted agents in clinical development. *Mol Pharm.* 2013;10(11):4055-4062.
14. Johnson DA, Katz PO, Armstrong D, et al. The Safety of Appropriate Use of Over-the-Counter Proton Pump Inhibitors: An Evidence-Based Review and Delphi Consensus. *Drugs.* 2017;77(5):547-561.
15. Joerger M. Treatment regimens of classical and newer taxanes. *Cancer Chemother Pharmacol.* 2016;77(2):221-233.
16. Stage TB, Bergmann TK, Kroetz DL. Clinical Pharmacokinetics of Paclitaxel Monotherapy: An Updated Literature Review. *Clin Pharmacokinet.* 2018;57(1):7-19.
17. van Hagen P, Hulshof MC, van Lanschot JJ, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. *N Engl J Med.* 2012;366(22):2074-2084.
18. Heijkoop ST, Franckena M, Thomeer MG, Boere IA, Van Montfort C, Van Doorn HC. Neoadjuvant chemotherapy followed by radiotherapy and concurrent hyperthermia in patients with advanced-stage cervical cancer: a retrospective study. *Int J Hyperthermia.* 2012;28(6):554-561.
19. van den Hurk CJ, Mols F, Vingerhoets AJ, Breed WP. Impact of alopecia and scalp cooling on the well-being of breast cancer patients. *Psychooncology.* 2010;19(7):701-709.
20. Lacouture ME, Sibaud V, Gerber PA, et al. Prevention and management of dermatological toxicities related to anticancer agents: ESMO Clinical Practice Guidelines(). *Ann Oncol.* 2021;32(2):157-170.

21. Picard M. Management of Hypersensitivity Reactions to Taxanes. *Immunol Allergy Clin North Am.* 2017;37(4):679-693.
22. Joerger M. Prevention and handling of acute allergic and infusion reactions in oncology. *Ann Oncol.* 2012;23 Suppl 10:x313-319.
23. Greenberger PA, Patterson R, Tapio CM. Prophylaxis against repeated radiocontrast media reactions in 857 cases. Adverse experience with cimetidine and safety of beta-adrenergic antagonists. *Arch Intern Med.* 1985;145(12):2197-2200.
24. Cook J, Shuster S. Lack of effect of H2 blockade in chronic urticaria [proceedings]. *Br J Dermatol.* 1979;101 Suppl 17:21-22.
25. Greenberger P, Harris K, Patterson R. The effect of histamine-1 and histamine-2 antagonists on airway responses to histamine in the rhesus monkey. *J Allergy Clin Immunol.* 1979;64(3):189-196.
26. Prado CM. Body composition in chemotherapy: the promising role of CT scans. *Curr Opin Clin Nutr Metab Care.* 2013;16(5):525-533.
27. Prado CM, Lima IS, Baracos VE, et al. An exploratory study of body composition as a determinant of epirubicin pharmacokinetics and toxicity. *Cancer Chemother Pharmacol.* 2011;67(1):93-101.
28. Cespedes Feliciano EM, Chen WY, Lee V, et al. Body Composition, Adherence to Anthracycline and Taxane-Based Chemotherapy, and Survival After Nonmetastatic Breast Cancer. *JAMA Oncol.* 2019.
29. Hopkins JJ, Sawyer MB. A review of body composition and pharmacokinetics in oncology. *Expert Rev Clin Pharmacol.* 2017;10(9):947-956.
30. de Man FM, van Eerden RAG, Oomen-de Hoop E, et al. Efficacy and Toxicity of Weekly Carboplatin and Paclitaxel as Induction or Palliative Treatment in Advanced Esophageal Cancer Patients. *Cancers (Basel).* 2019;11(6).
31. Polee MB, Sparreboom A, Eskens FA, et al. A phase I and pharmacokinetic study of weekly paclitaxel and carboplatin in patients with metastatic esophageal cancer. *Clin Cancer Res.* 2004;10(6):1928-1934.
32. Chirieac LR, Swisher SG, Ajani JA, et al. Posttherapy pathologic stage predicts survival in patients with esophageal carcinoma receiving preoperative chemoradiation. *Cancer.* 2005;103(7):1347-1355.
33. Toxopeus ELA, de Man FM, Krak N, et al. Association between Paclitaxel Clearance and Tumor Response in Patients with Esophageal Cancer. *Cancers (Basel).* 2019;11(2).
34. Krause DS, Van Etten RA. Tyrosine kinases as targets for cancer therapy. *N Engl J Med.* 2005;353(2):172-187.
35. Dy GK, Adjei AA. Understanding, recognizing, and managing toxicities of targeted anticancer therapies. *CA Cancer J Clin.* 2013;63(4):249-279.
36. Jiao Q, Bi L, Ren Y, Song S, Wang Q, Wang YS. Advances in studies of tyrosine kinase inhibitors and their acquired resistance. *Mol Cancer.* 2018;17(1):36.
37. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med.* 2008;359(4):378-390.
38. Brose MS, Nutting CM, Jarzab B, et al. Sorafenib in radioactive iodine-refractory, locally advanced or metastatic differentiated thyroid cancer: a randomised, double-blind, phase 3 trial. *Lancet.* 2014;384(9940):319-328.
39. Escudier B, Eisen T, Stadler WM, et al. Sorafenib for treatment of renal cell carcinoma: Final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial. *J Clin Oncol.* 2009;27(20):3312-3318.
40. Agency. EM. https://www.ema.europa.eu/.

41. Lipworth AD, Robert C, Zhu AX. Hand-foot syndrome (hand-foot skin reaction, palmar-plantar erythrodysesthesia): focus on sorafenib and sunitinib. *Oncology.* 2009;77(5):257-271.
42. Zimmerman EI, Gibson AA, Hu S, et al. Multikinase Inhibitors Induce Cutaneous Toxicity through OAT6-Mediated Uptake and MAP3K7-Driven Cell Death. *Cancer Res.* 2016;76(1):117-126.
43. Robbins N, Koch SE, Tranter M, Rubinstein J. The history and future of probenecid. *Cardiovasc Toxicol.* 2012;12(1):1-9.
44. Daimon M, Kato T, Kaino W, et al. Thyroid dysfunction in patients treated with tyrosine kinase inhibitors, sunitinib, sorafenib and axitinib, for metastatic renal cell carcinoma. *Jpn J Clin Oncol.* 2012;42(8):742-747.
45. van Doorn L, Eskens FA, Visser TJ, van der Lugt A, Mathijssen RH, Peeters RP. Sorafenib induced thyroiditis in two patients with hepatocellular carcinoma. *Thyroid.* 2011;21(2):197-202.
46. Makita N, Iiri T. Tyrosine kinase inhibitor-induced thyroid disorders: a review and hypothesis. *Thyroid.* 2013;23(2):151-159.
47. Zimmerman MA, Ghobrial RM, Tong MJ, et al. Recurrence of hepatocellular carcinoma following liver transplantation: a review of preoperative and postoperative prognostic indicators. *Arch Surg.* 2008;143(2):182-188; discussion 188.
48. Neves KB, Montezano AC, Lang NN, Touyz RM. Vascular toxicity associated with anti-angiogenic drugs. *Clin Sci (Lond).* 2020;134(18):2503-2520.
49. de Jesus-Gonzalez N, Robinson E, Moslehi J, Humphreys BD. Management of antiangiogenic therapy-induced hypertension. *Hypertension.* 2012;60(3):607-615.
50. Moslehi JJ. Cardiovascular Toxic Effects of Targeted Cancer Therapies. *N Engl J Med.* 2016;375(15):1457-1467.
51. Lankhorst S, Baelde HJ, Clahsen- van Groningen MC, Smedts FM, Danser AH, van den Meiracker AH. Effect of high salt diet on blood pressure and renal damage during vascular endothelial growth factor inhibition with sunitinib. *Nephrol Dial Transplant*. 2016;31(6):914-921.

PART I

CHEMOTHERAPY

CHAPTER 2

CAPECITABINE AND THE RISK OF FINGERPRINT LOSS

JAMA Oncology. 2017;3(1):122-123

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and Ron H.J. Mathijssen

INTRODUCTION

Anticancer treatments are frequently accompanied by cutaneous side effects: capecitabine treatment induces hand-foot syndrome (HFS) in 50% to 60% of patients, whereas hand-foot skin reaction (HFSR) has been reported in 19% to 34% of patients treated with the tyrosine kinase inhibitors (TKIs) sunitinib malate or sorafenib tosylate.[1] Ultimately, these cutaneous adverse events are believed to result in the loss of fingerprints, which to our knowledge, has been described anecdotally for patients treated with capecitabine[2-6] and can cause serious identification problems. We assessed the association of HFS and HFSR with fingerprint quality.

METHODS

This prospective cohort study was performed at the Erasmus MC Cancer Institute, Rotterdam, the Netherlands, and included 337 ten-fingerprint sets from 150 patients. The principal inclusion criterion was a planned daily treatment with capecitabine (as monotherapy or combination therapy) or a TKI. Previous treatment with these drugs was not allowed. Fingerprints were taken from all patients' fingers using a digital fingerprint scanner (MorphoLivescan; Morpho) before treatment, within 6 to 10 weeks after the start of treatment and after treatment discontinuation. At the same time, digital photographic images (Nikon Corporation) were made of the palms and fingers of patients to detect abnormalities that could affect the fingerprints. Three dactyloscopists and a detective from the Netherlands National Police Agency visually assessed fingerprints and images, respectively. The baseline fingerprints were compared with the fingerprints during treatment and were scored on overall quality of friction ridge details and the suitability for individualization purposes. A 5-point scale was used on which slight improvement was scored as 1, no changes as 2, slightly decreased quality as 3, major loss of quality as 4, and total loss of fingerprint quality as 5. The scores were averaged, and, subsequently, these results were dichotomized to severe quality loss (score 4-5) or no severe changes in fingerprints (score 1-3). The severity levels of HFS and HFSR were graded according the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.03. Groups were compared using a χ^2 test. The institutional review board of the Erasmus MC Cancer Institute approved the study protocol, and written informed consent was obtained from all patients.

RESULTS

Between July 5, 2013 and July 12, 2015, we recorded 337 ten-fingerprint sets with corresponding digital images from 150 patients. A total of 112 patients, predominantly having colorectal cancer (n = 49) or hepatocellular carcinoma (n = 31), provided fingerprints at baseline and during treatment, of which 66 patients were treated with capecitabine and 46 patients with the TKIs sorafenib (n = 30), pazopanib hydrochloride (n = 10), or sunitinib (n = 6). Within 8 weeks of treatment, severe quality loss of fingerprints (**Figure)** was noticed in 9 patients (14%) treated with capecitabine and in 1 patient (2%) treated with the TKI sunitinib. In addition, HFS and HFSR were observed in 46 patients (70%) treated with capecitabine and in 21 patients (46%) treated with the TKIs. The grades for HFS and HFSR were not associated with the incidence of severe fingerprint quality loss ($P = .43$ and $P = .41$, respectively). Severe fingerprint quality loss recovered completely within 2 to 4 weeks after treatment discontinuation in all 3 patients who were able to provide posttreatment fingerprints.

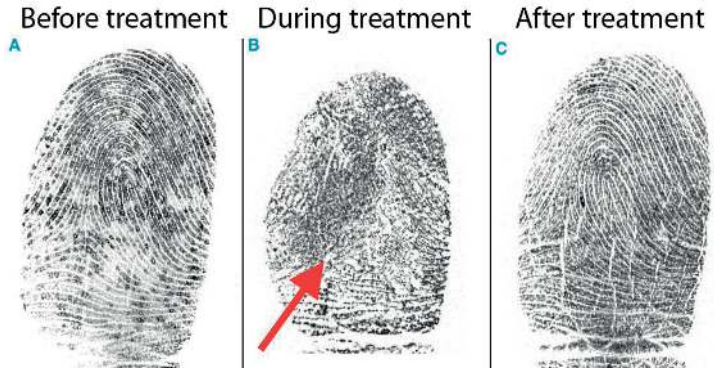

Figure. Detailed fingerprint patterns of 1 fingertip
Detailed fingerprint patterns from 1 patient, obtained before (A), during (B), and after (C) treatment with capecitabine. The arrowhead points to the complete loss of friction ridge details, which has recovered completely after treatment discontinuation.

DISCUSSION

Severe fingerprint quality loss is a frequent adverse event during capecitabine treatment. We demonstrated that HFS is not associated with the loss of fingerprints, which seems to be reversible after treatment discontinuation. Still, the fingerprint loss may cause significant difficulties for patients in their daily lives because this adverse effect of capecitabine treatment has caused identification problems at state borders.[2,3,6] Moreover, fingerprints are increasingly used for identification on personal electronic devices,

such as smartphones and computer laptops. Although fingerprint loss has no clinical significance, physicians should be aware of its major consequences in daily lives of the affected patients.

ADDITIONAL CONTRIBUTIONS

John Riemen and Bart Kraus provided technical advice and contributed in borrowing the fingerprint scanner from Morpho. In addition, John Turfboer, Anne Borgman van Vliet, and Liesbeth Rensen the dactyloscopists performed the fingerprint analyses. None received financial compensation for their work.

REFERENCES

1. Balagula Y, Wu S, Su X, Feldman DR. F, Lacouture ME. The risk of hand foot skin reaction to pazopanib, a novel multikinase inhibitor: a systematic review of literature and meta-analysis. *Invest New Drugs*. 2012;30:1773-1781.
2. Garcia-Saénz JA, Martin M, Díaz-Rubio E. Elementary, My Dear Watson. *J Clin Oncol*. 2007;25:1815-1816.
3. Wong M, Choo SP, Tan EH. Travel warning with capecitabine. *Ann Oncol.* 2009; 20:1281-1286.
4. Al-Ahwal MS. Chemotherapy and fingerprint loss: Beyond Cosmetic. *Oncologist*. 2012;17:291-293.
5. Rovere RK, de Lima AS. Forbidden to Drive -a New Chemotherapy Side Effect. *Klin Onkol.* 2015;28:370-372.
6. Chavarri-Guerra Y, Soto-Perez-de Celis E. Images in clinical medicine. Loss of fingerprints. *N Engl J Med.* 2015;372:e22.

CHAPTER 3

EFFECT OF THE PROTON PUMP INHIBITOR ESOMEPRAZOLE ON THE SYSTEMIC EXPOSURE OF CAPECITABINE: RESULTS OF A RANDOMIZED CROSSOVER TRIAL

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ABSTRACT

Background

Retrospective data suggest that gastric acid reduction by proton pump inhibitors (PPIs) impairs the dissolution and subsequent absorption of capecitabine, and thus potentially reduces the capecitabine exposure. Therefore, we examined prospectively the effect of esomeprazole on the pharmacokinetics of capecitabine.

Methods

In this randomized crossover study, patients with cancer were assigned to 2 sequence groups, each consisting of 3 phases: capecitabine with esomeprazole administration 3 hours before (phase A), capecitabine alone (phase B), and capecitabine concomitant with cola and esomeprazole co-administration 3 hours before (phase C). The primary end point was the relative difference (RD) in exposure to capecitabine assessed by the area under the plasma concentration-time curve from zero to infinity ($AUC_{0\text{-}inf}$) and analyzed by a linear mixed effect model.

Results

Twenty-two evaluable patients were included in the analysis. After esomeprazole, there was a 18.9% increase in $AUC_{0\text{-}inf}$ of capecitabine (95% confidence interval (CI), -10.0% to 57.0%, $P = 0.36$). In addition, capecitabine half-life was significantly longer after esomeprazole (median 0.63 hours vs. 0.46 hours, $P = 0.005$). Concomitant cola did not completely reverse the effects observed after esomeprazole (RD 3.3% (95% CI, -16.3 to 27.4%, $P = 1.00$).

Conclusion

Capecitabine exposure is not negatively influenced by esomeprazole cotreatment. Therefore, altered capecitabine pharmacokinetics do not explain the assumed worse clinical outcome of PPI-cotreated patients with cancer.

INTRODUCTION

Capecitabine, an oral prodrug of the active metabolite 5-fluorouracil (5-FU), is a frequently used antimetabolic agent in solid tumors, including breast cancer, gastroesophageal cancer, and colorectal cancer. It is most frequently administered in a 2 weeks-on, 1 week-off, schedule. After oral administration, capecitabine is rapidly and completely absorbed from the gastrointestinal tract as an intact molecule and is metabolized to 5-FU via a 3-step enzymatic cascade.[1] First to 5'-deoxy-5-fluorocytidine (5'-DFCR) by carboxylesterase (primarily in the liver), then to 5'-deoxy-5-fluorouridine (5'-DFUR) by cytidine deaminase (in tumor cells and liver), and finally to the active drug 5-FU by thymidine phosphorylase.[1]

A potential problem with orally administered agents is the variability in absorption due to various factors, such as food and/or comedication.[2-4] With capecitabine administered after food, a reduced exposure was demonstrated, however, with a minimal effect on the exposure to 5-FU.[4] In a study with the aluminium and magnesium containing antacid Maalox co-administered with capecitabine, an increased exposure to capecitabine was seen with minimal impact on the metabolite 5'-deoxy-5-fluorocytidine and no effect on other metabolites.[5] Hence, these specific interactions are not considered to be of clinical relevance.

Recent research has pointed toward a clinically relevant interaction between capecitabine and proton pomp inhibitors (PPIs). Capecitabine used concomitantly with several PPIs compared to the same regimens without PPIs resulted in a study of Chu et al. in patients with gastroesophageal cancer, in a significant reduction in median progression-free survival of 4.2 months vs 5.7 months ($P = < 0.001$) and median overall survival 9.2 months vs. 11.3 months, ($P = 0.04$). Sun et al. showed in patients with early stage colorectal cancer treated with capecitabine concomitant with PPI therapy a decrease in 5-year recurrence-free survival (74% vs. 83%, $P = 0.03$).[6,7]

The authors have speculated that changes in the stomach pH value following PPI administration reduce dissolution and absorption of capecitabine in the gastrointestinal tract.[6,7] These conclusions unfortunately were not supported by pharmacokinetic (PK) data of capecitabine or 5-FU. Given the potential impact of this specific interaction,[8] we prospectively assessed the systemic exposure to capecitabine and 5-FU with or without PPI (esomeprazole) co-administration. In addition, we investigated whether this potential PK interaction could be reversed by addition of the acidic beverage cola, as previously demonstrated by our group with the tyrosine kinase inhibitor erlotinib.[9]

METHODS

Trial design and outcome

This randomized two-armed, three-phase, crossover, interventional study was performed between February 2018 and December 2020 at the Erasmus MC Cancer Institute Rotterdam, The Netherlands. The study was approved by the local ethics committee of the Erasmus Medical Center (number MEC 17-552) and competent authority. The study was registered at the European Clinical Trials Database (EudraCT 2017-004465-27) and the Dutch trial registry (www.trialregister.nl; number NL6849).

In order to assess the effect of PPIs on the absorption of capecitabine, the primary outcome was to evaluate the area under the plasma concentration-time curve (AUC) of capecitabine alone as compared to capecitabine used with the PPI esomeprazole, and compared with capecitabine used with esomeprazole and cola. The secondary outcome was to study the maximum concentration (C_{max}) and time to C_{max} (T_{max}) of capecitabine, and to determine the AUC, C_{max} and T_{max} of 5-FU.

Participants and treatment

Adult patients (aged ≥ 18 years) with a confirmed diagnosis of a solid tumor planned for capecitabine treatment according to standard of care (as monotherapy or in combination with oxaliplatin or bevacizumab) and an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2, who provided written informed consent, were eligible to participate in the study. Prior treatment with capecitabine without a documented history of ≥ grade 3 toxicity was allowed. Patients actively treated for diabetes mellitus, patients who could not abstain from grapefruit juice, dietary supplements, or medication which could interact with capecitabine or esomeprazole (Nexium), and/or patients who could not interrupt gastric acid-suppressive therapy for a period of 8 days and - if necessary - were unwilling to switch to esomeprazole 40 mg once daily during the study period, were excluded.

Additionally, patients with a known impaired drug absorption (e.g., achlorhydria), a complete deficiency of dihydropyrimidine dehydrogenase activity, use of strong CYP 2C19/3A4 inducers and/or inhibitors, and pregnant and lactating women were also excluded.

Patients were treated with capecitabine twice daily for 2 weeks followed by a 1-week rest period in 3-week cycles [10] and were dosed between 2,000 mg and 3,500 mg daily[11] according to the physician's discretion. In addition, *DPYD* genotyping for variants *2A, c.2846A>T, c.1679T>G and c.1236G>A was performed, which is considered standard practice in the Netherlands.[12] Because capecitabine has linear PKs[1] dose adjustments (e.g., due to toxicity) were allowed after the first 8 study days of a cycle and by the start of a new cycle.

Patients used the morning dose of capecitabine with esomeprazole (40 mg once daily) for 4 consecutive days (phases A and C) or capecitabine alone (phase B) within 30 minutes after a meal according to the package insert.[13] During phase A and phase C, the morning dose of capecitabine was administered 3 hours after esomeprazole intake, presuming a maximally elevated intragastric pH at the time of capecitabine intake.[14] During phase C, the capecitabine morning dose was administered concomitantly with 250 mL of cola (Coca Cola Classic), whereas in phases A and B, capecitabine was administrated with water. All patients were asked to fill in a diary to check for compliance and toxicities during each study period. Adverse events were classified based on the Common Terminology Criteria for Adverse Events, version 4.03.[15] The incidence of adverse events was obtained from electronic case records and patient diaries. Adverse events which were present at baseline were only registered if they worsened during treatment. To take possible sequence and time effects into account, patients were randomized into two sequence groups: sequence phase A-B-C or phase C-B-A.

Capecitabine pharmacokinetics

Patients were admitted to the hospital on day 8 of a course for a PK blood sampling day. Blood samples were collected at predefined time points just before capecitabine intake, and at 0.25 hours, 0.5 hours, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 8 hours (in total, 9 time points per PK day) after the first oral morning capecitabine dose during each of the study phases.

Details on the processing of the blood, the measurement of capecitabine, and 5-FU[16] are further outlined in the **Methods S1**. Predefined PK endpoints were the AUC from the pre-administration time point until infinity ($AUC_{0\text{-}inf}$), C_{max}, T_{max} and the elimination half-life at which $AUC_{0\text{-}inf}$ and C_{max} were dose corrected to 1,500 mg capecitabine (PK parameter * (standard dose (1,500 mg)/administered dose). The parameters were determined using WinNonlin version 8.3 (Phoenix, Certara, Princeton, NJ, USA) for both capecitabine and 5-FU.

STATISTICAL ANALYSIS

A difference in the systemic exposure to capecitabine of 25% was considered to be clinically relevant.

It was assumed that the within-patient SD was 27%.[1] For capecitabine, the AUC of the 3 sampling days were compared "pair wisely" to each other. Therefore, the Bonferroni correction was applied to correct for multiple testing resulting in a 2-sided alpha of 0.0167. Given a power of 80%, the sample size calculation resulted in a required number of 22

evaluable patients.[17,18] Patients were considered evaluable when they completed all the three study phases.

Analyses of $AUC_{0\text{-}inf}$, were performed on log-transformed values. Estimates for the mean differences in (log) $AUC_{0\text{-}inf}$ were obtained using a linear mixed effect model with treatment, sequence, and period as fixed effects and patient within sequence as a random effect.[19] Variance components were estimated based on restricted maximum likelihood methods and the Kenward-Roger method of computing the denominator degrees of freedom was used.

The mean differences were exponentiated to provide point estimates of the ratio of geometric means and the Bonferroni-corrected 95% confidence intervals (CIs; i.e., 98.333% CIs were calculated) for these ratios, which can be interpreted as relative differences in percentages (RD = (geometric mean ratio-1)*100%). Because the aim was to show bioequivalence of the PK parameters of capecitabine alone and the combination of capecitabine, esomeprazole, and cola, a Bonferroni-corrected 90% CI (i.e., 96.667% CI) was determined for the comparison of these 2 phases. Bioequivalence is shown if this CI of the geometric mean ratio lies within 0.80 and 1.25.

The secondary PK outcomes C_{max} of capecitabine and the AUC and C_{max} of 5-FU were analyzed in a similar way as the AUC, whereas T_{max}, and elimination terminal half-life was analyzed by means of the Wilcoxon signed rank test. Analyses were performed using Stata (StataCorp version 16.1, 2020. Statistical Software, College Station, TX, USA).

RESULTS

Participants

Between January 2018 and December 2020, 32 patients were enrolled into the study **(Figure 1).**

In total, 22 patients (phase A-B-C, n = 13; phase C-B-A, n = 9) completed all study phases and were evaluable for analysis. Patient characteristics are summarized in **Table 1**.

Effect of esomeprazole on the pharmacokinetics of capecitabine and 5-FU

The dose-corrected PK parameters $AUC_{0\text{-}inf}$ and C_{max} of capecitabine and its active metabolite 5-FU are shown in **Figure 2** and summarized for all the study phases in **Table 2**. After esomeprazole co-administration, the geometric mean $AUC_{0\text{-}inf}$ and C_{max} of capecitabine increased with 18.9 % (95% CI, -10.0% to 57.0%, P = 0.36) and 9.9% (95% CI, -33.0% to 80.1%, P = 1.00), respectively. Esomeprazole led to a delayed median T_{max} (2 hours vs. 1 hour, P = 1.00) and a longer median plasma half-life of capecitabine (0.63h vs. 0.46h, P = 0.005; **(Figure 3)**.

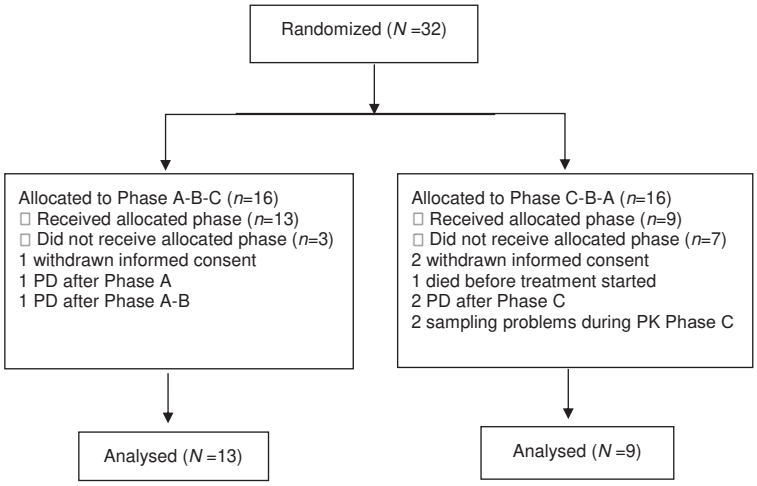

Figure 1. Consort flow diagram. Phase A: Capecitabine with esomeprazole, 3 hours before capecitabine intake for 4 days (days 5-8). Phase B: Capecitabine alone. Phase C: Capecitabine intake with 250 mL of cola and esomeprazole, 3 hours before capecitabine intake (days 5-8). PD, progressive disease; PK, pharmacokinetic.

The differences in capecitabine PKs after esomeprazole were slightly reversed by concomitant cola use: the geometric mean ratio of $AUC_{0\text{-inf}}$ of capecitabine + esomeprazole + cola vs. capecitabine alone was 1.04 with Bonferroni corrected 90% CI ranging from 0.84-1.28. No sequence nor period effects were seen for any of the comparisons of the $AUC_{0\text{-inf}}$ and C_{max} (results not shown).

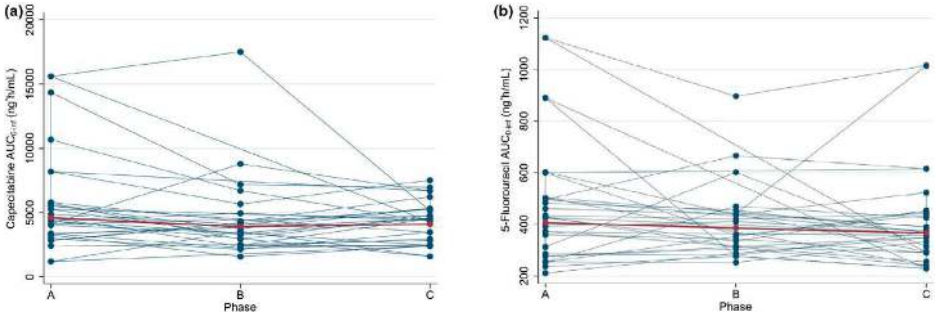

Figure 2. Scatter plots illustrating the AUC $_{0\text{-inf}}$ of -capecitabine (a) and 5-FU (b) per subject for each study phase; AUC $_{0\text{-inf}}$ was dose-corrected to 1,500 mg capecitabine. Phase A (capecitabine with esomeprazole, 3 hours prior) phase B (capecitabine alone) and phase C (capecitabine intake with concomitant 250 mL of cola and esomeprazole 3 hours prior capecitabine intake). The blue lines connect the values for each individual patient. The bold red line depicts the geometric means. The estimated parameters of patients were dose corrected to 1,500 mg capecitabine. 5-FU, 5-fluorouracil; AUC $_{0\text{-inf}}$ = area under the curve from zero to infinity.

Table 1. Patient characteristics

	Phase A-B-C	Phase C-B-A	Total
Characteristics	**(*n* = 13)**	**(*n* = 9)**	**(*N* = 22)**
Gender			
Female	2 (15%)	3 (33%)	5 (23%)
Male	11 (85%)	6 (66%)	17 (77%)
Age (years), median [IQR]	56 [51-63]	59 [53-61]	58 [52-63]
ECOG Performance Status			
0	1 (8%)	1 (11%)	2 (9%)
1	12 (92%)	8 (89%)	20 (91%)
Ethnic origin			
White	12 (92%)	9 (100%)	21 (95%)
Black	1 (8%)	0	1 (5%)
Tumor type			
Colorectal	10 (76%)	8 (89%)	18 (82%)
Esophagus/gastric	3 (23%)	0	3 (14%)
Parathyroid carcinoma	0	1 (11%)	1 (4%)
Metastatic disease	12 (92%)	8 (89%)	20 (90%
Prior oncological surgery			
Hemicolectomy	7 (54%)	4 (44%)	11 (50%)
DPYD status based on 4 genotypes			
Normal metabolizer	13 (100%)	9 (100%)	22 (100%)
Type of treatment regimen			
Capecitabine - monotherapy	3 (23%)	2 (22%)	5 (23%)
Capecitabine - oxaliplatin	7 (54%)	5 (56%)	12 (54%)
Capecitabine - bevacizumab	3 (23%)	2 (22%)	5 (23%)
Capecitabine cumulative daily dosing			
4,000 mg	2 (15%)	2 (22%)	4 (18%)
3,500 mg	8 (62%)	5 (56%)	13 (59%)
3,000 mg	2 (15%)	1 (11%)	3 (14%)
2,000 mg	1 (8%)	1 (11%)	2 (9%)

Data were expressed as *N* %. *DPYD*, gene encoding dihydropyrimidine dehydrogenase; ECOG, Eastern Cooperative Oncology Group; IQR, interquartile range.

Adverse events

The most common all-grade capecitabine-related adverse events observed were fatigue (50%) and nausea (9%). Grade ≥ 3 adverse events were not observed. In phase A and phase C there was a low grade (grade 1) headache (n = 6) as a possible side effect of esomeprazole.[20] All adverse events during the study periods are detailed in **Table S1**.

Table 2. Capecitabine and 5-FU pharmacokinetic results; $AUC_{0\text{-}inf}$ and C_{max} were dose-corrected to 1,500 mg capecitabine

PK parameter	Capecitabine + Esomeprazole 3h prior (phase A)	Capecitabine alone (phase B)	Capecitabine + cola concomitant + Esomeprazole 3h prior (phase C)	Relative difference phase A vs phase B (95%CI)	P-value	Relative difference phase C vs phase B (90%CI)	P-value
Capecitabine							
$AUC_{0\text{-}inf}$, ng*h/mL (CV%))	4601.6 (63.9)	3899.9 (58.5)	4098.5 (41.5)	18.9 % (-10.0% to 57.0%)	0.36	3.3% (-16.3% to 27.4%)	1.00
C_{max}, ng/mL (CV%))	3040.6 (89.2)	2832.1 (79.0)	2731.2 (47.1)	9.9% (-33.0% to 80.1%)	1.00	-5.0% (-33.6% to 35.9%)	1.00
T_{max}, median hours (IQR))	2.0 (1.0-3.0)	1.0 (1.0-2.0)	1.0 (0.5-2.0)		1.00		1.00
$T_{1/2}$, median hours (IQR))	0.63 (0.52-0.84)	0.46 (0.36-0.55)	0.51 (0.44-0.67)		0.005		0.06
5 FU							
$AUC_{0\text{-}inf}$, ng*h/mL (CV%))	406.7 (43.4)	385.9 (32.5)	366.4 (35.6)	7..8% (-12.3% to 32.4%)	1.00	-5.3% (-15.8% to 6.5%)	0.90
$C_{max,}$ (ng/mL (CV%))	181.5 (58.0)	198.6 (45.8)	168.2 (38.1)	-4.33% (-27.6% to 26.4%)	1.00	-15.4% (-30.0% to 2.2%)	0.17
$T_{max,}$ median hours (IQR))	2.0 (2.0-3.0)	2.0 (1.0-3.0)	2.0 (1.0-2.0)		1.00		1.00
$T_{1/2v}$ (median hours (IQR))	0.88 (0.71-1.02)	0.76 (0.70-0.79)	0.86 (0.75-1.01)		0.08		0.001

$AUC_{0\text{-}inf}$, area under the curve timepoint 0 hours to infinity (expressed as geometric mean ng*h/mL (CV)); CI, confidence interval; C_{max}, maximum concentration (expressed as geometric ng/mL (CV)); CV, coefficient of variation expressed in percentage; IQR, interquartile range; PK, pharmacokinetic; T_{max}, time until maximum concentration (expressed as median hours (IQR)); $T_{1/2}$ = terminal half-life (expressed as median hours (IQR)).

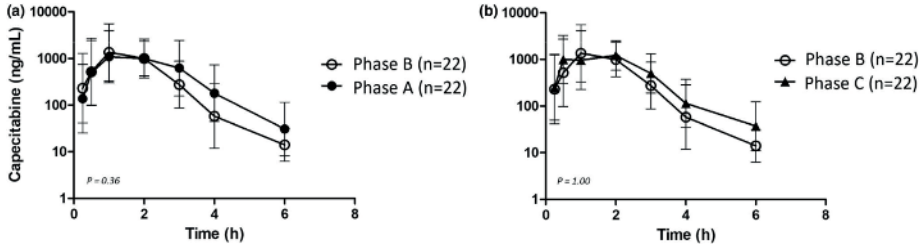

Figure 3. Concentration-time curves of capecitabine during each study phase. Capecitabine with esomeprazole, 3 hours prior (phase A, n = 22) compared to capecitabine alone (phase B, n = 22) and capecitabine intake with concomitant 250 mL of cola and esomeprazole 3 hours prior capecitabine intake (phase C, n=22) compared to capecitabine alone (phase B). Data at t = 8 hours are not shown because capecitabine concentrations were below the limit of quantification for most patients. The estimated parameters of patients were dose corrected to 1500 mg capecitabine. The difference in capecitabine $AUC_{0\text{-inf}}$ between phase A and phase B was not statistically significant (P = 0.36). Median capecitabine half-life was longer in phase A (0.63 hours) than in phase B (0.46 hours, *P* = 0.005). $AUC_{0\text{-inf}}$ = area under the curve from zero to infinity.

DISCUSSION

In this study, we prospectively assessed the role of esomeprazole co-administration on the systemic exposure of capecitabine and its active metabolite 5-FU and found a prolonged half-life of capecitabine following co-administration with esomeprazole. The addition of cola partly reversed the observed effects of esomeprazole co-administration on capecitabine PKs. We observed that the variability in capecitabine exposure was larger than was expected based on literature data,[1] which explains why an almost 19% increase in capecitabine exposure was not statistically significant. Nevertheless, the increase in capecitabine exposure after esomeprazole we found contradicts the theories that PPIs reduce capecitabine absorption and effect.[6,7]

These results might be caused by a prolonged absorption of capecitabine after cotreatment with PPIs and has previously also been observed after a single dose of capecitabine with concomitant Maalox.[5] As mentioned before, previous retrospective studies have shown a negative clinical impact on progression-free survival and overall survival of co-administration of a PPI with capecitabine.[6,7] One of the assumed PK mechanisms to explain this observation is diminished intestinal absorption of capecitabine due to decreased dissolution in a less acidic environment. This potentially relevant interaction is included in widely used drug interaction databases such as Micromedex and Lexicomp.[21] Given the higher, rather than lower, exposure to capecitabine after esomeprazole co-administration (i.e., the most potent gastric acid reducing PPI) observed in this study, we conclude that these observed differences in clinical outcome are not pharmacokinetically driven. Moreover, the likelihood of a drug interaction at absorption level has recently been challenged as the proposed dissociation constant of capecitabine is much

higher than previously assumed.[22] This probably explains why a decrease in capecitabine absorption has not been observed in PK interaction studies with Maalox[5] and rabeprazole[23] or in patients with a previous gastrectomy.[24] It has been proposed that PPIs might reduce gastrointestinal motility, but evidence on this subject is conflicting and it remains questionable whether cola would reverse this effect.[25,26] As the metabolism of capecitabine and its metabolites is not mediated by CYP2C19, the CYP2C19 inhibiting PPIs are not expected to cause any changes in capecitabine metabolism.

In our study, the observed statistically significant prolonged half-life of capecitabine following esomeprazole co-administration does not seem to represent inhibition of capecitabine metabolism since the effect was not observed when cola was concomitantly administered. There is no evidence or rationale of esomeprazole inhibiting capecitabine metabolism, let alone of cola reversing that inhibition. If the prolonged half-life after esomeprazole represents a true biological effect, it would be at the absorption level where the acidity of cola would completely reverse the effects of prolonged absorption, but this does not comply with previous evidence that capecitabine does not exhibit flip-flop PKs.[24] Last, at the cellular level, we cannot exclude that PPIs reduce the intratumoral exposure to (or activation of) the active capecitabine metabolites.

In absence of an evident PK explanation, the negative association between PPIs and survival after capecitabine might be caused by pharmacodynamic effects. This might be a direct pharmacodynamic interaction at the cellular level, but this is not supported by previous *in vitro* studies,[23] as no effect of rabeprazole on the inhibitory effects of capecitabine metabolites on colon cancer cell line proliferation was found. Alternatively, indirect pharmacodynamic mechanisms might cause the interaction, as PPIs are known to inhibit the absorption of several vitamins and minerals, such as magnesium, which has been associated with adverse cancer outcome.[27]

Alternatively, and most relevantly, the potential drug interaction between capecitabine and PPIs has only been described in one retrospective and one *post hoc* analysis[6,7] and therefore needs to be questioned. Moreover, in a recent third analysis from the phase III AXEPT trial in patients with colorectal cancer,[28] patients using PPIs did not have worse survival on capecitabine and irinotecan than those not on PPI cotreatment. In contrast, using PPIs was associated with better survival after a 5-FU containing regimen in that study. These conflicting results cause that no hard conclusions can be drawn on the existence of a true interaction between capecitabine and PPIs.

In conclusion, we have shown that capecitabine exposure is not negatively influenced by esomeprazole cotreatment. Therefore, altered capecitabine PKs do not explain the assumed worse clinical outcome of PPI cotreated patients with cancer. Because we cannot exclude a pharmacodynamic drug-drug interaction, prospective studies are warranted to truly confirm that there exists a drug-drug interaction between capecitabine and PPIs and, if present, to elucidate the mechanisms behind this interaction.

REFERENCES

1. Reigner B, Blesch K, Weidekamm E. Clinical pharmacokinetics of capecitabine. *Clin Pharmacokinet.* 2001;40(2):85-104.
2. Parsad S, Ratain MJ. Food Effect Studies for Oncology Drug Products. *Clin Pharmacol Ther.* 2017;101(5):606-612.
3. Scripture CD, Figg WD. Drug interactions in cancer therapy. *Nat Rev Cancer.* 2006;6(7):546-558.
4. Reigner B, Verweij J, Dirix L, et al. Effect of food on the pharmacokinetics of capecitabine and its metabolites following oral administration in cancer patients. *Clin Cancer Res.* 1998;4(4):941-948.
5. Reigner B, Clive S, Cassidy J, et al. Influence of the antacid Maalox on the pharmacokinetics of capecitabine in cancer patients. *Cancer Chemother Pharmacol.* 1999;43(4):309-315.
6. Chu MP, Hecht JR, Slamon D, et al. Association of Proton Pump Inhibitors and Capecitabine Efficacy in Advanced Gastroesophageal Cancer: Secondary Analysis of the TRIO-013/LOGiC Randomized Clinical Trial. *JAMA Oncol.* 2017;3(6):767-773.
7. Sun J, Ilich AI, Kim CA, et al. Concomitant Administration of Proton Pump Inhibitors and Capecitabine is Associated With Increased Recurrence Risk in Early Stage Colorectal Cancer Patients. *Clin Colorectal Cancer.* 2016;15(3):257-263.
8. Hussaarts K, van Leeuwen RWF, Mathijssen RHJ. Factors Affecting the Association of Proton Pump Inhibitors and Capecitabine Efficacy in Advanced Gastroesophageal Cancer. *JAMA Oncol.* 2018;4(2):263-264.
9. van Leeuwen RW, Peric R, Hussaarts KG, et al. Influence of the Acidic Beverage Cola on the Absorption of Erlotinib in Patients With Non-Small-Cell Lung Cancer. *J Clin Oncol.* 2016;34(12):1309-1314.
10. Koukourakis GV, Zacharias G, Tsalafoutas J, Theodoridis D, Kouloulias V. Capecitabine for locally advanced and metastatic colorectal cancer: A review. *World J Gastrointest Oncol.* 2010;2(8):311-321.
11. de Man FM, Veerman GDM, Oomen-de Hoop E, et al. Comparison of toxicity and effectiveness between fixed-dose and body surface area-based dose capecitabine. *Ther Adv Med Oncol.* 2019;11:1758835919838964.
12. Henricks LM, Lunenburg C, de Man FM, et al. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. *Lancet Oncol.* 2018;19(11):1459-1467.
13. https://www.ema.europa.eu/en/documents/product-information/capecitabine-accord-epar-product-information_en.pdf.
14. Hunfeld NG, Touw DJ, Mathot RA, et al. A comparison of the acid-inhibitory effects of esomeprazole and pantoprazole in relation to pharmacokinetics and CYP2C19 polymorphism. *Aliment Pharmacol Ther.* 2010;31(1):150-159.
15. US Department of Health and Human Services. Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03. June 14, 2010. Maryland, United States: US National Institute of Health.
16. Konings IR, Sleijfer S, Mathijssen RH, et al. Increasing tumoral 5-fluorouracil concentrations during a 5-day continuous infusion: a microdialysis study. *Cancer Chemother Pharmacol.* 2011;67(5):1055-1062.
17. Hills M, Armitage P. The two-period cross-over clinical trial. *Br J Clin Pharmacol.* 1979;8(1):7-20.
18. Schoenfeld D. Statistical considerations for a cross-over study where the outcome is a measurement. http://hedwig.mgh.harvard.edu/sample_size/js/js_crossover_quant.html, 2018.
19. Jones B. *Design and analysis of cross-over trials: second edition.* United States: Chapman & Hall/CRC; 2003.

20. Agency. EM. https://www.ema.europa.eu/.
21. Vinal D, Rodriguez-Salas N, Perez-Wert P, Higuera O, Ghanem I, Feliu J. Efficacy of capecitabine when used concomitantly with proton pump inhibitors in cancer patients: a systematic review. *Clin Transl Oncol.* 2020;22(8):1288-1294.
22. Cheng V, Lemos M, Hunter N, Badry N, Lemos J. Concomitant use of capecitabine and proton pump inhibitors - Is it safe? *J Oncol Pharm Pract.* 2019;25(7):1705-1711.
23. Sekido M, Fujita KI, Kubota Y, et al. Rabeprazole intake does not affect systemic exposure to capecitabine and its metabolites, 5'-deoxy-5-fluorocytidine, 5'-deoxy-5-fluorouridine, and 5-fluorouracil. *Cancer Chemother Pharmacol.* 2019;83(6):1127-1135.
24. Jacobs BAW, Deenen MJ, Joerger M, et al. Pharmacokinetics of Capecitabine and Four Metabolites in a Heterogeneous Population of Cancer Patients: A Comprehensive Analysis. *CPT Pharmacometrics Syst Pharmacol.* 2019;8(12):940-950.
25. Sanaka M, Yamamoto T, Kuyama Y. Effects of proton pump inhibitors on gastric emptying: a systematic review. *Dig Dis Sci.* 2010;55(9):2431-2440.
26. Michalek W, Semler JR, Kuo B. Impact of acid suppression on upper gastrointestinal pH and motility. *Dig Dis Sci.* 2011;56(6):1735-1742.
27. Gile J, Ruan G, Abeykoon J, McMahon MM, Witzig T. Magnesium: The overlooked electrolyte in blood cancers? *Blood Rev.* 2020;44.
28. Kim SY, Lee JS, Kang J, et al. Proton Pump Inhibitor Use and the Efficacy of Chemotherapy in Metastatic Colorectal Cancer: A Post Hoc Analysis of a Randomized Phase III Trial (AXEPT). *Oncologist.* 2021;26:e954-962.

Supplementary Table 1. Adverse events

	Capecitabine + esomeprazole 3h prior Phase A n (%)	Capecitabine alone Phase B n (%)	Capecitabine + cola concomitant + esomeprazole 3 h prior Phase C n (%)	Overall# N (%)
Gastrointestinal				
Anorexia				
All grades	0	3 (14)	0	0
Grade ≥ 3	0	0	0	0
Constipation				
All grades	0	1 (5)	3 (14)	0
Grade ≥ 3	0	0	0	0
Diarrhea				
All grades	3 (14)	1 (5)	3 (14)	0
Grade ≥ 3	0	0	0	0
Mucositis				
All grades	0	2	1 (5)	0
Grade ≥ 3	0	0	0	0
Nausea				
All grades	6 (27)	6 (27)	6 (27)	2 (9)
Grade ≥ 3	0	0	0	0
Vomiting				
All grades	2 (9)	2 (9)	2 (9)	1 (5)
Grade ≥ 3	0	0	0	0
Skin tissue disorders				
Hand-foot syndrome				
All grades	1 (5)	2 (9)	3 (14)	1 (5)
Grade ≥ 3	0	0	0	
General disorders				
Fatigue				
All grades	15 (68)	15 (68)	14 (63)	11 (50)
Grade ≥ 3	0	0	0	0
Pain				
Headache				
All grades	3 (14)	0	3 (14)	0
Grade ≥ 3	0	0	0	0
Abdominal				
All grades	1 (5)	1 (5)	0	0
Grade ≥ 3	0	0	0	0

	Capecitabine + esomeprazole 3h prior **Phase A** **n (%)**	**Capecitabine alone** **Phase B** **n (%)**	**Capecitabine + cola concomitant + esomeprazole 3 h prior** **Phase C** **n (%)**	**Overall#** **N (%)**
Blood value disorders				
Anemia				
All grades	2 (9)	2 (9)	2 (9)	1 (5)
Grade ≥ 3	0	0	0	0
AST/ALT increase				
All grades	2 (9)	1 (5)	1 (5)	1 (5)
Grade ≥ 3	0	0	0	0
Bilirubin increased				
All grades	2 (9)	1 (5)	1 (5)	1 (5)
Grade ≥ 3	0	0	0	0
Neutropenia				
All grades	1 (5)	1 (5)	0	0
Grade ≥ 3	0	0	0	0
Platelet count decreased				
All grades	1 (5)	1 (5)	2 (9)	1 (5)
Grade ≥ 3	0	0	0	0

Number of patients is scored as individual patients per phase.*Toxicity was graded according to the NCI-CTCAE classification (version 4.03)

Overall toxicity was defined as the number of patients during the whole study period (i.e., all the three phases). AST = aspartate aminotransferase; ALT = alanine aminotransferase, h = hour, N = numerous.

SUPPLEMENTARY METHODS

Detailed description assay capecitabine. Blood samples were collected in 4 mL lithium heparin blood collection tubes and processed into plasma (within 10 minutes) by centrifugation for 10 minutes at 2,500**g* (at 4^0 C). Plasma was transferred into polypropylene tubes (1,8 mL Nunc® tubes) and frozen immediately at -80°C until analysis. Capecitabine was extracted from 25 µL aliquots of human lithium heparinized plasma after the addition of 100 µL Internal Standard Working Solution (10 ng/mL capecitabine-d11 in acetonitrile). An aliquot of 5 µL of the clear supernatant was injected into the UPLC-MS/MS system. 5-FU was extracted from 30 µL aliquots of human lithium heparinized plasma after the addition of 10 µL Internal Standard Working Solution (500 ng/mL 5-fluorouracil-13C,15N2 in water) with 1,5 mL ethyl acetate. After evaporation of the organic phase and re-suspension of the residue in an aliquot of 50 µL of water/formic acid (100:0.02, v/v), an aliquot of 10 µL was injected into the UPLC-MS/MS system.

CHAPTER 4

EFFECT OF SCALP COOLING ON THE PHARMACOKINETICS OF PACLITAXEL

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ABSTRACT

Background

Chemotherapy-induced alopecia (CIA), a side effect with high impact, can be prevented by cooling the scalp during the administration of some cytotoxic drugs. However, the effects of this prolonged scalp cooling on the pharmacokinetics of chemotherapy have never been investigated.

Methods

In this study, we compared the pharmacokinetics of the widely used chemotherapeutic agent paclitaxel (weekly dose of 80-100 mg/m^2) in female patients with solid tumors using concomitant scalp cooling (n = 14) or not (n = 24). Blood samples were collected in all patients for pharmacokinetic analyses up to 6 hours after one course of paclitaxel administration. The primary endpoint was the clearance (L/h) of paclitaxel.

Results

Paclitaxel clearance– expressed as relative difference in geometric means– was 6.8% (90% CI, –16.7% to 4.4%) lower when paclitaxel was administered with concomitant scalp cooling versus paclitaxel infusions without scalp cooling. Within the subgroup of patients using the scalp cooling, paclitaxel clearance was not statistically significantly different between patients with CIA (alopecia grade 1 or 2) and those without CIA.

Conclusion

Hence, scalp cooling did not negatively influence the clearance of paclitaxel treatment.

INTRODUCTION

Chemotherapy-induced alopecia (CIA) is a commonly feared side effect of systemic anti-cancer treatment.[1] It can affect a patient's quality of life dramatically and is one of the most distressing and adverse aspects of anti-cancer treatment, particularly for women.[2] Scalp cooling is a well-known method to try to prevent CIA during the administration of cytotoxic drugs for solid tumors.[3,4] Using scalp cooling, liquid refrigerant is pumped as coolant through a cooling cap that is placed on the head of the patient. In general, scalp cooling is started 20–45 min prior to, during, and up to 20–150 min after the chemotherapy infusion.[5] Scalp cooling results in a locally decreased blood flow due to vasoconstriction, resulting in a lower chemotherapy concentration at the root of the hair follicles and thereby, hopefully, in hair preservation.[6] The pharmacokinetics and pharmacodynamics of several drugs are influenced by body temperature.[7] Deep scalp cooling (4 °C), lasting for 20–45 min before, continued during and lasting for up to 150 min after chemotherapy infusion, may potentially lead to a temperature reduction of the whole body. This drop in body temperature may lead to alterations in pharmacokinetics.[7] This is of clinical relevance as changes in pharmacokinetics may lead to under- or over-exposure to the drug of interest. In a previous study, a physiologically based pharmacokinetic model (PBPK) of doxorubicin was modified to include a scalp skin compartment. The results of the model showed that maximum and average concentrations of doxorubicin in the scalp skin compartment were reduced by a factor of 3.6 and 1.6, respectively, during scalp cooling. These effects were due to reduced tissue perfusion and can positively influence the survival of hair follicles. However, mass transfer characteristics were not considered.[8,9]

At present, there are no data available regarding the effects of scalp cooling on the pharmacokinetics of the cytotoxic drugs that are infused.

The severity of CIA, but also the success rate of scalp cooling, depends on the type of anti-cancer treatment used, its dose, method of administration and schedule of treatment.[10,11] Scalp cooling in patients treated with taxane-based chemotherapy such as paclitaxel, a widely used antineoplastic agent for the treatment of several cancers (e.g. breast, ovarian and esophageal cancer),[12-14] led to hair conservation in more than 50%, of patients, depending on the dose, compared with those who received no scalp cooling.[15] Scalp cooling is therefore offered as a part of standard treatment.The aim of the present study was to investigate the impact of scalp cooling on paclitaxel pharmacokinetics in women who were scheduled to start treatment with paclitaxel and opted for scalp cooling compared to women who did not, of which there is a historical cohort. Although scalp cooling is usually a good option to prevent hair loss, it is unclear why some patients still develop CIA despite scalp cooling.[2] Therefore, we also studied the

difference in paclitaxel pharmacokinetics between patients who developed alopecia compared to those who did not, despite scalp cooling.

MATERIALS AND METHODS

Study Design and Patient Population

The aim of the study was to compare the pharmacokinetics of weekly paclitaxel between female cancer patients (aged over 18 years) who did use scalp cooling (SC+) concomitantly and who did not scalp cooling (SC-). The pharmacological data of the SC+ patients were prospectively collected (MEC-2015-140, date of approval 25 January 2016, Dutch Trial Registry; www.trialregister.nl (accessed on 2 August 2021; NL5543) and the pharmacokinetic data of the 24 SC– patients came from a previous single center pharmacokinetic study (MEC-2003-264, date of approval 19 February 2004, Dutch Trial Registry; www.trialregister.nl (accessed on 2 August 2021; NL2187). Both studies were performed at the Erasmus MC Cancer Institute, Department of Medical Oncology, Rotterdam, the Netherlands. All participating patients were asked to sign a written informed consent form. The studies were conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board and the local Ethics Committee of the Erasmus MC Rotterdam. All patients were treated with paclitaxel infusions (combined with carboplatin) in a weekly dose of 80–100 mg/m^2. Paclitaxel was dissolved in 250 mL sodium chloride 0.9% and infused in 1 h (i.e., an infusion rate of 250 mL/h). In those patients who had a history of hypersensitivity reaction to paclitaxel, a standard stepwise increase in infusion rates was used: 15 min at 5 mL/h, followed by 15 min at 12.5 mL/h and then continued at the normal infusion rate of 250 mL/h. Premedication (dexamethasone, ranitidine and clemastine) was administrated just before the paclitaxel infusion and in accordance with local standards. Body temperature was only measured among the SC+ patients prior to the start of SC, at the start of the paclitaxel infusion (30 min after starting SC), 5 min prior to the end of the paclitaxel infusion and 180 min after ending the scalp cooling on three different body areas: in the mouth, in one ear and in one of the axillas. Scalp cooling was performed with a Paxman machine (PSC-2 Model, Paxman Coolers Limited, Huddersfield, UK).[16] Hair was wetted before the start of scalp cooling. Cooling to 4 °C started 30 min prior to the infusion of paclitaxel and the cooling continued until 60 min after the administration of paclitaxel.[10]

Pharmacokinetics of Paclitaxel

Blood samples for the pharmacological analyses were collected during one of the paclitaxel administrations, not necessarily the first administration, at four predefined time points: pre-dose, 55 (5 min prior to the end of the paclitaxel infusion), 90 and 360 min

after the start of the paclitaxel infusion.[17] In patients where paclitaxel was administered in a standard stepwise increase in infusion rates because of hypersensitivity during previously administered paclitaxel treatment, this was pre-dose, 85 (5 min prior to the end of the paclitaxel infusion), 120 and 360 min after the start of paclitaxel infusion. The samples were collected by venipuncture or cannula in 4.5 mL lithium heparin blood collection tubes and processed within 10 min by centrifugation for 10 min at 2500–3000 x *g* at 4 °C. Plasma was transferred into polypropylene tubes (1.8 mL Nunc Cryotube vials), which were stored at a temperature of minus 70 °C. Paclitaxel pharmacokinetics were measured in all plasma samples using a validated ultra-performance liquid chromatographic coupled to tandem mass spectrometry (UPLC-MS/MS) for precise quantification of paclitaxel plasma concentrations at the Laboratory of Translational Pharmacology of the Erasmus MC Rotterdam.[18] Non-linear mixed effects modeling was conducted using the software NONMEM.[19] A previously developed population pharmacokinetic model for paclitaxel,[15] with two compartments describing the disposition and linear elimination, was used as a starting point. However, the model was here expanded to a three-compartment model to fit the data. Scalp cooling was tested for its effect on the model PK parameters. The model was used to obtain paclitaxel clearances and volume of distribution for each subject.

Chemotherapy-Induced Alopecia (CIA)

Patients received multiple courses of paclitaxel. Only patients without alopecia were eligible to participate in the study. The severity of alopecia was scored at the start of paclitaxel treatment and just before each treatment cycle thereafter by a physician or nurse practitioner according to the Common Terminology Criteria for Adverse Events (CTCAE) grades, version 4.03.[20] CIA (according to CTCAEv4) is defined as grade 1 or 2 alopecia; grade 1 is defined as hair loss of <50% of an individual's hair under normal conditions, not obvious from a distance but only upon close inspection, for which a different hairstyle may be required to cover the hair loss but a wig or hairpiece is not necessary; grade 2 is defined as hair loss of >50% of an individual's hair under normal conditions that is apparent to others and for which a wig or hairpiece is necessary if the patient desires to camouflage the hair loss.

Statistical Analysis

The primary objective of our study was to investigate whether the clearance of paclitaxel was equivalent between patients who were treated with weekly paclitaxel with scalp cooling and without scalp cooling. The secondary objective was to determine the relation between paclitaxel clearance, temperature and CIA within the subgroup of patients using scalp cooling. A sample size of 18 patients per group was required to demonstrate equivalence of SC+ and the SC–, with 80% power and a two-sided significance level

of 0.05 and an original equivalence limit of one standard deviation (SD) based on the control group. However, since there were 24 controls available instead of the 18 patients required, the SC+ group could be reduced to 14 patients without a loss of power. Based on advancing insight, it was decided to use the standard bioequivalence limits of 0.80 and 1.25 for the 90% CI of the ratio of the geometric means of paclitaxel clearance to draw conclusions about equivalence. Therefore, the analysis of clearance was performed on log-transformed values, as this parameter was assumed to follow a lognormal distribution.[21] Estimates for the mean difference in (log) plasma clearance and its 90% confidence interval (CI) were obtained by using the two-sample t-test. The mean difference and the 90% CI were exponentiated to provide the point estimate of the geometric mean ratio and the 90% CI for this ratio that can be interpreted as a relative difference (RD) in percentages by using the following equation: RD = (geometric mean ratio–1) x 100%. The difference in paclitaxel clearance between patients who developed hair loss versus those who did not was analyzed similarly to the analysis of clearance. However, as the aim here was to study whether there was a difference, the 95% CI was used for this analysis. The differences in temperature over time were analyzed by location by means of a mixed model with a random effect for each patient. Patient characteristics from the prospective study and the control cohort were presented as medians and interquartile ranges (IQR) or as numbers with percentages. Clearance was described per study and hair loss group by means of the geometric mean and the coefficient of variation (CV). All statistical analyses were performed using Stata version 16.1 (StataCorp, College Station, TX, USA).

RESULTS

Patients Characteristics

Between January 2016 and December 2020, a total of 21 female patients with solid tumors were enrolled in the scalp cooling study. Seven patients were excluded due to incomplete blood sampling. Hence, 14 patients were evaluable for the main analyses. These patients were treated in accordance with the study protocol with scalp cooling during one cycle of paclitaxel treatment dosed at 80 mg/m^2 (36%) or 90 mg/m^2 (64%) depending on the indication. Three patients were treated with the standard stepwise increase in infusion rates because of hypersensitivity during previously administered paclitaxel treatment. The median paclitaxel infusion duration was 1.08 h. The post infusion cooling time was 60 min for all patients after which the scalp cooling was removed. Baseline demographic and clinical characteristics of the SC+ group and the SC- group are summarized in **Table 1**.

Table 1. Baseline characteristics

Baseline Characteristics	Scalp Cooling (SC+) (*n* = 14)	No Scalp Cooling (SC–) (*n* = 24)
Female, *n* (%)	14	24
Age (years) median [IQR]	51 [46–62]	61 [54–65]
Paclitaxel treatment dose, *n* (%)		
80 mg/m^2	5 (36)	10 (42)
90 mg/m^2	9 (64)	8 (37)
100 mg/m^2	0	4 (21)
Paclitaxel dose (mg), median [IQR]	150 [143–160]	153 [143–170]
Infusion time (h), median [IQR]	1.08 [1.00–1.31]-	1.01 [0.92–1.58]
BSA (m^2) median [IQR}	1.71 [1.63–1.87]	1.80 [1.63–1.93]
Indication, *n* (%)		
Breast cancer	5 (36)	11 (46)
Cervix cancer	9 (64)	5 (21)
Esophageal cancer	0	3 (12)
Ovarian cancer	0	5 (21)

Abbreviations: BSA = body surface area, IQR = interquartile range; n = number of patients.

Effect of Scalp Cooling on Paclitaxel Pharmacokinetics

Paclitaxel concentrations of the pharmacokinetic profile of patients using scalp cooling (n = 14) and patients not using scalp cooling (n = 24) are shown in **Figure 1**.

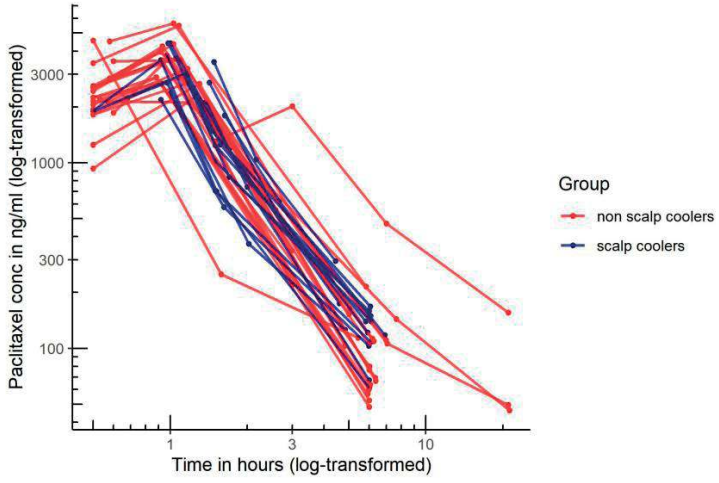

Figure 1. Paclitaxel concentration time profiles of the pharmacokinetic profile of patients using scalp cooling (n = 14, blue line) and patients not using scalp cooling (n = 24, red line)

Pharmacokinetic results of 14 SC+ patients and 24 SC– patients are depicted in Table 2. Paclitaxel clearance was 6.8% (90% CI, –16.7% to 4.4%) lower when paclitaxel was administered with concomitant scalp cooling compared to paclitaxel administration without scalp cooling. The distribution of paclitaxel was 5.9% (90% CI,–2.3% to 14.8%) higher with concomitant scalp cooling compared to paclitaxel administration without scalp cooling. The scalp cooling did not have a significant effect on the model PK parameters.

Table 2. Paclitaxel clearance and Vd of patients with scalp cooling (SC+) versus without scalp cooling (SC–) during one course of paclitaxel administration.

PK Parameter	SC+ (with Scalp Cooling) *n* = 14	SC– (without Scalp Cooling) *n* = 24	SC+ Versus SC–
Clearance *, L/h (CV%)	405.9 (18.2%)	435.5 (21.1%)	-
Relative difference (90% CI)	-	-	–6.8% (–16.7 to 4.4)
Vd *, L (CV%)	234.0 (16.2%)	221.0 (13.0%)	-
Relative difference (90% CI)	-	-	5.9 % (–2.3 to 14.8%)

* Clearances and Vd are expressed as geometric means of individual estimates. Abbreviations: CV = coefficient of variation; CI = confidence interval, Vd = volume of distribution.

Temperature Course during Scalp Cooling

The course of the temperature during the scalp cooling of 14 patients, measured on three different locations, is shown in Figure 2. No significant difference in time could be observed in the mouth (overall *P* -value = 0.238). For both ear and axilla, a significant difference was found between baseline and the 30 min point (*P* = 0.001 and *P* = 0.039, respectively). Furthermore, a difference was also found between baseline and the 55 min point in the ear (*P* = 0.003).

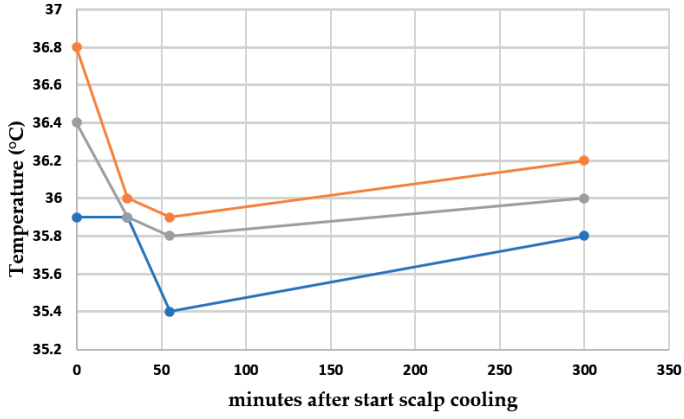

Figure 2. Course of median temperature during the scalp cooling of 14 patients in an ear, the mouth and an axilla Locations of temperature measurements: orange line: an ear; gray line: the mouth; and blue line: an axilla.

Chemotherapy-Induced Alopecia (CIA) during Scalp Cooling

Despite adequate scalp cooling during paclitaxel treatment, seven patients reported CIA during scalp cooling grade 1 (n = 4) and grade 2 (n = 3), whereas the other seven patients reported no CIA during scalp cooling. There was no difference in the median dosage of paclitaxel. More patients with than without CIA, despite scalp cooling, had a decrease of >1 ^{0}C in at least one of the measurement sites (see **Table 3**).

Table 3. Chemotherapy-induced alopecia (CIA) during scalp cooling

Patient Temperature during SC+	CIA during SC+ CTCAE Grade 1–2 Alopecia *n* = 7	No CIA during SC+ CTCAE Grade 0 Alopecia *n* = 7
Age (years) median [IQR]	50 [47.0–55.5]	59 [46.0–67.5]
Number of courses of paclitaxel administration, median [IQR]	6 [6–18]	6 [6–18]
Paclitaxel dose (mg/m^2), median [IQR]	90 [80–90]	90 [80–90]
Paclitaxel dose (mg), median [IQR]	150 [140–170]	150 [130–160]
Concomitant use of carboplatin	5/7	4/7
Ear temperature (°C) baseline versus end of scalp cooling, median [IQR]	36.6 [36.0–36.9] versus 36.2 [34.6–36.6]	36.9 [36.6–37.3] versus 36.6 [35.8–37.5]
Mouth temperature (°C) baseline versus end of scalp cooling, median [IQR]	35.8 [34.8–36.5] versus 35.7 [35.1–36.3]	36.5 [36.3–36.9] versus 36.4 [35.8–37.0]
Axilla temperature (°C) baseline versus end of scalp cooling, median [IQR]	35.6 [35.5–36.1] versus 35.6 [35.2–36.0]	36.0 [35.9–36.5] versus 36.3 [35.7–36.6]
% of patients with a decrease of >1 °C from baseline in at least one of the measurement sites	40%	24%

Abbreviations CIA = chemotherapy-induced alopecia; CTCAE = Common Terminology Criteria for Adverse Events; IQR = interquartile.

Paclitaxel Clearance with or without CIA after Scalp Cooling

Within the SC+ group, paclitaxel clearance and Vd was not significantly statistically different between patients with CIA (alopecia grade 1 or 2) and those without CIA (**Table 4**).

Table 4. Paclitaxel clearance and volume of distribution of patients with CIA versus without CIA in the scalp cooling (SC+) subgroup during one course of paclitaxel administration

PK Parameter	SC+ with CIA *n* = 7	SC+ without CIA *n* = 7	SC+ with CIA versus SC+ without CIA
Clearance *, L/h (CV%)	410.2 (20.5%)	401.6 (17.1%)	-
Relative difference (95% CI)	-	-	2.2% (−17.8% to 27.1%)
Vd, L (CV%)	234.9 (16.6%)	233.1 (17.2%)	-
Relative difference (95% CI)	-	-	0.2% (−17.1% to 22.5%)

*Clearances and Vd are expressed as geometric means. Abbreviations: CIA = chemotherapy-induced alopecia; CV = coefficient of variation; CI = confidence interval, Vd = volume of distribution.

DISCUSSION

In the present study, we demonstrated that clearance as measure for systemic exposure and the volume of distribution of paclitaxel did not reduce or increase as a result of scalp cooling. Although the decrease in body temperature during scalp cooling at each site measured was small, the decrease was statistically significant different during scalp cooling for the measurement in the ear and axilla. There was no significant difference in temperature until 3 h after scalp cooling was discontinued. Finally, half of the patients developed some form of hair loss despite scalp cooling. However, this was not associated with paclitaxel clearance. Mild hypothermia (body cooling to 32 to 34 °C for 12 to 48 h) can alter the pharmacokinetic parameters of several drugs.[7] Although the mechanism(s) behind changes in drug levels due to hypothermia has not been fully elucidated, impaired hepatic metabolism is likely, possibly via its effect on cytochrome P450 metabolism. In a study in healthy volunteers, for example, the clearance of midazolam as an index of CYP3A4/5 metabolism decreased by 11% for every degree Celsius decrease in a core temperature of 36.5 °C.[22]

In our analysis, a population PK model consisting of a central compartment with two peripheral compartments connecting to it was used to obtain clearances and volumes of distribution of each subject. Unlike PBPK models, these compartments have no anatomic or physiological significance. However, they can still be used to investigate the influence of subject characteristics (i.e., scalp cooling) on the predicted subject PK parameters describing the whole-body drug disposition.[23] Considering the scalp skin drug disposition in particular would require more data, and it is outside the scope of our study.

To the best of our knowledge, this is the first study investigating the influence of scalp cooling on the pharmacokinetic outcome of anti-cancer drugs. We hypothesized that the decrease in body temperature as a result of scalp cooling may influence the pharmacokinetics of paclitaxel. Although a small decrease in body temperature was found after 50 min of scalp cooling, which did not fully return to baseline after 5 h, the absolute decrease was limited: 60% of the women had a temperature drop of less than just 1 °C. This drop in temperature may be too small to demonstrate a difference in clearance of paclitaxel.

Scalp cooling is usually a good option to prevent hair loss for anti-cancer drugs with a short half-life or a rapid systemic distribution, such as paclitaxel.[11] However, it is unclear why some patients still develop CIA despite scalp cooling during such chemotherapy administration. In our study, half of the women were found to have some form of hair loss, of whom more than 40% developed grade 2 alopecia (although no one developed full baldness). This is somewhat higher than mentioned in previous studies, probably due to differences in definition of CIA between these studies.[11] We found no clear dif-

ference between the patients who developed CIA and those who did not. If any, the mean temperatures were slightly lower among those women without CIA compared to those with CIA. It is important to emphasize that CIA was also not due to differences in paclitaxel clearance. Further research is needed to identify possible explanations to better advise future patients about the chance of hair preservation with scalp cooling.

Some potential shortcomings of our study need to be mentioned. To answer our research question, the pharmacokinetic data from the controls (SC-) were collected prospectively as a part of a separate study. However, the method used to calculate paclitaxel clearance was similar in both studies, allowing for the pooling of results. Since patients had received paclitaxel at different doses (range 80–100 mg/m^2), the clearance of paclitaxel was used as the primary endpoint, as this result is unaffected by dose differences. Although the dose of paclitaxel strongly determines the success rate of scalp cooling for hair preservation, within the dose range of 80–90 mg/m^2, this had no effect on CIA in our study. In three patients, the total infusion time of paclitaxel was somewhat longer than the standard 60 min infusion time due to a history of hypersensitivity reaction to paclitaxel. This also may have a slight effect on the pharmacokinetics. However, infusion time was taken into consideration when calculating the clearance for each patient.

CONCLUSIONS

Our data showed that scalp cooling concomitant with paclitaxel did not reduce nor increase the clearance of paclitaxel. Therefore, it is unlikely that scalp cooling influences paclitaxel efficacy.

Finally, despite scalp cooling, half of the patients in our study developed a form of hair loss. Importantly, neither an association with difference in paclitaxel clearance nor a change in hair loss was found. Further research is warranted to optimize hair preservation in patients treated with paclitaxel.

REFERENCES

1. Dunnill CJ, Al-Tameemi W, Collett A, Haslam IS, Georgopoulos NT. A Clinical and Biological Guide for Understanding Chemotherapy-Induced Alopecia and Its Prevention. *Oncologist.* 2018;23(1):84-96.
2. van den Hurk CJ, Mols F, Vingerhoets AJ, Breed WP. Impact of alopecia and scalp cooling on the well-being of breast cancer patients. *Psychooncology.* 2010;19(7):701-709.
3. van den Hurk CJ, van den Akker-van Marle ME, Breed WP, van de Poll-Franse LV, Nortier JW, Coebergh JW. Impact of scalp cooling on chemotherapy-induced alopecia, wig use and hair growth of patients with cancer. *Eur J Oncol Nurs.* 2013;17(5):536-540.
4. Massey CS. A multicentre study to determine the efficacy and patient acceptability of the Paxman Scalp Cooler to prevent hair loss in patients receiving chemotherapy. *Eur J Oncol Nurs.* 2004;8(2):121-130.
5. Lacouture ME, Sibaud V, Gerber PA, et al. Prevention and management of dermatological toxicities related to anticancer agents: ESMO Clinical Practice Guidelines. *Ann Oncol.* 2021;32(2):157-170.
6. Breed W, van den Hurk CJG, Peerboom M. Presentation, Impact and Prevention of Chemotherapy-induced Hair Loss. *Expert Rev Dermatol.* 2011;6(1):109-125.
7. van den Broek MP, Groenendaal F, Egberts AC, Rademaker CM. Effects of hypothermia on pharmacokinetics and pharmacodynamics: a systematic review of preclinical and clinical studies. *Clin Pharmacokinet.* 2010;49(5):277-294.
8. Gustafson DL, Rastatter JC, Colombo T, Long ME. Doxorubicin pharmacokinetics: Macromolecule binding, metabolism, and excretion in the context of a physiologic model. *J Pharm Sci.* 2002;91(6):1488-1501.
9. Janssen FEM. Modelling physiological and biochemical aspects of scalp cooling. *Technische Universiteit Eindhoven.* 2007.
10. van den Hurk CJ, Peerbooms M, van de Poll-Franse LV, Nortier JW, Coebergh JW, Breed WP. Scalp cooling for hair preservation and associated characteristics in 1411 chemotherapy patients - results of the Dutch Scalp Cooling Registry. *Acta Oncol.* 2012;51(4):497-504.
11. Komen MM, Smorenburg CH, van den Hurk CJ, Nortier JW. Factors influencing the effectiveness of scalp cooling in the prevention of chemotherapy-induced alopecia. *Oncologist.* 2013;18(7):885-891.
12. Stage TB, Bergmann TK, Kroetz DL. Clinical Pharmacokinetics of Paclitaxel Monotherapy: An Updated Literature Review. *Clin Pharmacokinet.* 2018;57(1):7-19.
13. van Hagen P, Hulshof MC, van Lanschot JJ, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. *N Engl J Med.* 2012;366(22):2074-2084.
14. Heijkoop ST, Franckena M, Thomeer MG, Boere IA, Van Montfort C, Van Doorn HC. Neoadjuvant chemotherapy followed by radiotherapy and concurrent hyperthermia in patients with advanced-stage cervical cancer: a retrospective study. *Int J Hyperthermia.* 2012;28(6):554-561.
15. Nangia J, Wang T, Osborne C, et al. Effect of a Scalp Cooling Device on Alopecia in Women Undergoing Chemotherapy for Breast Cancer: The SCALP Randomized Clinical Trial. *JAMA.* 2017;317(6):596-605.
16. Paxman Coolers Ltd. Paxman PSC-2 brochure. Internet: http:// ebookbrowse.com/psc2-manual-pdf-d234169346. Accessed on 9 September 2012. 2012.
17. Henningsson A, Sparreboom A, Sandstrom M, et al. Population pharmacokinetic modelling of unbound and total plasma concentrations of paclitaxel in cancer patients. *Eur J Cancer.* 2003;39(8):1105-1114.

18. de Graan AJ, Elens L, Sprowl JA, et al. CYP3A4*22 genotype and systemic exposure affect paclitaxel-induced neurotoxicity. *Clin Cancer Res.* 2013;19(12):3316-3324.
19. Bauer RJ. NONMEM Tutorial Part I: Description of Commands and Options, with Simple Examples of Population Analysis. *CPT Pharmacometrics Syst Pharmacol.* 2019.
20. US Department of Health and Human Services. Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03. June 14, 2010. Maryland, United States: US National Institute of Health.
21. European Medicines Agency, Guideline on the Investigation of Bioequivalence, 20th January 2010. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf.
22. Hostler D, Zhou J, Tortorici MA, et al. Mild hypothermia alters midazolam pharmacokinetics in normal healthy volunteers. *Drug Metab Dispos.* 2010;38(5):781-788.
23. Mould DR, Upton RN. Basic concepts in population modeling, simulation, and model-based drug development. *CPT Pharmacometrics Syst Pharmacol.* 2012;1:e6.

CHAPTER 5

THE ADDED VALUE OF H_2-ANTAGONISTS IN PREMEDICATION REGIMENS DURING PACLITAXEL TREATMENT

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ABSTRACT

Background

Ranitidine, a histamine 2-blocker, is the standard of care to prevent hypersensitivity reactions (HSRs) caused by paclitaxel infusion. However, the added value of ranitidine in this premedication regimen is controversial. Therefore, we compared the incidence of HSRs during paclitaxel treatment between a standard regimen including ranitidine and a regimen without ranitidine.

Methods

This prospective, pre-post interventional, non-inferiority study compared the standard premedication regimen (n = 183) with dexamethasone, clemastine and ranitidine with a premedication regimen without ranitidine (n = 183). The primary outcome was the incidence of HSR grade ≥3. Non-inferiority was determined by checking whether the upper bound of the two-sided 90% confidence interval (CI) for the difference in HSR rates excluded the +6% non-inferiority margin.

Results

In both the pre-intervention (with ranitidine) and post-intervention (without ranitidine) group 183 patients were included. The incidence of HSR grade ≥3 was 4.4% (n = 8) in the pre-intervention group and 1.6% (n = 3) in the post-intervention group: difference –2.7% (90% CI, –6.2 to 0.1).

Conclusion

As the upper boundary of the 90% CI does not exceed the predefined non-inferiority margin of +6%, it can be concluded that a premedication regimen without ranitidine is non-inferior to a premedication regimen with ranitidine.

INTRODUCTION

Paclitaxel is one of the most commonly used anticancer drugs worldwide. It is effective for the treatment of several malignancies, including breast, lung-, ovarian, head and neck, and oesophageal cancer. However, due to its hydrophobic properties, paclitaxel must be emulsified in Cremophor-EL (polyethoxylated castor oil and ethanol) which frequently leads to hypersensitivity reactions (HSRs) during paclitaxel infusion.[1] HSRs during paclitaxel infusion can range from mild erythematous rashes to life-threatening anaphylaxis.[2] To prevent HSRs, premedication regimens were introduced as standard of care during paclitaxel treatment and generally consists of the corticosteroid dexamethasone combined with a histamine 1 (H_1)-receptor antagonist (e.g., clemastine or diphenhydramine) and the histamine 2 (H_2)-receptor antagonist ranitidine.[3,4] Without premedication regimens, HSRs were seen in 25–42% of all patients using paclitaxel.[5,6] Since the introduction of premedication regimens as standard of care, the incidence of HSRs during paclitaxel infusion was significantly decreased, but nevertheless occur in ~20% of all patients in the range from mild to death. Severe HSRs during paclitaxel infusion, defined as grade ≥3 (as per Common Terminology Criteria for Adverse Events; CTCAE version 4.03) occur in ~4% of all patients despite premedication.[7-12]

In addition, studies show that ~97% of all HSRs present within the first 10 min from the start of infusion during the first or second paclitaxel cycle.[12-15]

Ranitidine is primarily registered for the treatment of gastro-duodenal reflux and ulcer disease. The use of an H_2-antagonist in the standard of care premedication regimen for paclitaxel was based on the standard regimen used for preventing HSRs during the use of urographic radiocontrast media.[16] It was believed that blockade of both the H_1- and the H_2-receptors decreased the proportion of patients who experienced an allergic reaction. However, the efficacy in preventing paclitaxel-associated HSRs has never been thoroughly studied and is therefore controversial. The use of an H_2-antagonist (cimetidine) during paclitaxel infusions was first described during a phase 1 trial by Wiernik et al.,[17] but the efficacy in the prevention of HSRs was not assessed. Moreover, it has been shown that cimetidine or ranitidine are not effective in the prevention of HSRs.[18-20] In addition, earlier reports showed that ranitidine itself can cause side effects such as abnormal liver enzyme levels, nausea, vomiting, skin rash and HSRs. Ranitidine-induced HSRs occur in 0.7% of all ranitidine infusions.[21,22] Despite these findings the use of an H_2-antagonist during paclitaxel infusion is still recommended as standard premedication to prevent paclitaxel induced HSRs. Therefore, we aimed to determine the added value of ranitidine in preventing clinically relevant HSRs by comparing the standard premedication regimen with ranitidine to an experimental premedication regimen without ranitidine.

METHODS

Study design

A single-centre, prospective, pre-post interventional, non-inferiority study was conducted at the Erasmus MC Cancer Institute in Rotterdam, the Netherlands. All paclitaxel-naive patients aged ≥18 years within the outpatient department who were planned to receive their first cycle of paclitaxel for systemic cancer treatment, were enrolled in the study. From October 2018 until 19 April 2019, patients received a premedication regimen with ranitidine. Between 19 April 2019 and December 2019 a second group of patients was included, they received a premedication regimen without ranitidine. Paclitaxel could be part of a combination regimen or given as monotherapy in either a weekly or 3-weekly cycle.

The standard premedication regimen with ranitidine was compared to an experimental premedication regimen without ranitidine. Patients in the pre-intervention group received the standard premedication regimen consisting of dexamethasone (10 mg intravenously (IV)), clemastine (2 mg IV) and ranitidine (50 mg IV). Patients in the post-interventional group received the experimental premedication regimen without the H_2-antagonist ranitidine. Patients in both groups were followed for a minimum of two cycles and a maximum of six cycles of paclitaxel infusions if no HSR would occur or until the occurrence of the first HSR within the first six cycles. During each infusion of paclitaxel, the occurrence of HSRs, defined as an immunological response to paclitaxel corresponding with CTCAE grade from 1 (minimal) to 5 (death), version 4.03, was registered. In case an HSR occurred, patients were treated according to local standards.

The primary endpoint was the incidence of HSRs grade ≥3 during paclitaxel treatment. Secondary objectives were to determine and compare the severity (any grade) of paclitaxel-induced HSR; to determine the number of paclitaxel dosages until first HSR occurrence (any grade) and to determine the cumulative dose of paclitaxel at the moment of HSR occurrence, all with and without ranitidine. All included patients gave informed consent. The study was approved by the medical ethical board of the Erasmus MC and registered at the Dutch trial registry (www.trialregister.nl; number NL8173).

Statistical analysis

Considering previous studies, 4% of all patients in both treatment groups were expected to experience an HSR grade ≥3.[8,10,11,13] A non-inferiority margin of the difference between the incidence was set at 6% (the HSR rates in the group without ranitidine should be no worse than 6% more than the rate in the group receiving ranitidine). A sample size of 366 (thus 183 patients per group) would be sufficient to confer 90% power at the one-sided significance level of 0.05 using a binomial test.[23]

A closed test procedure was applied to the primary outcome. First, the incidence of patients that experienced an HSR grade ≥3 for both groups, the difference between these incidences and the associated two-sided 90% confidence interval (CI) for the difference was estimated. Non-inferiority of leaving out ranitidine compared to treatment with ranitidine was accepted if the upper bound of the two-sided 90% CI (equal to one-sided 95% CI) around the estimated difference in the primary endpoint lied <6%. Similar analyses were performed for any grade HSR. Furthermore, for both clinically relevant, defined as CTCAE grade ≥3, and any grade HSRs univariate and multivariate logistic regression analysis were considered if the number of events was sufficient to perform multivariate analyses (i.e., if at least 20 events (HSRs) had occurred). If possible, variables which were significant in the univariate analysis were considered for the multivariate analysis. Additionally, the severity of paclitaxel-induced HSR was tabulated by study period and the exact χ^2 test for trend was used to compare study periods. The mean cumulative dose of paclitaxel received was computed for the cycles which were given before the one where HSR emerged and divided over the body surface, shown per group and per HSR grade. Data were analyzed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp, Armonk, NY) and R.[24]

RESULTS

Of 366 included patients, 183 patients received ranitidine (pre-intervention) as part of their paclitaxel premedication regimen and 183 patients did not receive ranitidine (post-intervention). The median age was 61 years (range 26-86 years) and 60.4% of all patients were women (Table 1). Most patients were diagnosed with esophageal (42.1%), breast (32.5%), lung (8.7%) and uterine cervical cancer (7.9%). Of all patients, 18.6% had ≥1 previously registered (non-paclitaxel) medication allergy before entering the study. Clinically relevant HSR grade ≥3 occurred in eight patients (4.4%) in the pre-interventional group with ranitidine compared to three patients (1.6%) in the post-interventional group without ranitidine (Table 2). The absolute risk difference between the two groups was –2.7% (90% CI, –6.2 to 0.1). Hence, non-inferiority was shown. Given the low number of events (<20 events (HSRs)), no additional logistic regression analyses were performed on this outcome.

HSR (any grade) during paclitaxel infusion occurred in 37 (20%) in the pre-interventional group with ranitidine and 22 (12%) in the post-interventional group without ranitidine (**Figure 1**). Regarding the comparison of– any grade– HSRs, a regimen without ranitidine showed to be non-inferior to the pre-intervention regimen with ranitidine (difference –8.2%, 95% CI, –15.0 to –1.4, $P = 0.046$). The severity of HSRs, the number of paclitaxel dosages and time to first HSR occurrence did not differ between the groups (**Table 2**).[9]

Table 1. Patient characteristics

	Pre-intervention group with ranitidine (*N* = 183)	Post-intervention group without ranitidine (*N* = 183)	*P*-value
Age (years), median (IQR1 – 3)	61 (51-70)	61 (51-70)	0.608[a]
Sex: *N* (%)			
Female	115 (62.8)	106 (57.9)	0.336 [b]
Male	68 (37.2)	77 (42.1)	
Tumour type: *N* (%)			
Uterine cervical	20 (10.9)	9 (4.9)	<0.001[c]
Lung	3 (1.6)	29 (15.8)	
Breast	60 (32.8)	59 (32.2)	
Ovarian	10 (5.5)	2 (1.1)	
Oesophageal	78 (42.6)	76 (41.5)	
Endometrial	5 (2.7)	4 (2.2)	
Others	7 (3.8)[d]	4 (2.2)[e]	
Allergies:			
Registered medication allergies[f], *N* (%)	37 (20.2)	31 (16.9)	0.420[b]
If medication allergies, mean number[g], median (IQR1-3, max)	1 (1-2, 5)	1 (1-2, 3)	0.243[a]
Registered food allergies[f], *N* (%)	3 (1.6)	3 (1.6)	1.000[b]
Comedication with effect on allergy symptoms, excluding chemotherapy- related medication: *N* (%)			
Corticosteroids[h]	18 (9.8)	18 (9.8)	1.000[b]
Beta blockers[i]	20 (10.9)	26 (14.2)	0.344[b]
Immunomodulatory agents[j]	2 (1.1)	0	0.499[c]
Anti-histamines[k]	9 (4.9)	9 (4.9)	1.000[b]

P-values belong to the groups (thus: *P*-value<0.0001 belongs to all tumour types, not only to uterine cervical). [a]Mann-Whitney U test. [b]χ^2 test. [c]Fisher's exact test. [d]Oropharynx, vaginal cancer, angiosarcoma, gastric cancer 3x, rectal cancer. [e]Thymus, prostate cancer, angiosarcoma, merkel-cell carcinoma. [f]Registered in electronic patient registration. Concerns all registered medication or food allergies before start of the first paclitaxel administration. [g]Applicable to patients where at least 1 medication allergy was registered in the electronic patient registration before the start of the first paclitaxel administration. [h]Corticosteroïds: Beclometason, betamethason, budesonide, cortison, dexamethason, fludrocortison, hydrocortison, methylprednisolon, prednisolon, prednison, triamcinolone en triamcinolonacetonide. [i]Beta blockers: acebutolol, atenolol, bisoprolol, carvedilol, celiprolol, esmolol, labetalol, landiolol, metoprolol, nebivolol, pindolol, propranolol. [j]Immunomodulatory agents: abatacept, adalimumab, alemtuzumab, anakinra, apremilast, aurothiobarnsteenzuur, azathioprine, baricitinib, basiliximab, belatacept, belimumab, benralizumab, brodalumab, canakinumab, certolizumab pegol, ciclosporine, dupilumab, eculizumab, everolimus, etanercept, fingolimod, glatirameer, golimumab, guselkumab, hydroxychloroquine, infliximab, interferon alfa 2a, interferon beta 1a, interferon beta 1b, interferon gamma 1b, ixekizumab, leflunomide, mepolizumab, mycofenolzuur, natalizumab, ocrelizumab, omalizumab, peginterferon alfa 2a, peginterferon beta 1a, pimecrolimus, pirfenidon, reslizumab, risankizumab, ropeginterferon alfa 2b, sarilumab, secukinumab, sirolimus, tacrolimus, temsirolimus, teriflunomide, thymocytenimmunoglobuline, tildrakizumab, tocilizumab, tofacitinib, ustekinumab and vedolizumab. [k]Anti-histamines: acrivastine, alimemazine, azelastine, cetirizine, chloorcyclizine, cinnarizine, clemastine, cyclizine, desloratadine, dimetindeen, ebastine, emedastine, fexofenadine, hydroxyzine, ketotifen, levocabastine, levocetirizine, loratadine, meclozine, mizolastine, olopatadine, oxomemazine, promethazine, rupatadine and tripelennamine.

Table 2. Characteristics of occurred hypersensitivity reactions

	Pre-intervention group with ranitidine (*N* = 183)	Post-intervention group without ranitidine (*N* = 183)	*P* value
Patients with HSR (any grade): *N*	37	22	
HSR per grade[a] *N* (%)			
Grade 1	4 (10.8)	1 (4.5)	
Grade 2	25 (67.6)	18 (81.8)	
Grade 3	6 (16.2)	3 (13.6)	0.825[b]
Grade 4	2 (5.4)	0 (0)	
Grade 5	0 (0)	0 (0)	
Occurrence of HSR during: *N* (%)			
Cycle 1	13 (35.1)	8 (36.4)	
Cycle 2	15 (40.5)	7 (31.8)	
Cycle 3	5 (13.5)	6 (27.3)	
Cycle 4	2 (5.4)	1 (4.5)	0.811[b]
Cycle 5	1 (2.7)	0 (0)	
Cycle 6	1 (2.7)	0 (0)	
Occurrence of first symptoms, no. of minutes after start of paclitaxel infusion: *N* (%)			
0-5 min	23 (62.1)	13 (59.1)	
5-15 min	10 (27.0)	8 (36.4)	
15-60 min	3 (8.1)	1 (4.5)	
60-120 min	0 (0)	0 (0)	1.000[b]
>120 min	0 (0)	0 (0)	
Unknown	1 (2.7)	0 (0)	
Cumulative dose of paclitaxel at the time of occurrence of HSR (mg/m^2)[c] median (Q1-Q3)			
HSR grade 1	0 (0–74.5)	0 (N/A: *N*=1)	
HSR grade 2	163.0 (0-184.9)	51.4 (0-120.4)	
HSR grade 3	87.2 (0-175.7)	0 (0-0)[d]	NA
HSR grade 4	0 (0-0)[d]	NA	
HSR grade 5	NA	NA	

HSR = hypersensitivity reaction; *NA* = not applicable. *P* values belong to groups (thus *P*-value 0.825 belongs to grade 1 till grade 5, not only grade 1; *P* values belong to cycle (thus *P*-value 0.811 belongs to cycle 1 to cycle 6, not only cycle 1; *P* values belong to groups (thus *P*-value 1.000 belongs to all occurences, not only to 0-5 min). [a]CTCAE v4.03 [b]χ^2 test for trend. [c]Cumulative dose in mg /m^2 administered in the cycles before the cycle in which the paclitaxel-induced HSR occurs. [d]Cumulative dose of 0 since HSR occurred during the first paclitaxel administration.

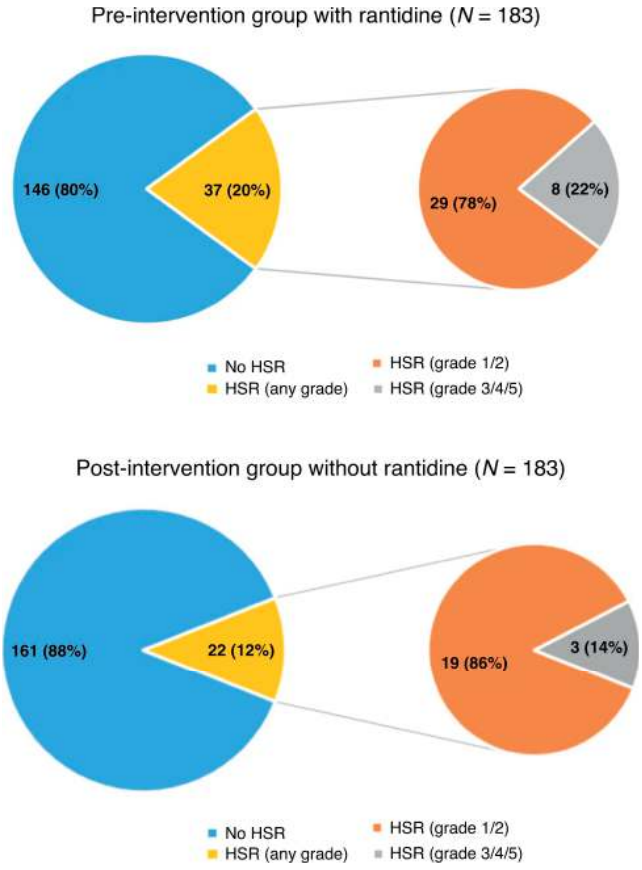

Figure 1. Distribution of patients Patients who experienced a hypersensitivity reaction (HSR) in the pre-intervention group with ranitidine and the post-intervention group without ranitidine. Data is presented as *N* (%).

Univariate logistic regression analyses showed that besides 'ranitidine premedication' versus 'no ranitidine premedication' (P = 0.035), sex (P = 0.034) and tumour type (P = 0.003) were also significantly related to HSR any grade. However, as sex could explain the differences in tumour type (a lower percentage of patients with breast cancer were included and thus fewer women were included) and no rationale could be given for the relation between tumour type and HSR, the multivariate analysis was performed with sex and 'ranitidine premedication' versus 'no ranitidine premedication'.[8,10-13,25-27] In the multivariate analysis 'ranitidine premedication' versus 'no ranitidine premedication' (P = 0.043) and sex (P = 0.042) remained statistically significant (**Table 3**) and thus showed that patients who were treated without ranitidine and males were at lower risk for developing an HSR any grade. Detailed clinical characteristics are described of patients with HSRs grade ≥3 during paclitaxel infusions in the groups with and without ranitidine in Table 4.

Table 3. Univariate- and multivariate analysis

	Univariate		Multivariate	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Ranitidine (without vs. with)	0.54 (0.30 – 0.96)	0.035	0.55 (0.31 – 0.98)	0.043
Age (continuous)	0.99 (0.97 – 1.01)	0.259		
Sex (male vs. female)	0.51 (0.28 – 0.95)	0.034	0.525 (0.28 – 0.98)	0.042
Tumour type		0.003		
Lung vs. gynecological[a]	0.18 (0.05 – 0.69)	0.012		
Breast vs. gynecological[a]	0.30 (0.14 – 0.64)	0.002		
Oesophageal vs. gynecologicala	0.25 (0.12 – 0.53)	<0.001		
Other vs. gynecological*	0.40 (0.08 – 2.03)	0.266		
Co-medication[b] (yes vs. no)	0.78 (0.37 – 1.46)	0.381		
Other previous medication allergies (yes vs. no)	1.63 (0.85 – 3.15)	0.143		

OR odds ratio. [a]Uterine cervical, ovarian and endometrial carcinoma. [b]Co-medication with effect on allergy symptoms, excluding chemotherapy- related medication: corticosteroids, beta blockers, immunomodulators and/or anti-histamines.

Table 4. Detailed characteristics of patients with HSRs grade ≥3 during paclitaxel infusions, RANISTOP study

	With/ without ranitidine	Tumor type	Age, (years)	HSR occurred during cycle no.	Dose, (mg/ m2)	Absolute dose (mg)	Symptoms	HSR grade‡	Time after start infusion until start symptoms (min)
1	With ranitidine	Cervix	49	1	175	300	Shortness of breath, cyanose, back pain, muscle pain, urticaria	4	0-5
2	With ranitidine	Esophagus	68	1	50	100	Back pain, vomiting, syncope, ECG changes	4	5-15
3	With ranitidine	Cervix	48	2	90	150	Shivering, abdominal pain, facial flushing	3	5-15
4	With ranitidine	Breast	69	2	80	150	Hypertension, flushing	3	0-5
5	With ranitidine	Ovary	62	2	175	350	Flushing, hypotension, dizziness, nausea	3	5-15
6	With ranitidine	Ovary	70	2	175	300	Abdominal pain, back pain, dyspnea, flushing, hypotension	3	0-5
7	With ranitidine	Cervix	57	1	90	130	Shortness of breath	3	5-15
8	With ranitidine	Breast	55	1	80	150	Shortness of breath, bronchospasm, chest pain	3	15-60
9	Without ranitidine	Esophagus	78	3	100	200	Chest pain, flushing, hypertension	3	0-5
10	Without ranitidine	Lung	61	1	200	400	Chest pain, flushing, back pain	3	5-15
11	Without ranitidine	Lung	66	1	200	350	Tachycardia, hypertension, red tingling hands	3	15-60

‡CTCAE v4.

DISCUSSION

To our knowledge, this is the first study that prospectively investigated the added value of ranitidine as part of standard of care premedication regimens in preventing paclitaxel induced HSRs. This study showed that a premedication regimen without ranitidine is non-inferior to the standard premedication regimen with ranitidine in preventing clinically relevant paclitaxel induced HSRs.

Based on a literature- and data study conducted at the Erasmus MC, we expected an incidence of HSRs any grade of ~20% and of clinically relevant HSRs (grade ≥3) of ~4% during paclitaxel infusion in the patient population with ranitidine.[3,11,25,26,28] In the RANISTOP study, we found incidences of HSRs and clinically relevant HSRs consistent with these findings. The lower incidence of HSRs in the post-interventional group without ranitidine may be partially explained by the fact that ranitidine itself may cause HSRs.[2]

The strengths of this study are the prospective study design and the broad inclusion criteria. These factors increase the representativeness of the data and the results are more likely to reflect daily clinical practice. The main limitation of this study was the non-randomized design. A non-randomized pre-post interventional trial design was chosen because of clinical feasibility in the sake of time and money. Moreover, as patients in this study received regular paclitaxel-based therapy with only a subtle change ('ranitidine premedication' versus 'no ranitidine premedication') in the pre-post regimen respectively, there were concerns about receiving the assigned treatment. Statistical analysis showed that there were no significant differences observed in patient characteristics between the group with ranitidine and the group without ranitidine except for tumour type. In the group without ranitidine a significantly higher percentage of lung cancer patients were seen but this difference can be attributed to an increasing number of NSCLC patients being treated with paclitaxel (in combination with carboplatin, bevacizumab and atezolizumab) as part of a novel treatment option. As a result, a lower number of patients with gynecologic tumours were seen in the group without ranitidine. However, literature showed that tumour type was not associated with an increased risk of paclitaxel-induced HSR.[10,11,15] Hence, we believe that the difference in HSR incidence between the groups with and without ranitidine is attributable to the removal of ranitidine. Besides, a relatively large non-inferiority margin of +6% was chosen in this study in order to set feasible goals in number of patients within a specific timeframe. A non-inferiority margin of +6% fits within the large variety of paclitaxel induced HSR-incidences seen in literature.[8,10,11,13] In addition, earlier reports showed that ranitidine itself can cause HSRs, which would result in a decrease in HSR incidence for the group without ranitidine. Moreover, as the upper bound of the two-sided 90% CI for the difference in HSR rates was only +0.1% the large margin did not affect the conclusions of this study. In this study, a differentiation between HSRs to ranitidine or paclitaxel or a second

chemotherapeutic agent was not included as most HSRs occurred within the first 15 minutes after starting the paclitaxel infusion and could almost certainly be attributed to paclitaxel (and not to second chemotherapeutic agents in the regimen).[12-15] However, an additional in-depth assessment of known HSRs (e.g., through an allergological test) would be an interesting addition to similar studies in the future to discriminate between HSRs to paclitaxel and ranitidine.

During this study, a worldwide recall of ranitidine was issued. This event has made the conclusions of this study even more relevant as this study provides confirmation that ranitidine can be safely omitted from the premedication regimen during paclitaxel infusion and that an alternative is not necessary in preventing HSRs. Therefore, it should be considered to remove ranitidine from the paclitaxel labels and guidelines addressing the prevention of paclitaxel-induced HSR.

In times of increasing healthcare costs and increasing workload, appropriate use of drugs is becoming more important. In the Netherlands, each year over 26,000 paclitaxel infusions are given to patients, resulting in the same amount of unnecessary ranitidine injections. The total costs of ranitidine per patient might be relatively low but considering the high number of patients that receive ranitidine this will inevitably result in a major reduction of healthcare costs. But probably more important is the time saving and effort saving through less pharmacy technician and nursing time and patients benefits such as shorter infusion time and fewer medication risks.

This study shows that premedication regimens during anticancer treatment should be evaluated more critically. Their recommended use might not always be evidence-based and therefore may not be effective. Thus, more research is needed on the effectiveness, safety and proper dose of other premedication and co-medication drugs during anticancer therapy.

In conclusion, this study showed that a premedication regimen without ranitidine was non inferior compared to a premedication regimen with ranitidine in preventing HSRs during paclitaxel infusion. The recent worldwide recall and subsequent shortages of ranitidine has made the conclusions of this study even more relevant as this study provides confirmation that ranitidine can be safely omitted from paclitaxel regimens and that an alternative is not necessary in preventing HSRs.

REFERENCES

1. Szebeni J, Alving CR, Savay S, et al. Formation of complement-activating particles in aqueous solutions of Taxol: possible role in hypersensitivity reactions. *Int Immunopharmacol.* 2001;1(4):721-735.
2. Park KH, Pai J, Song DG, et al. Ranitidine-induced anaphylaxis: clinical features, cross-reactivity, and skin testing. *Clin Exp Allergy.* 2016;46(4):631-639.
3. Joerger M. Prevention and handling of acute allergic and infusion reactions in oncology. *Ann Oncol.* 2012;23 Suppl 10:x313-319.
4. Taxol (paclitaxel) injection label. Accessed data fda 02/11/ 2020.
5. Choy H. Taxanes in combined modality therapy for solid tumors. *Crit Rev Oncol Hematol.* 2001;37(3):237-247.
6. Vogel WH. Infusion reactions: diagnosis, assessment, and management. *Clin J Oncol Nurs.* 2010;14(2): E10-21.
7. Common Terminology Criteria for Adverse Events, version 4.0. Published: May 28, 2009 https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03/Archive/CTCAE_4.0_2009-05 29_QuickReference_8.5x11.pdf. Accessed 03/20, 2020. 2009.
8. Markman M, Kennedy A, Webster K, Kulp B, Peterson G, Belinson J. Paclitaxel-associated hypersensitivity reactions: experience of the gynecologic oncology program of the Cleveland Clinic Cancer Center. *J Clin Oncol.* 2000;18(1):102-105.
9. Romano A, Torres MJ, Castells M, Sanz ML, Blanca M. Diagnosis and management of drug hypersensitivity reactions. *J Allergy Clin Immunol.* 2011;127(3 Suppl): S67-73.
10. Nokihara H, Yamamoto N, Ohe Y, Hiraoka M, Tamura T. Pharmacokinetics of Weekly Paclitaxel and Feasibility of Dexamethasone Taper in Japanese Patients with Advanced Non-small Cell Lung Cancer. *Clin Ther.* 2016;38(2):338-347.
11. Bookman MA, Kloth DD, Kover PE, Smolinski S, Ozols RF. Short-course intravenous prophylaxis for paclitaxel-related hypersensitivity reactions. *Ann Oncol.* 1997;8(6):611-614.
12. Weiss RB, Donehower RC, Wiernik PH, et al. Hypersensitivity reactions from taxol. *J Clin Oncol.* 1990;8(7):1263-1268.
13. Markman M, Kennedy A, Webster K, Peterson G, Kulp B, Belinson J. An effective and more convenient drug regimen for prophylaxis against paclitaxel-associated hypersensitivity reactions. *J Cancer Res Clin Oncol.* 1999;125(7):427-429.
14. Berger MJ, Vargo C, Vincent M, et al. Stopping paclitaxel premedication after two doses in patients not experiencing a previous infusion hypersensitivity reaction. *Support Care Cancer.* 2015;23(7):2019-2024.
15. Yahata H, Saito M, Sendo T, et al. Prophylactic effect of pemirolast, an antiallergic agent, against hypersensitivity reactions to paclitaxel in patients with ovarian cancer. *Int J Cancer.* 2006;118(10):2636-2638.
16. Greenberger PA, Patterson R, Simon R, Lieberman P, Wallace W. Pretreatment of high-risk patients requiring radiographic contrast media studies. *J Allergy Clin Immunol.* 1981;67(3):185-187.
17. Wiernik PH, Schwartz EL, Strauman JJ, Dutcher JP, Lipton RB, Paietta E. Phase I clinical and pharmacokinetic study of taxol. *Cancer Res.* 1987;47(9):2486-2493.
18. Greenberger PA, Patterson R, Tapio CM. Prophylaxis against repeated radiocontrast media reactions in 857 cases. Adverse experience with cimetidine and safety of beta-adrenergic antagonists. *Arch Intern Med.* 1985;145(12):2197-2200.

19. Cook J, Shuster S. Lack of effect of H2 blockade in chronic urticaria [proceedings]. *Br J Dermatol.* 1979;101 Suppl 17:21-22.
20. Greenberger P, Harris K, Patterson R. The effect of histamine-1 and histamine-2 antagonists on airway responses to histamine in the rhesus monkey. *J Allergy Clin Immunol.* 1979;64(3):189-196.
21. Demirkan K, Bozkurt B, Karakaya G, Kalyoncu AF. Anaphylactic reaction to drugs commonly used for gastrointestinal system diseases: 3 case reports and review of the literature. *J Investig Allergol Clin Immunol.* 2006;16(3):203-209.
22. Han TY, Jang WS, Yu M, Lee HK, Son SJ, Seo SJ. Anaphylactic reaction to ranitidine (Zantac(R)). *Int J Dermatol.* 2011;50(11):1397-1399.
23. HyLown.com. Power and sample size calculator (Accessed 03/20, 2020).
24. R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2019.
25. Koppler H, Heymanns J, Weide R. Dose reduction of steroid premedication for paclitaxel: no increase of hypersensitivity reactions. *Onkologie.* 2001;24(3):283-285.
26. Sasada S, Hirashima T, Nakamura Y, et al. Preliminary experience with a modified premedication protocol that included intravenous diphenhydramine and calcium bromide for the prophylaxis of paclitaxel-related hypersensitivity reactions. *Int J Clin Oncol.* 2007;12(4):274-278.
27. Hainsworth JD, Greco FA. Paclitaxel administered by 1-hour infusion. Preliminary results of a phase I/II trial comparing two schedules. *Cancer.* 1994;74(4):1377-1382.
28. Aoyama T, Takano M, Miyamoto M, et al. Is there any predictor for hypersensitivity reactions in gynecologic cancer patients treated with paclitaxel-based therapy? *Cancer Chemother Pharmacol.* 2017;80(1):65-69.

CHAPTER 6

THE INFLUENCE OF BODYCOMPOSITION ON THE SYSTEMIC EXPOSURE OF PACLITAXEL IN ESOPHAGEAL CANCER PATIENTS

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ABSTRACT

Changes in body composition are associated with chemotherapy-related toxicities and effectiveness of treatment. It is hypothesized that the pharmacokinetics (PK) of chemotherapeutics may depend on body composition. The effects of body composition on the variability of paclitaxel PK were studied in patients with esophageal cancer. Skeletal muscle index (SMI), visceral adipose tissue (VAT), and skeletal muscle density (SMD) were measured at the third lumbar vertebra on computed tomography (CT) scans performed before treatment. Paclitaxel PK data were collected from a prospective study performed between May 2004 and January 2014. Non-linear mixed-effects modeling was used to fit paclitaxel PK profiles and evaluate the covariates body surface area (BSA), SMI, VAT, and SMD using a significance threshold of $P < 0.001$. Paclitaxel was administered to 184 patients in a dose range of 50 to 175 mg/m^2. Median BSA was 1.98 m^2 (range of 1.4 to 2.8 m^2). SMI, VAT, and SMD were not superior to BSA in predicting paclitaxel PK. The additive value of SMI, VAT, and SMD to BSA was also negligible. We did not find evidence that paclitaxel dosing could be further optimized by correcting for SMI, VAT, or SMD.

INTRODUCTION

Paclitaxel is a highly lipophilic antineoplastic agent and is administered as an intravenous infusion. It is widely used for the treatment of lung, ovarian, breast, and esophageal cancer, amongst others.[1-3] Paclitaxel is currently dosed solely based on the body surface area (BSA) of the patient.

Despite this BSA-individualized dose, the interindividual variability (IIV) of paclitaxel pharmacokinetics (PK) remains high and consequently, the variability in clinical outcome (i.e., efficacy and toxicity) remains high as well. Apparently, a part of the total IIV can be explained by BSA,[4] which can be expected, as BSA is calculated from only height and weight as a surrogate for body composition. This may not take into account the actual differences affecting paclitaxel PK between patients [5-7]. Paclitaxel is poorly soluble in water and therefore the infusion fluid contains a micelle-forming agent, Cremophor EL®. [8] The clearance of paclitaxel in this micelle-forming formulation is significantly increased in obese patients.[9] In addition, the time-above-threshold-concentration of 0.05 µmol/L is related to both hematological toxicities and peripheral neuropathy.[1] While a low paclitaxel clearance puts patients at risk for drug-related toxicities, patients with a high clearance are at risk of suboptimal systemic drug levels, leading to a diminished therapeutic effect. Ideally, other covariates –or sets of covariates– than BSA would be used to predict paclitaxel exposure before treatment initiation. Skeletal muscle mass (i.e., skeletal muscle index, SMI), adipose tissue, and skeletal muscle density (SMD) (i.e., a measure for skeletal muscle quality and intramuscular fat infiltration) could potentially serve as predictive covariates, as they are associated with altered volumes of distribution, metabolism, and clearance of cytotoxic drugs.[10] Previous studies demonstrated a wide variation in muscle mass and visceral adipose tissue (VAT) in patients with identical BSA and/or body mass index (BMI), producing a heterogeneity in chemotherapy tolerance and treatment-related toxicity such as neutropenia.[6,11,12]

These findings suggest that SMI, VAT, and SMD may be superior to BSA or may add reliability in predicting drug exposure and could help optimize chemotherapy dosing strategies. More knowledge on SMI, VAT, and SMD influencing paclitaxel pharmacokinetics (PK) may therefore help to improve the individualization of paclitaxel dosing. Currently available population paclitaxel PK models lack actual bio-impedance measurements and merely apply different formulas using patients' weight and height rather than specific metabolic parameters such as SMI, VAT, or SMD.

Patients with esophageal cancer are prone to common symptoms such as malnutrition and weight loss, which can lead to skeletal muscle wasting and loss of adipose tissue. These patients may show a higher IIV of paclitaxel PK and be at an increased risk of toxicity. In this study, we investigated whether variation between patients in paclitaxel exposure can be explained by metabolic parameters such as SMI, VAT, and SMD.

MATERIALS AND METHODS

Patients

The patient cohort comprised 184 adult patients with esophageal cancer treated with paclitaxel at the Erasmus MC Cancer Institute, who were prospectively included in an institutional database (www.trialregister.nl; NL2187 (NTR2311) between May 2004 and January 2014.[13,14] All patients provided written informed consent for the mentioned trial, and only patients who received paclitaxel mono- or combination therapy were included. All patients with esophageal cancer received paclitaxel 50 mg/m^2 weekly in a neoadjuvant chemoradiotherapy regimen,[2] or as an induction or palliative treatment with paclitaxel 100 mg/m^2 weekly for a maximum of 6 weeks, followed by a 175 mg/m^2 dose every 3 weeks. From all patients, evaluable baseline computed tomography (CT) imaging of the abdomen was available.

Body Composition Measurements

BSA was calculated for each patient according the Mosteller method.[15] Body composition was assessed using each patient's pretreatment staging CT scan prior to the start of paclitaxel treatment. The cross-sectional skeletal muscle surface area (SMA) and VAT were measured at the third lumbar vertebra (L3) level at one contrast-enhanced transversal CT-image slice and were automatically calculated using the preset Hounsfield Units (HU) thresholds and expressed in square centimeters using the in-house developed FatSeg software program package version 2.4 (developed by the Biomedical Imaging Group Rotterdam, Rotterdam, the Netherlands) using MeVisLab (Mevis Medical Solutions, Bremen, Germany). The measured SMA in cm^2 was corrected for height squared (m^2) to determine the skeletal muscle index (SMI; cm^2/m^2). SMD was quantified as mean muscle attenuation as assessed between −29 and +150 HU.[16] L3 was chosen as an anatomical landmark based on its linear correlation to total body lean body mass.[17] CT scans were performed within 8–10 weeks before treatment initiation. All CT scans were assessed on identical slices by a trained observer to whom patient details were blinded.[18]

Paclitaxel Pharmacokinetics

The analyses for paclitaxel pharmacokinetics were performed according to previous studies.[13,14] According to protocol, three post-administration blood samples for PK analysis of paclitaxel were obtained up to 5 h after paclitaxel treatment using a formerly endorsed limited sampling strategy. The PK analysis was conducted in the first or in one of the following courses during one chemotherapy treatment cycle. Samples were collected in 4 mL lithium heparin (Li-He) blood collection tubes. Subsequent to sample collection, paclitaxel concentrations were quantitated by a validated high performance liquid chromatography/tandem mass spectrometry (HPLC/MS-MS) detection method.[19]

Paclitaxel plasma concentrations below the lower limit of quantitation (LLOQ) of 2 ng/mL were not reported. Cremophor EL®, the formulation vehicle for paclitaxel, causes a shift in the blood distribution and reduces the availability of the free circulating fraction of paclitaxel. As a result, the total fraction of paclitaxel does not behave in a linear pharmacokinetic way in contrast to its free fraction.

Pharmacokinetic Model Evaluation and Covariate Analysis

A previously validated population PK model for paclitaxel was used as a reference model.[20] This three-compartment model with nonlinear elimination included four covariates: BSA, gender, age, and total bilirubin. These covariates were proven to significantly correlate with the elimination capacity of paclitaxel (VM_{EL}). Firstly, we fitted the data to this model. Hereafter, we evaluated whether replacing BSA by other bio-impedance measures, including SMI, VAT, and SMD, improved the model fit, as depicted in Equation (1).

$$VM_{EL} = \Theta_1 * \left(\frac{BI}{BI_{median}}\right)^{\Theta_2} * \Theta_3{}^{GENDER} * \left(\frac{AGE}{AGE_{median}}\right)^{\Theta_4} * \left(\frac{BILI}{BILI_{median}}\right)^{\Theta_5} \quad (1)$$

where Θ_1 represents the typical population value for maximal elimination rate of paclitaxel; *BI* represents the bio-impedance measurement BSA, SMI, VAT, or SMD; and Θ_2 to Θ_5 represent the estimated influence of the respective bio-impedance measurements, gender, age, and total bilirubin on the maximal elimination rate. Finally, we investigated the effect of adding either VAT, SMD, or SMI to the four-covariate model. All continuous covariates were centered to the population median value. Graphical diagnostics, differences in Objective Function Value (OFV) and IIV in VM_{EL}, visual predictive check (VPC) with n = 1000, and parameter plausibility were used to evaluate whether actual bio-impedance measurements were superior or additive to the classical BSA approach. A significance threshold of $P < 0.001$, corresponding to a difference in OFV of >10.83 for one degree of freedom, was used to discriminate between the covariate models. Parameter precision was estimated using sampling importance resampling (SIR).[21]
Non-linear mixed effects modeling was conducted using NONMEM® (version 7.3.0, ICON Development Solutions, Ellicott City, MD, USA) and Perl-speaks-NONMEM (version 4.4.8). All analyses were performed with the first-order conditional estimation method with interaction. Piraña® (version 2.9.2) was used as interface and data management and graphical assessments were performed in R (version 3.0.1), e.g., using Xpose.

RESULTS

In total, 550 paclitaxel plasma concentrations were available from 184 patients for PK analyses, as depicted in **Table 1**. Paclitaxel was administered intravenously to 147 males and 37 females. The median age in the total patient cohort was 64 years (range of 40 to 83 years). One hundred and thirty-two patients received paclitaxel 50 mg/m^2 (72%), forty-five patients were treated with 100 mg/m^2, and 7 patients with 175 mg/m^2 paclitaxel.

Table 1. Patient characteristics

Parameters	Cohort
Number of patients (*n*)	184
Paclitaxel dose (mg/m^2), median (range)	70 (62–252)
Infusion time (h), median (range)	0.9 (0.3–1.5)
Number of samples (*n*)	550
Per patient, median (range)	3 (2–4)
BSA (m^2), median (range)	1.98 (1.42–2.76)
SMI (cm^2/m^2), median (range)	48.5 (30.9–83.4)
VAT (cm^2), median (range)	165 (0.67–502)
SMD (HU), median (range)	37 (14–56)
Gender, male, *n* (%)	147 (80)
Age, median (range)	64 (40–83)
Indication, *n* (%) esophageal cancer	184 (100)
Paclitaxel treatment, *n* (%)	
Induction/palliative (3 weekly 175 mg/m^2)	7 (4)
Induction/palliative (weekly 100 mg/m^2)	45 (24)
Neoadjuvant (weekly 50 mg/m^2)	132 (72)
Bilirubin, total (µmol/L), median (IQR)	7 (5–9)

BSA = body surface area, HU = Hounsfield Units, IQR = interquartile range, SMD = skeletal muscle density, SMI = skeletal muscle index, VAT = visceral adipose tissue.

The previously developed four-covariate model [22] including BSA, was able to fit the paclitaxel exposure data and showed plausible parameter estimates. However, a few parameters could not be well estimated at the moment of paclitaxel dosing for which the previously reported values [22] were used. This was the case for the peripheral distribution volume and the effect of total bilirubin on paclitaxel elimination. For the final BSA model, the "goodness-of-fit" data of observed versus predicted paclitaxel exposure and the visual predictive check (VPC) results are depicted in **Figures 1** and **2**, respectively.

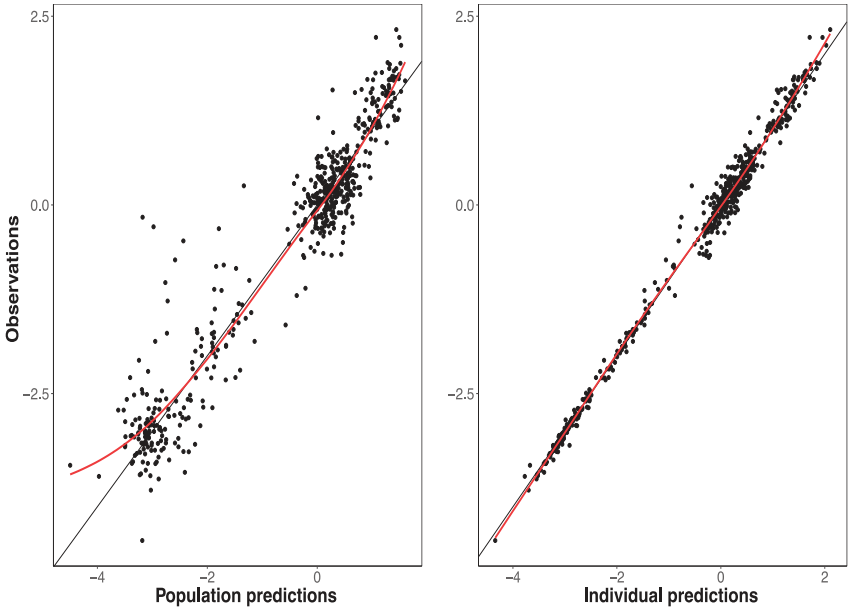

Figure 1. Goodness-of-fit plots presenting: BSA model predictions (left panel) or individual Bayesian predictions (right panel) versus observed paclitaxel concentrations, depicted using log transformed data.

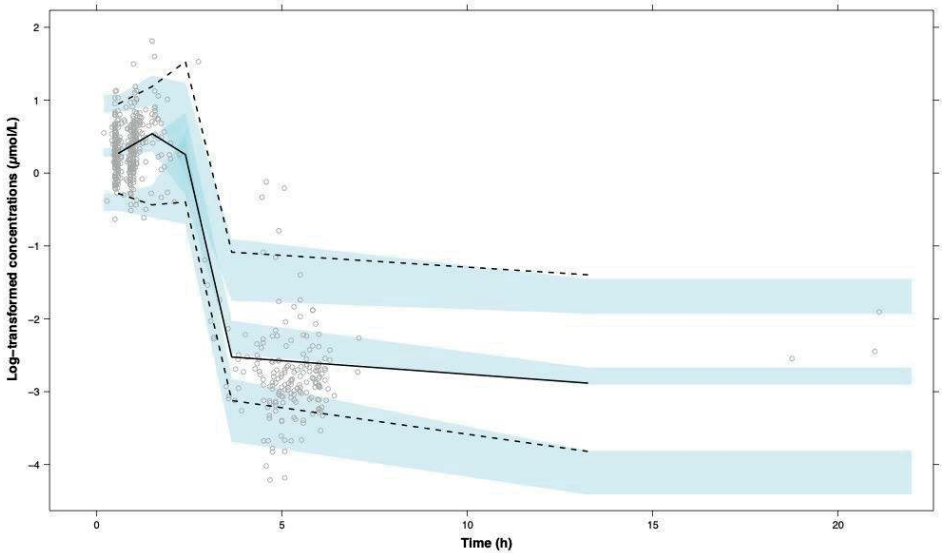

Figure 2. Visual predictive check plot of the BSA model using n = 1000 and log-transformed paclitaxel plasma concentrations. Dots represent observed paclitaxel concentrations, the black line represents the observed median concentrations, the dashed lines are the observed 5th and 95th percentiles, and the light blue areas represent the 95% confidence intervals of the median, 5th, and 95th percentiles.

Replacing the covariate BSA by the actual bio-impedance measurements SMI, VAT, or SMD did not improve model fit (difference Objective Function Value (dOFV) +29, +34, and +25, respectively). Besides, the IIV of the elimination capacity of paclitaxel was increased (+4.3% for SMI, +5.5% for SMD, and +3.1% for VAT, respectively), as shown in **Table 2**. The influence of either BSA, SMI, VAT, or SMD on the estimated VM_{EL} of paclitaxel is depicted in Figure 3. In this model, BSA is positively correlated with the elimination capacity of paclitaxel (VM_{EL}). For model specifications, see Pharmacokinetic Model Evaluation and Covariate Analysis).

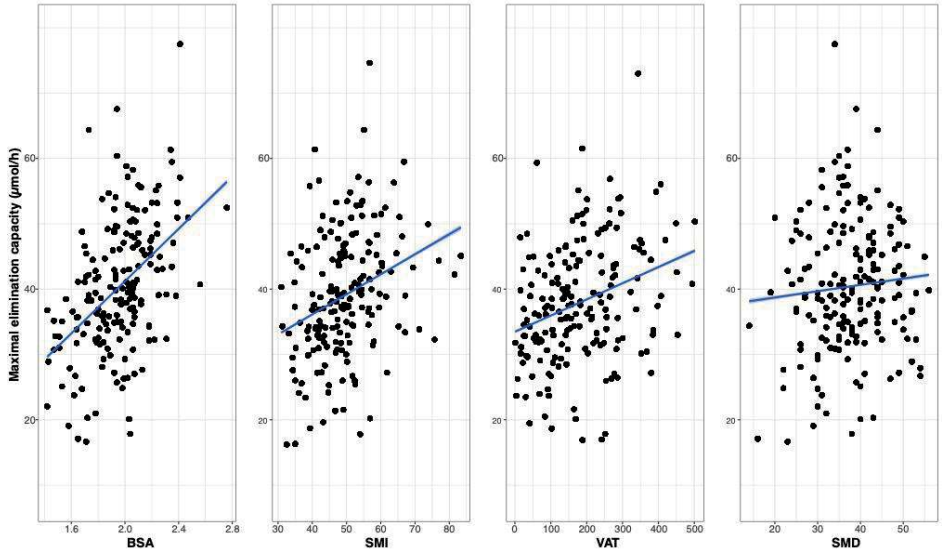

Figure 3. Data simulation of the impacts of BSA, SMI, VAT, and SMD on the maximal elimination capacity of paclitaxel.

Furthermore, adding either covariate SMI, VAT, or SMD to the previously established covariate model including BSA did not reach our significance threshold of $P < 0.001$ (dOFV of −0.1, −0.1, and +3.4 respectively; data not shown). Data evaluation using a 2-compartmental model, in which no basic PK parameters needed to be fixed, led to similar results and did not alter our conclusion on the impact of BSA, SMI, VAT, and/or SMD (data not shown).

Table 2. Population pharmacokinetic parameters of paclitaxel of the covariate model of BSA, SMI, VAT, and SMD.

Parameter (Unit)	Covariate Model BSA		Covariate Model SMI		Covariate Model VAT		Covariate Model SMD	
	dOFV	REF	dOFV	+29	dOFV	+34	dOFV	+25
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
VM_{EL} (µmol/h)	31.6	26.7–37.4	32.4	26.7–40.6	31.9	26.0–39.0	31.6	34.7–38.9
V_1 (L)	24.0	20.8–27.6	24.7	21.3–28.4	25.0	21.8–28.4	25.2	22.0–28.8
V_3 (L)	267	NA	267	NA	267	NA		
KM_{EL} (µmol/L)	0.40	0.29–0.52	0.51	0.37–0.73	0.49	0.33–0.68	0.55	0.36–0.74
VM_{TR} (µmol/h)	179	138–225	153	119–199	148	111–188	144	113–193
KM_{TR} (µmol/L)	1.91	1.37–2.67	1.80	1.46–1.98	1.75	1.24–2.39	1.73	1.15–2.36
K_{21} (h^{-1})	2.34	1.85–2.96	2.16	1.69–2.72	2.09	1.64–2.65	2.15	1.70–2.69
Q (L/h)	20.7	18.0–23.3	19.8	17.0–23.0	19.6	17.0–22.3	19.9	16.6–23.0
Body composition on VM_{EL}	1.25	1.03–1.51	0.34	0.24–0.44	0.09	0.06–0.12	0.03	0.02–0.04
Age on VM_{EL}	−0.30	−0.39–0.22	−0.30	−0.32–0.30	−0.50	−0.65–0.34	−0.28	−0.36–0.20
Gender on VM_{EL}	1.07	0.96–1.19	1.20	1.06–1.34	1.23	1.09–1.37	1.30	1.15–1.47
Bilirubin on VM_{EL}	−0.17	NA	−0.17	NA	−0.17	NA	−0.17	NA
Interindividual variability								
VM_{EL} (%)	24.3	20.8–28.3	28.6	24.9–33.3	27.4	24.0–32.4	29.8	25.5–34.9
V_1 (%)	39.1	33.2–45.3	38.1	31.3–43.7	37.7	31.9–45.4	37.5	30.6–43.5
Q (%)	62.0	52.3–72.9	62.8	52.8–74.0	64.3	52.8–75.6	62.4	50.7–74.3
Residual variability								
σ_{prop} (%)	22.5	20.1–25.5	22.4	20.2–25.5	22.6	20.0–25.5	22.4	19.8–25.3

The data represent the following: BSA = body surface area, dOFV = difference Objective Function Value, K_{21} = rate constant of the distribution from the first peripheral compartment to the central compartment, NA = not applicable, Q = intercompartmental clearance between the central and second peripheral compartment, σ_{prop} = proportional residual error, SMD = skeletal muscle density, SMI = skeletal muscle index, VAT = visceral adipose tissue, V_1 = volume of central compartment, V_3 = volume of the second peripheral compartment, VM_{EL} = maximal elimination rate, VM_{TR} = maximal transport rate from the central to the first peripheral compartment, KM_{EL} = plasma concentration at half VM_{EL}, and KM_{TR} = plasma concentration at half VM_{TR}.

DISCUSSION

To our knowledge this is the first study that assessed the direct correlation between PK of paclitaxel and the body composition parameters SMI, VAT, and SMD from cross-sectional CT images. Variation in paclitaxel exposure in relation to these body composition parameters was investigated. BSA was previously found to only have a clinically relevant impact on VM_{EL}.[20,22,23] Hence, we evaluated the influence of SMI, VAT, and SMD on VM_{EL}. We found that the parameters SMI, VAT, and SMD did not give a significantly better model fit than BSA nor did they lead to a decrease in IIV of VM_{EL}. Thus, these actual bio-impedance measurements were not superior to BSA in predicting paclitaxel PK. Moreover, the added value of these actual bio-impedance measurements to BSA also appeared negligible. Thus, the relatively high IIV of paclitaxel exposure could not be attributed to differences in SMI, VAT, or SMD. Therefore, according to our model, conventional BSA-based dosing of paclitaxel remains the best approach to dose paclitaxel and minimize paclitaxel IIV.

Recently, several studies suggested a correlation of SMI, VAT, and/or SMD with taxane-related toxicity. One example is a study in a cohort of 151 early breast cancer patients treated with anthracycline and docetaxel or paclitaxel in which patients with a low SMI had significantly more adverse events.[24] Another study correlated visceral adipose tissue with safety parameters in 1395 patients with non-metastatic breast cancer treated with an anthracycline and docetaxel and/or paclitaxel and found that patients with larger visceral adiposity had a lower cumulative dose suggesting a lower tolerability for the treatment.[11] These observations can be explained by the influence of adipose tissue on the taxane pharmacokinetic profile, and pharmacokinetics was correlated with body composition. However, pharmacokinetic data was lacking in these studies to support this hypothesis. Our findings indicate that actual bio-impedance measurements from CT scans cannot explain variability in paclitaxel PK.

A possible explanation for the low predictive value of SMI, VAT, and SMD in our study may be that the CT scans and the PK sampling were not performed on the same day. While BSA was always available on the actual PK day the CT scan was performed before treatment initiation within 8–10 weeks. Another possible explanation is that the total number of PK samples is too small or the study population is too homogeneous to demonstrate the potential influence of these measured body size parameters as compared to BSA. In addition, our study has several limitations. It should be noted that our cohort consisted of patients with esophageal cancer and that the most of them (n = 132) were treated with the well-tolerable paclitaxel dosing schedule of 50 mg/m^2 in a curative setting. Furthermore, not all blood samples were collected during the first treatment cycle resulting in different paclitaxel dosages, especially in the induction/palliative setting.

Since we cannot explain paclitaxel interindividual variability in pharmacokinetics, one may want to consider therapeutic drug monitoring (TDM). This has recently been extensively studied by Joerger et al. in a randomized controlled trial. Although paclitaxel TDM did not improve clinical outcome or severe neutropenia, it did improve tolerability in terms of paclitaxel associated neuropathy.[25] This extended cohort analysis in patients with esophageal cancer showed that SMI, VAT, and SMD were not superior to BSA in predicting paclitaxel pharmacokinetics. These parameters should therefore not be used for paclitaxel dosing. Our results do not support an alternative for BSA-based paclitaxel dosing.

REFERENCES

1. Stage TB, Bergmann TK, Kroetz DL. Clinical Pharmacokinetics of Paclitaxel Monotherapy: An Updated Literature Review. *Clin Pharmacokinet.* 2018;57(1):7-19.
2. van Hagen P, Hulshof MC, van Lanschot JJ, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. *N Engl J Med.* 2012;366(22):2074-2084.
3. Toxopeus EL, Talman S, van der Gaast A, et al. Induction chemotherapy followed by surgery for advanced oesophageal cancer. *Eur J Surg Oncol.* 2015;41(3):323-332.
4. Baker SD, Verweij J, Rowinsky EK, et al. Role of body surface area in dosing of investigational anticancer agents in adults, 1991-2001. *J Natl Cancer Inst.* 2002;94(24):1883-1888.
5. Mathijssen RH, de Jong FA, Loos WJ, van der Bol JM, Verweij J, Sparreboom A. Flat-fixed dosing versus body surface area based dosing of anticancer drugs in adults: does it make a difference? *Oncologist.* 2007;12(8):913-923.
6. Prado CM, Lima IS, Baracos VE, et al. An exploratory study of body composition as a determinant of epirubicin pharmacokinetics and toxicity. *Cancer Chemother Pharmacol.* 2011;67(1):93-101.
7. Bins S, Ratain MJ, Mathijssen RH. Conventional dosing of anticancer agents: precisely wrong or just inaccurate? *Clin Pharmacol Ther.* 2014;95(4):361-364.
8. Nieuweboer AJ, Hu S, Gui C, et al. Influence of drug formulation on OATP1B-mediated transport of paclitaxel. *Cancer Res.* 2014;74(11):3137-3145.
9. Sparreboom A, Wolff AC, Mathijssen RH, et al. Evaluation of alternate size descriptors for dose calculation of anticancer drugs in the obese. *J Clin Oncol.* 2007;25(30):4707-4713.
10. Prado CM. Body composition in chemotherapy: the promising role of CT scans. *Curr Opin Clin Nutr Metab Care.* 2013;16(5):525-533.
11. Cespedes Feliciano EM, Chen WY, Lee V, et al. Body Composition, Adherence to Anthracycline and Taxane-Based Chemotherapy, and Survival After Nonmetastatic Breast Cancer. *JAMA Oncol.* 2019.
12. Hopkins JJ, Sawyer MB. A review of body composition and pharmacokinetics in oncology. *Expert Rev Clin Pharmacol.* 2017;10(9):947-956.
13. de Graan AJ, Elens L, Smid M, et al. A pharmacogenetic predictive model for paclitaxel clearance based on the DMET platform. *Clin Cancer Res.* 2013;19(18):5210-5217.
14. de Graan AJ, Loos WJ, Friberg LE, et al. Influence of smoking on the pharmacokinetics and toxicity profiles of taxane therapy. *Clin Cancer Res.* 2012;18(16):4425-4432.
15. Mosteller RD. Simplified calculation of body-surface area. *N Engl J Med.* 1987;317(17):1098.
16. Goodpaster BH, Thaete FL, Kelley DE. Composition of skeletal muscle evaluated with computed tomography. *Ann N Y Acad Sci.* 2000;904:18-24.
17. Shen W, Punyanitya M, Wang Z, et al. Total body skeletal muscle and adipose tissue volumes: estimation from a single abdominal cross-sectional image. *J Appl Physiol (1985).* 2004;97(6):2333-2338.
18. van Vugt JL, Levolger S, Gharbharan A, et al. A comparative study of software programmes for cross-sectional skeletal muscle and adipose tissue measurements on abdominal computed tomography scans of rectal cancer patients. *J Cachexia Sarcopenia Muscle.* 2017;8(2):285-297.
19. Sparreboom A, de Bruijn P, Nooter K, Loos WJ, Stoter G, Verweij J. Determination of paclitaxel in human plasma using single solvent extraction prior to isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Sci Appl.* 1998;705(1):159-164.
20. Crombag MBS, de Vries Schultink AHM, Koolen SLW, et al. Impact of Older Age on the Exposure of Paclitaxel: a Population Pharmacokinetic Study. *Pharm Res.* 2019;36(2):33.

21. Dosne AG, Bergstrand M, Harling K, Karlsson MO. Improving the estimation of parameter uncertainty distributions in nonlinear mixed effects models using sampling importance resampling. *J Pharmacokinet Pharmacodyn*. 2016;43(6):583-596.
22. Joerger M, Kraff S, Huitema AD, et al. Evaluation of a pharmacology-driven dosing algorithm of 3-weekly paclitaxel using therapeutic drug monitoring: a pharmacokinetic-pharmacodynamic simulation study. *Clin Pharmacokinet*. 2012;51(9):607-617.
23. Kraff S, Nieuweboer AJ, Mathijssen RH, et al. Pharmacokinetically based dosing of weekly paclitaxel to reduce drug-related neurotoxicity based on a single sample strategy. *Cancer Chemother Pharmacol*. 2015;75(5):975-983.
24. Shachar SS, Deal AM, Weinberg M, et al. Body Composition as a Predictor of Toxicity in Patients Receiving Anthracycline and Taxane-Based Chemotherapy for Early-Stage Breast Cancer. *Clin Cancer Res*. 2017;23(14):3537-3543.
25. Joerger M, von Pawel J, Kraff S, et al. Open-label, randomized study of individualized, pharmacokinetically (PK)-guided dosing of paclitaxel combined with carboplatin or cisplatin in patients with advanced non-small-cell lung cancer (NSCLC). *Annals of Oncology*. 2016;27(10):1895-1902.

CHAPTER 7

TISSUE TYPE DIFFERENCES IN ABCB1 EXPRESSION AND PACLITAXEL TISSUE PHARMACOKINETICS IN PATIENTS WITH ESOPHAGEAL CANCER

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ABSTRACT

Background

Data from previous work suggests that there is no correlation between systemic (plasma) paclitaxel exposure and efficacy in patients treated for esophageal cancer. In this trial, we investigated ABC efflux transporter expression and intratumoral pharmacokinetics of paclitaxel to identify changes which could be a first sign of chemoresistance.

Methods

Patients with esophageal cancer treated with paclitaxel and carboplatin (±concomitant radiotherapy) were included. During the first and last cycle of weekly paclitaxel, blood samples and biopsies of esophageal mucosa and tumor tissue were taken. Changes in paclitaxel exposure and expression of ABCB1 (P-glycoprotein) over time were studied in both tumor tissue and normal appearing esophageal mucosa.

Results

ABCB1 was significantly higher expressed in tumor tissue compared to esophageal tissue, during both the first and last cycle of paclitaxel (cycle 1: $P < 0.01$; cycle 5/6: $P = 0.01$). Interestingly, ABCB1 expression was significantly higher in adenocarcinoma than in squamous cell carcinoma ($P < 0.01$). During the first cycle, a trend towards a higher intratumoral paclitaxel concentration was observed compared to esophageal mucosa concentration (RD: 43%; 95% CI,−3% to 111%; $P = 0.07$). Intratumoral and plasma paclitaxel concentrations were significantly correlated during the first cycle ($AUC_{0\text{-}48h}$: $r = 0.72$; $P < 0.01$).

Conclusion

Higher ABCB1 expression in tumor tissue, and differences between histological tumor types might partly explain why tumors respond differently to systemic treatment. Resistance by altered intratumoral paclitaxel concentrations could not be demonstrated because the majority of the biopsies taken at the last cycle of paclitaxel did contain a low amount of tumor cells or no tumor.

INTRODUCTION

Esophageal cancer is the 7th most common cause of cancer-related mortality worldwide.[1] Paclitaxel in combination with carboplatin and radiotherapy is highly effective in the curative setting of esophageal cancer, and in combination with carboplatin alone it has shown moderate efficacy both during induction chemotherapy and in palliative setting of this tumor type.[2-5] Nonetheless, a substantial part of the patients with esophageal cancer do not benefit from this treatment or show progression of disease short after treatment has stopped.[5-7] Paclitaxel acts by the inhibition of cell proliferation, by promoting the stabilization of cellular microtubules and the concentration-dependent induction of multipolar spindles which eventually leads to apoptosis.[8-10]

Paclitaxel is also known for its induction of drug resistance,[11] although the exact mechanisms are unknown. Major factors probably causing paclitaxel resistance are alterations in stability of the microtubule network, reduced function of apoptotic proteins (e. g. B-cell leukemia/lymphoma 2 (Bcl-2), cellular tumor antigen (p53)), and overexpression of transmembrane efflux-pumps of the ATP-binding cassette (ABC) subfamily.[11-13] ABC-efflux transporters are essential in the protection of the cell against xenobiotics.[14] ABCB1 (P-glycoprotein) is one of the subtypes in the ABC-efflux transporter family.[14] ABCB1 is expressed in the plasma membrane of human cells and is known for its diversity in substrates that can be transported via this efflux transporter.[14] Overexpression of ABCB1 contributes to chemotherapy resistance of cancer cells *in vitro* and was related to worse survival of cancer patients in several studies.[11,14-18] *In vivo* studies demonstrated that inhibition or induction of ABCB1 in multidrug resistant tumor cells influences the intratumoral paclitaxel exposure.[13,19] Nevertheless, intratumoral pharmacokinetics of chemotherapeutical agents, and the relation between intratumoral chemotherapy exposure and ABC efflux transporter activity remains largely unknown, especially in the clinical setting.

In contrast to tissue pharmacokinetics, the systemic pharmacokinetics of paclitaxel are well known and characterized by a large inter-individual variability.[20,21] Moreover, commonly seen hematological toxicity and peripheral neuropathy have been linked with the time above a specific paclitaxel plasma concentration (i.e., >0.05 μM).[22,23] To determine the best dose for an individual patient it is often suggested to tailor the dose of paclitaxel based on the systemic pharmacokinetic exposure. This strategy improved the risk-benefit profile of non-small cell lung cancer patients treated with paclitaxel.[24] However, this is probably only a surrogate for the intratumoral exposure.[25] Additionally, in a previous study no correlation between systemic paclitaxel clearance and esophageal cancer response was shown.[7]

Currently, knowledge about the intratumoral concentrations of paclitaxel, the influence of intratumoral paclitaxel concentration on the effectiveness of the treatment and the

correlation between ABC efflux transporters and intratumoral paclitaxel is lacking. Therefore, there is an urgent need to investigate and elucidate the intratumoral paclitaxel pharmacokinetics.
In this exploratory study we assessed both ABC efflux transporter expression, and intratumoral and esophageal mucosa paclitaxel concentrations over time, to identify changes in paclitaxel concentrations and/or differences between tissue types which could potentially be a sign of the development of drug resistance in esophageal carcinoma.

METHODS

We performed a single center pharmacokinetic study in patients diagnosed with esophageal cancer for whom treatment with weekly paclitaxel and carboplatin was indicated. The study was performed between October 2017 and September 2019 at the Erasmus MC Cancer Institute, Rotterdam, the Netherlands. The Medical Ethics Committee and the board of directors of the Erasmus MC approved the study protocol. The study was performed in accordance with the International Conference on Harmonization Good Clinical Practice guidelines, the Declaration of Helsinki, and all applicable regulations. The trial is registered at the Dutch Trial Registry (www. trialregister.nl number NL5990). All patients provided written informed consent before any study related procedure was pursued.

Patients

Patients, 18 years or older, were eligible if they were diagnosed with a histologically proven malignancy of the esophagus that was safely accessible by upper endoscopy. They were treated with weekly paclitaxel and carboplatin with or without concomitant radiotherapy in a standard regimen (**Supplementary Methods 1**).[2,3,5] Patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Patients were excluded if the tumor caused esophageal stenosis prohibiting upper endoscopy, if they previously received radiotherapy on the esophagus, if they had a history of bleeding diathesis, or if they used medication or supplements which could interact with paclitaxel during the study period.

Study Design

The primary objective of the study was to demonstrate a 25% reduction of the intratumoral concentration of paclitaxel in the last cycle of weekly paclitaxel compared to the first cycle of paclitaxel in esophageal cancer patients. Secondary objectives of our study were to: 1. compare intratumoral paclitaxel concentrations with paclitaxel concentra-

tions in normal appearing esophageal mucosa, 2. compare paclitaxel concentrations in non-tumoral mucosa per study cycle, 3. correlate intratumoral concentrations of paclitaxel with systemic paclitaxel pharmacokinetics per study cycle, 4. to investigate ABCB1 expression over time, 5. compare ABCB1 expression between tumor tissue and non-tumoral esophageal mucosa tissue , and 6. compare ABCB1 expression between different histological types of esophageal cancer.

All included patients were seen at the outpatient clinic prior to each chemotherapy cycle. During cycle 1 and the last cycle (i.e., cycle 5 or 6), patients were admitted to the hospital to perform blood withdrawals for pharmacokinetic purposes and to undergo an upper endoscopy to obtain biopsies of tumor and normal appearing esophageal mucosa for pharmacokinetic purposes and pathological assessments. Patients were evaluable for the primary endpoint if the biopsies were successfully obtained during the first and the last cycle of their weekly paclitaxel treatment.

Biopsy Procedure

Upper endoscopy was planned at 4 h after the start of paclitaxel administration. Sedation with midazolam and fentanyl was allowed during the endoscopy procedure. During the procedure, a total of 2-4 biopsies of the tumor --with a mean diameter of 6 mm-- were taken by an experienced and dedicated gastroenterologist. Biopsies of normal appearing esophageal mucosa (visual inspection by the gastroenterologist) were taken at least 5 cm proximal or distally from the visible tumor area. These biopsies were of the same size and same numbers as the tumor biopsies. Half of the biopsies were directly frozen in liquid nitrogen and stored at < -70°C for pharmacokinetic analysis. The other half of the biopsies were formalin-fixed for pathological assessment. If the gastroenterologist could not identify a macroscopic tumor during the last treatment cycle, samples were taken at the same location as during the first cycle.

Pharmacokinetic Analysis

Plasma samples were taken before start of the paclitaxel infusion, 30 min after start of administration, 5 min prior to the end of infusion, and 1.5 and 3 h after the end of the administration of paclitaxel. The timing of blood sampling as well as tissue sampling were comparable when the anti-allergic infusion regimen was used. Blood samples were collected in 4 mL lithium heparin tubes and plasma was collected after centrifugation at 2,500**g* (4°C) for 10 min and stored at < -70°C until analysis. Paclitaxel concentrations were measured using a validated liquid chromatography-mass spectrometry method.[26] Systemic exposure was expressed as area under the curve from pre-infusion to 48 h ($AUC_{0\text{-}48h}$) and estimated using a previously developed population PK model developed in NONMEM.[20] The analysis took the anti-allergic infusion regimen into account. Tissue biopsies were homogenized in 400 µL of blank human plasma with a tissue-lyser (Qia-

gen, Germany) and a stainless-steel bead (5mm) for 90 s at 60Hz. Homogenized tissue samples were further processed as plasma samples as described above.

Pathological Analysis

To determine the expression of ABCB1 an automated immunostainer (the Ventana Benchmark ULTRA, Ventana Medical Systems Inc., Arizona, United States) was used. Sequential 4 µm thick (FFPE) sections were stained for ABCB1 using Optiview universal DAB detection Kit (#760-700, Ventana). In brief, following deparaffinization and heat-induced antigen retrieval with CC1 (#950-500, Ventana) for 32 min the tissue samples were incubated with the ABCB1 antibody (Company: Novusbio; Type: anti mouse; Clone: OTI1A7; Lot number: W001; Dilution: 1/9600) or another 32 min at 37˚C. Incubation was followed by hematoxylin II counter stain for 8 min and then a blue coloring reagent for 8 min according to the manufactures instructions (Ventana). Positive controls were used on every slide. After immunohistochemical staining the percentage of positive stained cells of interest and the intensity of the staining per biopsy were evaluated (by R.A.G.v.E. and M.D.). The biopsies were scored according to the immunoreactive score (IRS) descripted to Remmele and Stegner.[27]

Statistical Analysis

This study was powered to detect a 25% decrease of the intratumoral concentrations of paclitaxel in the last treatment cycle compared to the first treatment cycle. Since we had no information on forehand on the variability of the intratumoral paclitaxel concentrations, we assumed an intrapatient standard deviation of 30% in intratumoral paclitaxel concentrations. Given a power of 80% and two-sided significance level of 5%, at least 14 evaluable patients were required for the primary objective. Log-transformation was used for data regarding tissue (tumor and normal appearing esophagus mucosa tissue) paclitaxel concentrations and $AUC_{0\text{-}48h}$, since we assumed that these data followed a lognormal distribution. A paired *t*-test was used to compare tissue paclitaxel concentrations, and systemic exposure (i.e., $AUC_{0\text{-}48h}$) for the total study population. Mean differences with corresponding 95% confidence intervals (CI) were exponentiated to calculate the geometric mean ratio with 95% CI for these ratios. Geometric mean (GEM) ratios represent relative differences (RD) as a percentage. Comparisons between the first cycle and last cycle were made for intratumoral concentrations, healthy esophageal mucosa tissue concentrations, and plasma $AUC_{0\text{-}48h}$ using paired *t*-tests. The intratumoral paclitaxel concentration was also compared with normal appearing esophageal tissue concentration during cycle 1 and the last cycle using the same test. The intratumoral concentrations observed in adenocarcinoma and squamous cell carcinoma were compared to each other using an independent *t*-test. To compare the ABC efflux transporter expression between the types of tissues and the cycles of chemotherapy the Wilcoxon

signed-rank test was used. The correlation between systemic pharmacokinetics and tissue paclitaxel concentrations was estimated using Pearson's correlation coefficients. The correlation between immunohistochemical expression and intratumoral paclitaxel concentrations was estimated using Spearman's correlation coefficient given the ordinal immunohistochemical data used for this analysis. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

RESULTS

Patient Characteristics

In total 15 patients were included, of whom 14 patients were evaluable. One patient withdrew informed consent after the first cycle of chemotherapy and gastroscopy within the study. **Table 1** displays all baseline characteristics. The tumors were predominantly located in the distal esophagus (79%). Nine out of the 14 patients (67%) were diagnosed with an adenocarcinoma, while the remaining patients were diagnosed with a squamous cell carcinoma of the esophagus. The majority of the included patients were male (93%) and were treated with paclitaxel (50mg/m^2), carboplatin (AUC2) and concomitant radiotherapy (78%).

Table 1 Baseline characteristics

Characteristics (n = 14)	No. (%)
Gender	
Male	13 (93%)
Female	1 (7%)
Age (years)	
Median [IQR]	70 [64 – 76]
BMI (kg/m^2)	
Median [IQR]	27.7 [25.3 – 33.8]
BSA (m^2)	
Median [IQR]	2.1 [1.85 – 2.20]
Tumor location	
Mid esophageal tumor[a]	3 (21%)
Distal esophageal tumor[b]	11 (79%)
Histological subtype	
Adenocarcinoma	9 (64%)
Squamous cell carcinoma	5 (36%)
Treatment regimen	
CTx	3 (21%)
dCRT	2 (14%)
nCRT	9 (64%)

[a]*Mid esophageal tumor is defined as tumor located at 24–32 cm from the teeth row.*
[b]*Distal esophageal tumor is located between 32 and 40 cm form the teeth row.*
Abbreviations: BMI, body mass index; BSA, body surface area; CTx, chemotherapy; dCRT, definitive chemoradiotherapy; IQR, interquartile range; nCRT, neoadjuvant chemoradiotherapy; No., number of cases.

Tissue Biopsies

The time between start of infusion and biopsies was comparable between cycle 1 (median 4.8h; IQR 4.3-5.1h) and the last cycle (median 4.3h; IQR 3.7-4.8h). A summary of the location of the analyzed biopsies and pathological assessments is presented in **Supplementary Table S1**. The amount of tissue obtained during the biopsy procedure differed between normal esophageal mucosa and tumor tissue, and between cycles (cycle 1 tumor tissue: median 6.4 mg (IQR: 4.3-7.9 mg); cycle 1 esophageal mucosa: median 2.9 mg (IQR: 2.5-4.3 mg); last cycle tumor tissue: median 5.6 mg (IQR: 2.0-7.1 mg); last cycle esophageal mucosa: median 2.0 mg (IQR: 0.99-2.3 mg)). All biopsies of the tumor at the first cycle contained cancer cells (median cancer cell percentage 60%; IQR 30% - 85%). Biopsies of normally appearing esophageal mucosa during cycle 1 nonetheless contained tumor cells in two patients: subject 4 (20% tumor cells) and subject 15 (30% tumor cells). Of the tumor biopsies taken at the last treatment cycle, only the biopsies of six patients (43%) contained tumor cells, which is probably a positive result of the treatment. Of these six biopsies containing tumor cells, 5 samples contained maximum 10% tumor cells and one sample contained 80% tumor cells. The esophageal mucosa samples taken at the last cycle were all tumor cell negative, except one which contained 1% tumor cells. Necrosis was present in a minority of the biopsies, i.e., in 5 tumor samples and 1 normal mucosal sample during cycle 1 and in 4 tumor samples and 3 normal mucosal samples during the last cycle. In patients treated with concomitant radiotherapy, tumor samples showed limited necrosis percentages but instead showed active inflammation or ulceration.

Tissue Pharmacokinetics

Paclitaxel could be measured in all biopsy samples. One sample (esophageal mucosa cycle 5; subject 12) was excluded from all analyses due to a low amount of tissue (i.e., 0.04 mg) resulting in an unreliable quantification of the paclitaxel concentration. No statistical analyses were performed involving the tumor samples taken during the last cycle given the low amount of tumor cells observed in these biopsies. During the first cycle, a trend towards a higher intratumoral paclitaxel concentration was seen compared to the esophageal mucosa paclitaxel concentration (RD: 43.44%; 95% CI, -2.60%-111.22%; $P = 0.07$; **Table 2**) (excluding Barrett's esophagus biopsies; RD: 58%; 95% CI, 3%-145%; $P = 0.04$). The GEM paclitaxel concentration in normal esophageal mucosa during the first cycle was 2.03 ng/mg (95% CI: 1.38-2.98 ng/mg) while the intratumoral GEM paclitaxel concentration was 2.91 ng/mg (95% CI, 2.22-3.83 ng/mg). The intratumoral paclitaxel concentration in adenocarcinoma samples was not significantly different from the concentrations measured in squamous cell carcinoma samples during the first cycle (RD: -11%; 95% CI, -53%-70%; $P = 0.70$; **Table 2**). The paclitaxel concentration in esophageal mucosa during the last cycle of chemotherapy (GEM: 1.89 ng/mg (95% CI, 1.26-2.85 ng/

mg)) was not significantly different from the concentration measured during the first cycle in esophageal mucosa (RD: -10%; 95% CI, -47%-53%; $P = 0.68$; **Table 2**).

Table 2. Comparisons of tissue pharmacokinetics

	Relative difference	95% confidence interval	*p*-value
Esophageal PTX last cycle vs. esophageal PTX first cycle	–10%	–47% to 53%	0.68
Tumoral PTX first cycle vs. esophageal PTX first cycle	43%	–3% to 111%	0.07
Adenocarcinoma PTX first cycle vs squamous cell carcinoma PTX first cycle	–11%	–53% to 70%	0.70

PTX, paclitaxel

Immunohistochemical Staining

A summary of all immunohistochemical scores per biopsy is presented in **Table 3.** **Figure 1** depicts the H&E staining and the ABCB1 staining of a general representable biopsy of an adenocarcinoma (**Figures 1A,B**), squamous cell carcinoma (**Figures 1C,D**), healthy esophageal mucosa tissue (**Figures. 1E,F**) and Barrett's esophagus (**Figures 1E,F**). During the first cycle, ABCB1 expression in esophageal tumors was significantly higher than in normal esophagus mucosa biopsies ($P < 0.01$). The majority of the normal esophageal tissue biopsies did not express ABCB1 (11 out of 13 (85%)) according to the IRS score during this cycle. The other two esophageal tissue biopsies expressed ABCB1 mildly (n = 1) and strongly (n = 1 (8%)), respectively, of which the latter biopsy was taken from a Barrett's esophagus (**Figure 1F**). ABCB1 staining of tumor samples was strongly positive in all 9 adenocarcinoma samples taken during cycle 1, whereas ABCB1 was expressed significantly less in the squamous cell carcinoma samples ($P < 0.01$), i.e., moderately (n = 2), weakly (n = 1) or not at all (n = 1). Nine tumor samples were evaluable for immunohistochemistry at the last cycle: the 8 evaluable adenocarcinoma samples all remained strongly positive for ABCB1. The single evaluable squamous cell carcinoma sample expressed ABCB1 moderately which also corresponds with the ABCB1 expression observed during the first cycle of chemotherapy (**Table 3**). From the esophageal tissue biopsies taken after the last cycle, 9 biopsies (69%) were negative for ABCB1, while 1 sample (8%) was mildly positive, 2 samples (16%) moderately positive and 1 (Barrett's esophagus) sample (i.e., biopsy subject 15) (8%) was strongly positive for ABCB1 expression. In line with the results seen during the first cycle, the expression of ABCB1 during the last cycle was also significantly higher in tumor samples compared to healthy esophageal tissue ($P = 0.01$).

Table 3. Immunohistochemical score of ABCB1 per biopsy

Subject	Cycle	Tumor				Esophagus			
		Percentage positive cells	Score positive cells	Intensity score	IRS score	Percentage positive cells	Score positive cells	Intensity score	IRS score
1	Last cycle	no tumor	0	no tumor	NA	0%	0	0	0
2	First cycle	100%	4	3	12	0%	0	0	0
3	First cycle	40%	2	3	6	0%	0	0	0
3	Last cycle	40%	2	3	6	0%	0	0	0
4	First cycle	no tumor	0	no tumor	NA	0%	0	0	0
4	Last cycle	no tumor	0	no tumor	NA	0%	0	0	0
5	First cycle	5%	1	3	3	1%	1	3	3
5	Last cycle	no tumor	0	no tumor	NA	0%	0	0	0
6	First cycle	100%	4	3	12	0%	0	0	0
6	Last cycle	100%	4	3	12	1%	1	3	3
7	First cycle	60%	3	3	9	0%	0	0	0
7	Last cycle	100%	4	3	12	0%	0	0	0
8	First cycle	5%	1	1	1	0%	0	0	0
8	Last cycle	no tumor	0	no tumor	NA	0%	0	0	0
9	First cycle	100%	4	3	12	no tissue	no tissue	no tissue	NA
9	Last cycle	100%	4	3	12	0%	0	0	0
10	First cycle	100%	4	3	12	0%	0	0	0
10	Last cycle	100%	4	3	12	50%	2	2	4
11	First cycle	100%	4	3	12	0%	0	0	0
11	Last cycle	100%	4	3	12	0%	0	0	0
12	First cycle	100%	4	3	12	0%	0	0	0
12	Last cycle	100%	4	3	12	0%	0	0	0
13	First cycle	20%	2	2	4	0%	0	0	0
13	Last cycle	no tumor	0	no tumor	NA	no tissue	no tissue	no tissue	NA
14	First cycle	100%	4	3	12	0%	0	0	0
14	Last cycle	100%	4	3	12	10%	2	3	6
15	First cycle	100%	4	3	12	100%	4	3	12
15	Last cycle	100%	4	3	12	100%	4	3	12

The IRS (immunoreactive score) indicates different categories of ABCB1 expression (i.e., IRS 0–1 = negative for ABCB1; IRS 2–3 = mild ABCB1 expression; IRS 4–8 = moderate ABCB1 expression; IRS 9–12 = strong ABCB1 expression).

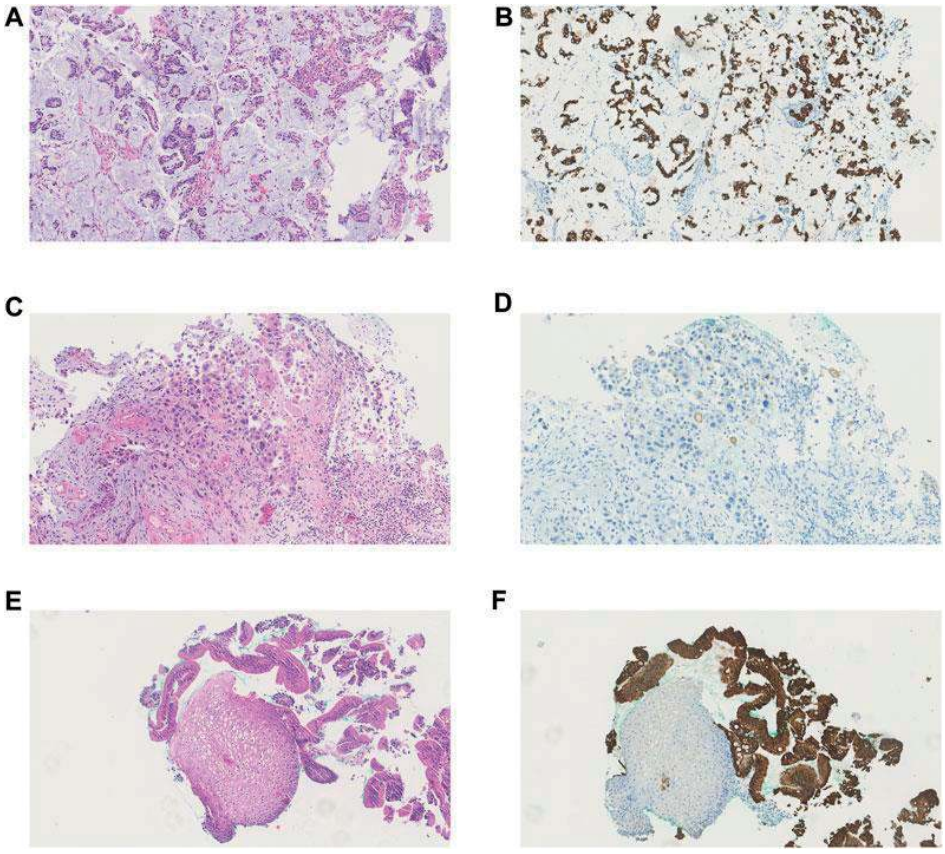

Figure 1. Haematoxylin and eosin (H&E) staining and immunohistochemical staining of ABCB1 in different types of investigated tissue. **(A)** H&E staining of adenocarcinoma; **(B)** ABCB1 immunohistochemical staining of adenocarcinoma; **(C)** H&E staining of squamous cell carcinoma; **(D)** ABCB1 immunohistochemical staining of squamous cell carcinoma; **(E)** H&E staining of healthy esophageal mucosa and Barrett esophagus; **(F)** ABCB1 immunohistochemical staining of healthy esophageal mucosa and Barrett esophagus.

Plasma Pharmacokinetics

The geometric mean $AUC_{0\text{-}48h}$ of paclitaxel was 2,898 ng·h/mL (95% CI, 2,171–3,868 ng·h/mL) during the first cycle, and was similar (2,946 ng·h/mL (95% CI, 2,186–3,969 ng·h/mL)) during the last cycle (RD: 1.66%; 95%CI, -5.41%-9.25%; $P = 0.631$).

Correlation Between Tissue- and Plasma Pharmacokinetics

No correlation could be determined between plasma pharmacokinetics (i.e., $AUC_{0\text{-}48h}$) or the plasma concentration measured around the biopsy procedure (C_{4h})) and the paclitaxel concentration in esophageal mucosa during the first and last cycle of paclitaxel. Interestingly, the intratumoral paclitaxel concentration was strongly positively correlated

to the plasma pharmacokinetics ($AUC_{0\text{-}48h}$: $r = 0.72$; $P < 0.01$ and C_{4h}: r = 0.70; $P < 0.01$) during cycle 1 (**Figures 2A,B**).

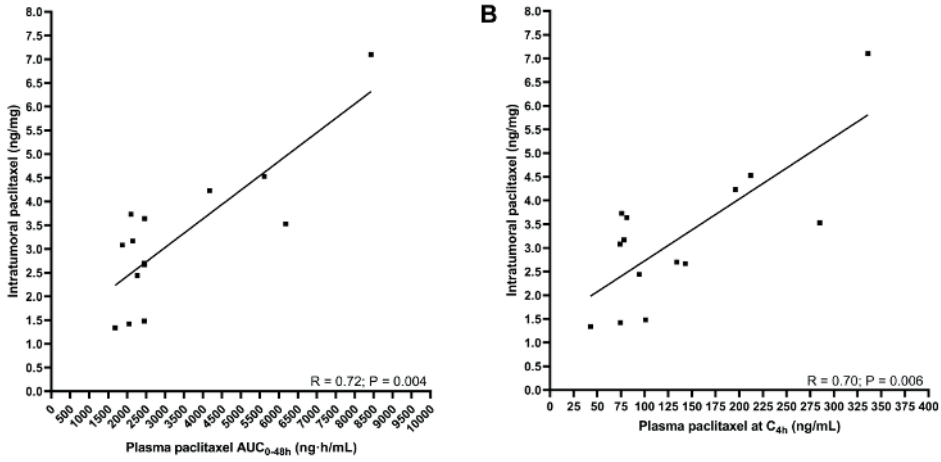

Figure 2. The correlation between intratumoral pharmacokinetics and plasma pharmacokinetics of paclitaxel. **(A)** intratumoral paclitaxel concentration and $AUC_{0\text{-}48h}$ **(B)** intratumoral paclitaxel concentration and concentration at 4h after start infusion.

DISCUSSION

In this study, we demonstrated that ABCB1 efflux transporter expression is significantly higher in adenocarcinoma of the esophagus compared to squamous cell carcinoma of the esophagus. Moreover, the expression of ABCB1 by esophageal carcinomas is higher compared to normal-appearing esophageal mucosa. We could not demonstrate an alteration of intratumoral paclitaxel as first sign of resistance due to the low tumor cell percentage in the (second) biopsies. Nevertheless, we may have (partly) explained the the effectivity of this taxane in esophageal cancer since the paclitaxel concentration in non-tumoral esophageal mucosa is lower than in tumor tissue, and a strong correlation between plasma pharmacokinetics and intratumoral paclitaxel concentration was seen. We have tried to identify pharmacokinetic mechanisms of resistance to paclitaxel in esophageal cancer. A major factor contributing to the occurrence of paclitaxel resistance in solid tumors is overexpression of ABC efflux transporters, which could potentially lower the intratumoral drug concentration.[11-13] Previous studies have reported expression of ABCB1 in adenocarcinoma of the esophagus, as well as in squamous cell carcinoma, while no expression of ABCB1 was described in esophageal mucosa [28 29] In line with these results, we have demonstrated that ABCB1 expression was higher in esophageal carcinoma than in normal esophageal mucosa. However, we have also demonstrated a significantly higher expression of ABCB1 in adenocarcinoma than in squamous cell

carcinoma of the esophagus. Interestingly, in the CROSS trial a significantly higher complete response rate in patients with squamous cell carcinoma of the esophagus than in those with esophageal adenocarcinoma was found.[3] Further, in the long-term data of the CROSS trial also a clinically relevant difference (adenocarcinoma: median overall survival of 43 months versus squamous cell carcinoma: 82 months median overall survival) between the two histological types seems to exist.[4] Therefore, it could be speculated that ABCB1 expression might have contributed to the differences in complete response rate and median survival between the two histological types. Several other studies investigating different regimens of repeated preoperative chemotherapy and radiotherapy in esophageal carcinoma could not identify a survival difference between those two histological subtypes.[30-32] This difference could possibly be explained by the fact that those studies administered cisplatin and fluoropyrimidines as chemotherapeutical agents which are both not substrates of ABCB1.[33]

The difference in ABCB1 expression between the different types of tissues could also be used to improve treatment with carboplatin and paclitaxel in esophageal cancer. A higher ABCB1 tissue expression is expected to result in a lower tissue drug concentration that could lead to a lower efficacy of the drug. Several studies demonstrated that in cell lines overexpressing ABC efflux transporters, inhibition of ABC transporters results in higher intratumoral paclitaxel exposure.[18,19] Increasing the intratumoral paclitaxel exposure by inhibition of ABCB1 expression might enhance the efficacy of the treatment, and thereby reducing a substantial part of patients who do not benefit from the treatment with paclitaxel and carboplatin. Since normal esophageal mucosa does not express ABCB1, it is not expected that inhibition of this ABC efflux transporter results in an increased chemotherapeutical exposure in the healthy esophageal mucosa.[28] Nevertheless, previous research demonstrated that the use of MDRT (multidrug resistance transporters) inhibitors are complicated by several factors.[34] The first generation of these inhibitors are characterized by the high doses needed with only limited efficacy, the severe toxicity profile of those compounds and the pharmacokinetic effects on other drugs.[34] Since these drugs affect drug transporters they have an (potentially negative) effect on the absorption, distribution, metabolism and elimination of others drugs used in patients.[34] Furthermore, it is always important to realize that these transporters are also expressed at other sites than tumors. ABCB1 transporters are also expressed by liver tissue and kidney tissue which could increase paclitaxel related toxicity in those organs which is undesirable given their essential function (EMBL-EBI Expression Atlas, 2021). Newer generations of MDRT inhibitors are characterized by milder toxicity profiles and reduced effects on the overall pharmacokinetics properties and therefore also the pharmacokinetics of other drugs.[34] Nonetheless, the efficacy of these newer generation of MDRT inhibitors remained also limited which might be caused by heterogeneity of

the tumor cells regarding ABCB1 expression, drug penetration, and other simultaneous existing resistance mechanisms.[34]

Contrary to the aforementioned expected influence of ABCB1 expression on tissue paclitaxel exposure, the intratumoral paclitaxel concentration is higher than the paclitaxel concentration in esophageal mucosa despite the higher ABCB1 expression in tumor tissue. One of the factors that might explain the discrepancy between the expectations and the observed results could be tumor vessel permeability. The permeability of vessels in the tumor is higher compared to healthy esophageal tissue that could make it more easily for paclitaxel to distribute into the tumor tissue.[35] The fact that we identified a strong correlation between systemic paclitaxel pharmacokinetics and intratumoral pharmacokinetics could also point to a high vessel permeability in the tumor. Moreover, in line with our findings, it was previously demonstrated that the intratumoral cisplatin concentration in tumor tissue of patients diagnosed with esophagus carcinoma and treated with cisplatin and 5-fluorouracil (5-FU) was higher compared to the concentration in healthy esophagus tissue.[36] Increased permeability of tumor tissue may also be induced by fractionated radiotherapy.[37,38] In line with the described effects of radiotherapy, the intratumoral doxorubicin distribution was improved by radiotherapy.[39]

Alterations over time in ABCB1 expression or intratumoral paclitaxel concentrations might also be a first sign of resistance of the tumor. Nonetheless, we could not identify an alteration in ABCB1 expression over time. This may the result of the relatively short treatment period in our study. In addition, we used a low chemotherapy dose. In contrast, Di Nicolantonio et al. did observe a significant increase in mRNA levels of ABCB1 in paired samples of adenocarcinoma of the esophagus after chemotherapy in an *in vitro* experiment and therefore may not be concordant with our clinical study results.[40] Moreover, Langer et al. also reported no alterations in ABCB1 expression after neoadjuvant chemotherapy in their clinical study.[41]

A comparison between the intratumoral paclitaxel concentration during the first cycle and last cycle was hampered by a low amount of tumor cells observed in tumor biopsies taken during the last cycle. Previous studies demonstrated that up to 28% of the patients who undergo chemoradiotherapy a complete pathological response is observed after completion of their treatment.[3,42] Therefore, it is most likely that the low amount of tumor cells observed in the biopsies taken during the last cycle is a treatment effect of the chemoradiotherapy. Due to this low tumor cell percentage in the tumor biopsies, it could be doubted if the paclitaxel concentrations measured represents the intratumoral paclitaxel concentration. Given that biopsies are homogenized before paclitaxel quantification, it is likely that the paclitaxel concentration measured represents the concentration inside the most dominant type of tissue which is probably non tumorous tissue in the intended tumor biopsies of the last cycle. Therefore, we could not investigate the

alteration in intratumoral paclitaxel concentrations over time which could be a sign of chemotherapy resistance.

Previously, it has also been attempted to investigate the intratumoral paclitaxel pharmacokinetics via several mathematical models which predict the distribution of the drug inside the tumor.[43] However, these models have limited accuracy probably due to simplification of the multiple factors involved in intratumoral drug distribution and can therefore not replace tumor biopsies for intratumoral pharmacokinetic analysis.[43] However, bioanalytical methods should be further improved so that even if a low amount of tumor tissue has been obtained, the intratumoral paclitaxel could be accurately measured without the influence of paclitaxel in the surrounding tissue on the measured intratumoral paclitaxel concentration. Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry might be a tool to achieve such an analytical improvement.

In conclusion, we found a significantly higher ABCB1 expression in esophageal adenocarcinomas than in squamous cell carcinomas, which might be causally related to a better treatment effectivity of paclitaxel in the latter. Resistance by reduced intratumoral paclitaxel concentrations could not be demonstrated because of the low tumor percentage at the last cycle of paclitaxel. Further research investigating the ABCB1 expression in esophageal carcinoma and esophageal mucosa tissue is warranted to elucidate the relationship between response and ABCB1 status.

REFERENCES

1. Global Burden of Disease Cancer C, Fitzmaurice C, Akinyemiju TF, et al. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-Years for 29 Cancer Groups, 1990 to 2016: A Systematic Analysis for the Global Burden of Disease Study. *JAMA Oncol.* 2018;4(11):1553-1568.
2. Polee MB, Sparreboom A, Eskens FA, et al. A phase I and pharmacokinetic study of weekly paclitaxel and carboplatin in patients with metastatic esophageal cancer. *Clin Cancer Res.* 2004;10(6):1928-1934.
3. van Hagen P, Hulshof MC, van Lanschot JJ, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. *N Engl J Med.* 2012;366(22):2074-2084.
4. Shapiro J, van Lanschot JJB, Hulshof M, et al. Neoadjuvant chemoradiotherapy plus surgery versus surgery alone for oesophageal or junctional cancer (CROSS): long-term results of a randomised controlled trial. *Lancet Oncol.* 2015;16(9):1090-1098.
5. de Man FM, van Eerden RAG, Oomen-de Hoop E, et al. Efficacy and Toxicity of Weekly Carboplatin and Paclitaxel as Induction or Palliative Treatment in Advanced Esophageal Cancer Patients. *Cancers (Basel).* 2019;11(6).
6. Chirieac LR, Swisher SG, Ajani JA, et al. Posttherapy pathologic stage predicts survival in patients with esophageal carcinoma receiving preoperative chemoradiation. *Cancer.* 2005;103(7):1347-1355.
7. Toxopeus ELA, de Man FM, Krak N, et al. Association between Paclitaxel Clearance and Tumor Response in Patients with Esophageal Cancer. *Cancers (Basel).* 2019;11(2).
8. Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. *Nat Rev Cancer.* 2004;4(4):253-265.
9. Weaver BA. How Taxol/paclitaxel kills cancer cells. *Mol Biol Cell.* 2014;25(18):2677-2681.
10. Zasadil LM, Andersen KA, Yeum D, et al. Cytotoxicity of paclitaxel in breast cancer is due to chromosome missegregation on multipolar spindles. *Sci Transl Med.* 2014;6(229):229ra243.
11. Barbuti AM, Chen ZS. Paclitaxel Through the Ages of Anticancer Therapy: Exploring Its Role in Chemoresistance and Radiation Therapy. *Cancers (Basel).* 2015;7(4):2360-2371.
12. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat Rev Cancer.* 2002;2(1):48-58.
13. Huisman MT, Chhatta AA, van Tellingen O, Beijnen JH, Schinkel AH. MRP2 (ABCC2) transports taxanes and confers paclitaxel resistance and both processes are stimulated by probenecid. *Int J Cancer.* 2005;116(5):824-829.
14. Schinkel AH, Jonker JW. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. *Adv Drug Deliv Rev.* 2003;55(1):3-29.
15. Trock BJ, Leonessa F, Clarke R. Multidrug resistance in breast cancer: a meta-analysis of MDR1/gp170 expression and its possible functional significance. *J Natl Cancer Inst.* 1997;89(13):917-931.
16. Schaich M, Soucek S, Thiede C, Ehninger G, Illmer T, Group SAS. MDR1 and MRP1 gene expression are independent predictors for treatment outcome in adult acute myeloid leukaemia. *Br J Haematol.* 2005;128(3):324-332.
17. Haber M, Smith J, Bordow SB, et al. Association of high-level MRP1 expression with poor clinical outcome in a large prospective study of primary neuroblastoma. *J Clin Oncol.* 2006;24(10):1546-1553.
18. Stordal B, Hamon M, McEneaney V, et al. Resistance to paclitaxel in a cisplatin-resistant ovarian cancer cell line is mediated by P-glycoprotein. *PLoS One.* 2012;7(7):e40717.

19. Tiwari AK, Sodani K, Dai CL, et al. Nilotinib potentiates anticancer drug sensitivity in murine ABCB1-, ABCG2-, and ABCC10-multidrug resistance xenograft models. *Cancer Lett.* 2013;328(2):307-317.
20. Henningsson A, Sparreboom A, Sandstrom M, et al. Population pharmacokinetic modelling of unbound and total plasma concentrations of paclitaxel in cancer patients. *Eur J Cancer.* 2003;39(8):1105-1114.
21. de Graan AJ, Elens L, Smid M, et al. A pharmacogenetic predictive model for paclitaxel clearance based on the DMET platform. *Clin Cancer Res.* 2013;19(18):5210-5217.
22. Gianni L, Kearns CM, Giani A, et al. Nonlinear pharmacokinetics and metabolism of paclitaxel and its pharmacokinetic/pharmacodynamic relationships in humans. *J Clin Oncol.* 1995;13(1):180-190.
23. Mielke S, Sparreboom A, Steinberg SM, et al. Association of Paclitaxel pharmacokinetics with the development of peripheral neuropathy in patients with advanced cancer. *Clin Cancer Res.* 2005;11(13):4843-4850.
24. Joerger M, Huitema AD, van den Bongard DH, Schellens JH, Beijnen JH. Quantitative effect of gender, age, liver function, and body size on the population pharmacokinetics of Paclitaxel in patients with solid tumors. *Clin Cancer Res.* 2006;12(7 Pt 1):2150-2157.
25. Mathijssen RH, Loos WJ, Verweij J. Determining the best dose for the individual patient. *J Clin Oncol.* 2011;29(33):4345-4346.
26. Sparreboom A, de Bruijn P, Nooter K, Loos WJ, Stoter G, Verweij J. Determination of paclitaxel in human plasma using single solvent extraction prior to isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Sci Appl.* 1998;705(1):159-164.
27. Remmele W, Stegner HE. [Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue] Vorschlag zur einheitlichen Definition eines Immunreaktiven Score (IRS) fur den immunhistochemischen Ostrogenrezeptor-Nachweis (ER-ICA) im Mammakarzinomgewebe. *Pathologe.* 1987;8(3):138-140.
28. Atlas E-EE. Results for ABCB1 and *Homo sapiens.* Available at://www.ebi.ac.uk.gxa/home. . 2021.
29. Vrana D, Hlavac V, Brynychova V, et al. ABC Transporters and Their Role in the Neoadjuvant Treatment of Esophageal Cancer. *Int J Mol Sci.* 2018;19(3).
30. Cooper JS, Guo MD, Herskovic A, et al. Chemoradiotherapy of locally advanced esophageal cancer: long-term follow-up of a prospective randomized trial (RTOG 85-01). Radiation Therapy Oncology Group. *JAMA.* 1999;281(17):1623-1627.
31. Reynolds JV, Muldoon C, Hollywood D, et al. Long-term outcomes following neoadjuvant chemoradiotherapy for esophageal cancer. *Ann Surg.* 2007;245(5):707-716.
32. Xi M, Xu C, Liao Z, et al. The impact of histology on recurrence patterns in esophageal cancer treated with definitive chemoradiotherapy. *Radiother Oncol.* 2017;124(2):318-324.
33. Marzolini C, Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther.* 2004;75(1):13-33.
34. Choi YH, Yu AM. ABC transporters in multidrug resistance and pharmacokinetics, and strategies for drug development. *Curr Pharm Des.* 2014;20(5):793-807.
35. Pasqualini R, Arap W, McDonald DM. Probing the structural and molecular diversity of tumor vasculature. *Trends Mol Med.* 2002;8(12):563-571.
36. Troger V, Francois E, Frenay M, Namer M, Milano G. Analysis of tissue platinum distribution in patients with cancer of the oesophagus. *Eur J Cancer.* 1991;27(3):256-259.

37. Debbage PL, Seidl S, Kreczy A, Hutzler P, Pavelka M, Lukas P. Vascular permeability and hyperpermeability in a murine adenocarcinoma after fractionated radiotherapy: an ultrastructural tracer study. *Histochem Cell Biol.* 2000;114(4):259-275.
38. Ng QS, Goh V, Milner J, Padhani AR, Saunders MI, Hoskin PJ. Acute tumor vascular effects following fractionated radiotherapy in human lung cancer: In vivo whole tumor assessment using volumetric perfusion computed tomography. *Int J Radiat Oncol Biol Phys.* 2007;67(2):417-424.
39. Potiron V, Clement-Colmou K, Jouglar E, et al. Tumor vasculature remodeling by radiation therapy increases doxorubicin distribution and efficacy. *Cancer Lett.* 2019;457:1-9.
40. Di Nicolantonio F, Mercer SJ, Knight LA, et al. Cancer cell adaptation to chemotherapy. *BMC Cancer.* 2005;5:78.
41. Langer R, Specht K, Becker K, et al. Comparison of pretherapeutic and posttherapeutic expression levels of chemotherapy-associated genes in adenocarcinomas of the esophagus treated by 5-fluorouracil- and cisplatin-based neoadjuvant chemotherapy. *Am J Clin Pathol.* 2007;128(2):191-197.
42. Shapiro J, van Hagen P, Lingsma HF, et al. Prolonged time to surgery after neoadjuvant chemoradiotherapy increases histopathological response without affecting survival in patients with esophageal or junctional cancer. *Ann Surg.* 2014;260(5):807-813; discussion 813-804.
43. Popilski H, Stepensky D. Mathematical modeling analysis of intratumoral disposition of anticancer agents and drug delivery systems. *Expert Opin Drug Metab Toxicol.* 2015;11(5):767-784.

SUPPLEMENTARY METHODS 1.

Treatment

To be eligible for this study patients could be treated with different schedules of chemotherapy. Choice for either of these schedules was made by the treating physician based on indication. The following schedules of paclitaxel were used in the study, depending on indication:

- Weekly paclitaxel 50 mg/m^2 in a 1-hour infusion was given together with carboplatin area under the curve (AUC) of 2 mg/mL/min concomitant with a total 3D conformal radiation dose of 41.4 Gy or 50.4 Gy given in 23 fractions or 28 fractions of 1.8 Gy each, with 5 fractions administered per week for 5 (in the neoadjuvant setting) or 6 (as definitive chemoradiotherapy) consecutive weeks depending on the indication (Shapiro et al., 2015).
- Weekly paclitaxel 100 mg/m^2 in a 1-hour infusion, in combination with carboplatin targeting an AUC of 4 mg/mL/min, for 6 consecutive weeks (Polee et al., 2004).

If patients experienced an allergic reaction during paclitaxel infusion, paclitaxel was given in a 1.75-hour infusion during the remaining cycles according to local anti-allergic regimen, consisting of:

- 0- 15 min Paclitaxel infusion at 15 mL/hour
- 15- 30 min 50 mL NaCl 0.9% + 2 mg clemastine
- 30- 45 min Paclitaxel infusion at 84 mL/hour
- 45- 105 min If no allergic reaction follows continue infusion of paclitaxel at 500 mL/hour

Supplementary Table 1. Pharmacokinetic tissue sample characteristics

Subj.	Regimen	Tissue type	Cycle	Location PK[α] (cm)	Weight (mg)	Location PA[β] (cm)	Tumor cells (%)	Necrosis (%)	Cycle	Location PK[α] (cm)	Weight (mg)	Location PA[β] (cm)	Tumor cells (%)	Necrosis (%)
1	CTx	Tumor	1	34	6.69	-	-	-	5	35	0.97	35	0	100
1	CTx	Esophageal mucosa	1	26	4.99	-	-	-	5	28	5.34	28	0	0
3	CTx	Tumor	1	38	9.66	36	80	20	6	39	15.2	39	0	-
3	CTx	Esophageal mucosa	1	30	2.94	29	0	0	6	30	1.97	30	-	-
4	dCRT	Tumor	1	32	7.34	32	10	10	6	28	1.46	28	0	10
4	dCRT	Esophageal mucosa	1	27	4.3	27	30	20	6	26	1.15	27	0	50
5	nCRT	Tumor	1	32	7.75	30	70	0	5	30	6.9	30	0	0
5	nCRT	Esophageal mucosa	1	23	0.57	24	0	0	5	25	0.33	25	-	-
6	CTx	Tumor	1	37	4.07	35	5	0	6	38	1.25	39	80	10
6	CTx	Esophageal mucosa	1	25	3.79	25	0	0	6	28	2.24	28	1	10
7	nCRT	Tumor	1	34	4.22	35	50	0	5	33	6.42	33	5	0
7	nCRT	Esophageal mucosa	1	28	2.92	28	0	0	5	28	2.52	28	0	0
8	nCRT	Tumor	1	37	6.18	37	90	10	5	37	7.11	37	0	0
8	nCRT	Esophageal mucosa	1	30	4.33	30	0	0	5	30	1.93	30	0	0
9	nCRT	Tumor	1	31	4.2	30	60	0	5	30	4.83	30	1	0
9	nCRT	Esophageal mucosa	1	28	2.35	25	0	0	5	23	2.52	23	0	0
10	nCRT	Tumor	1	35	7.67	35	100	0	5	39	7.7	39	10	0
10	nCRT	Esophageal mucosa	1	30	5.27	29	0	0	5	35	1.98	35	0	0
11	nCRT	Tumor	1	37	8.44	37	70	30	5	37	3.88	37	5	0
11	nCRT	Esophageal mucosa	1	32	1.14	32	-	-	5	32	0.56	32	-	0
12	nCRT	Tumor	1	34	4.35	34/35	100	0	5	39	6.52	35	10	0
12	nCRT	Esophageal mucosa	1	29	2.53	30	0	0	5	30	0.04	30	-	-
13	dCRT	Tumor	1	19	9.1	19	30	60	5	22	2.17	22	0	-
13	dCRT	Esophageal mucosa	1	32	2.83	32	-	-	5	27	1.13	27	0	-
14	nCRT	Tumor	1	38	4.81	38	60	0	5	37	7.24	37	0	0
14	nCRT	Esophageal mucosa	1	33	2.8	33	0	0	5	33	1.27	33	0	50
15	nCRT	Tumor	1	26	4.52	26	30	0	5	26	3.28	26	0	0
15	nCRT	Esophageal mucosa	1	20	2.72	20	20	0	5	20	2.11	20	0	0

[α] = Pharmacokinetic (PK) biopsy, [β]= Pathological (PA) biopsy. Abbreviations: CTx = chemotherapy, dCR = definitive chemoradiotherapy, nCRT = neoadjuvant chemoradiotherapy.

SUPPLEMENTARY REFERENCES

Shapiro J, van Lanschot JJB, Hulshof M, et al. Neoadjuvant chemoradiotherapy plus surgery versus surgery alone for oesophageal or junctional cancer (CROSS): long-term results of a randomised controlled trial. *Lancet Oncol.* 2015;16(9)**:**1090-1098.

Polee MB, Sparreboom A, Eskens FA, et al.A phase I and pharmacokinetic study of weekly paclitaxel and carboplatin in patients with metastatic esophageal cancer. *Clin Cancer Res.* 2004;10(6)**:**1928-1934.

PART II

TARGETED ORAL ANTICANCER THERAPY

CHAPTER 8

INFLUENCE OF PROBENECID ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF SORAFENIB

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ABSTRACT

Background

Prior studies have demonstrated an organic anion transporter 6 (OAT6)-mediated accumulation of sorafenib in keratinocytes. The OAT6 inhibitor probenecid decreases sorafenib uptake in skin and might, therefore, decrease sorafenib-induced cutaneous adverse events. Here, the influence of probenecid on sorafenib pharmacokinetics and toxicity was investigated.

Methods

Pharmacokinetic sampling was performed in 16 patients on steady-state sorafenib treatment at days 1 and 15 of the study. Patients received sorafenib (200–800 mg daily) in combination with probenecid (500 mg two times daily (b.i.d.)) on days 2–15. This study was designed to determine bioequivalence with geometric mean area under the curve from zero to twelve hours ($AUC_{0-12\,h}$) as primary endpoint.

Results

During concomitant probenecid, sorafenib plasma $AUC_{0-12\,h}$ decreased by 27% (90% CI, −38% to −14%; $P < 0.01$). Furthermore, peak and trough levels of sorafenib, as well as sorafenib concentrations in skin, decreased to a similar extent in the presence of probenecid. The metabolic ratio of sorafenib-glucuronide to parent drug increased (+29%) in the presence of probenecid. A decrease in systemic sorafenib concentrations during probenecid administration seems to have influenced cutaneous concentrations. Since sorafenib-glucuronide concentrations increased compared with sorafenib and sorafenib-*N*-oxide, probenecid may have interrupted enterohepatic circulation of sorafenib by inhibition of the organic anion transporting polypeptides 1B1 (OATP1B1).

Conclusion

Sorafenib treatment with probenecid is, therefore, not bioequivalent to sorafenib monotherapy. A clear effect of probenecid on sorafenib toxicity could not be identified in this study.

INTRODUCTION

Over the last two decades, systemic anti-cancer treatment options have been expanded from traditional cytotoxic chemotherapy to targeted agents, including tyrosine kinase inhibitors (TKIs). TKIs offer a number of important advantages over conventional cytotoxic chemotherapy like the oral administration of the drugs, but they are still afflicted by some major problems, including large interindividual pharmacokinetic variability, a narrow therapeutic window, and debilitating adverse events.[1] Cutaneous adverse events are among the most frequently observed toxicities with many TKIs, and their intensity can significantly affect both quality of life and health care economics.[2]

A particularly painful complication seen most frequently during the early weeks of use with TKIs, such as sorafenib, sunitinib, and regorafenib, is called hand-foot skin reaction (HFSR), in which painful hyperkeratotic plaques develop predominantly over sites of pressure or friction.[3,4] The clinical incidence of HFSR varies among TKIs with a particularly high incidence (20% ≥grade 3) being observed with sorafenib,[5] an orally administered multikinase inhibitor, registered for treatment of advanced hepatocellular carcinoma and advanced renal cell carcinoma as well as iodine-refractory advanced thyroid cancer.[3,4,6,7] Furthermore, it is investigated as a treatment option for acute myeloid leukemia.[8] The pathogenesis of TKI-induced HFSR remains currently unknown, and the only effective treatment options involve either dose reduction or discontinuation of therapy, which theoretically may have negative effects on disease management.[9,10] However, previous *in vitro* and in vivo research showed that sorafenib can accumulate in human epidermal keratinocytes mediated by the organic anion transporter 6 (OAT6),[11] and that sorafenib-induced skin toxicity can be prevented by cotreatment with the OAT6 inhibitor probenecid without negatively influencing the antitumor properties of sorafenib.[11] Probenecid is an uricosuric agent indicated for the maintenance treatment of hyperuricemia associated with gout and gouty arthritis. It was also used as an adjuvant for therapy with certain antibiotics, such as penicillin, ampicillin, or methicillin, because it elevates and prolongs their plasma levels by inhibition of renal excretion.[12] Probenecid is usually well tolerated at a dose of 500 mg two times daily and is usually taken for (many) months. Probenecid is also known as a pan- uridine diphosphate glucuronosyltransferase (UGT) inhibitor, used in drug registration studies and, therefore, could potentially influence pharmacokinetics of several drugs, including sorafenib that undergoes cytochrome P450 3A4 (CYP3A4)-mediated oxidation into its active metabolite (pyridine-*N*-oxide) and UGT1A9-mediated glucuronidation into sorafenib glucuronide.[13-15] Furthermore, probenecid is known to alter the activity of several drug transporters like OAT and the organic anion transporting polypeptides (OATP), which play a main role in renal and hepatic excretion.[16] However, the extent of this possible effect is not yet determined in clinical studies and the safety of the combination of these drugs is currently unknown.

As part of an ongoing project to develop translationally useful prevention strategies for sorafenib-induced HFSR, in the current study, we evaluated the pharmacokinetics (PK) and safety of sorafenib when concomitantly used with probenecid.

PATIENTS AND METHODS

This non-randomized, cross-over study was performed between November 2017 and November 2019 at the Erasmus University MC Cancer Institute. The study was approved by the local ethics committee of the Erasmus University MC (METC 17-490, date of approval 16-11-2017) and competent authority and was registered at the European Clinical Trials Database (EudraCT 2017-002470-40) and the Dutch trial registry (www.trialregister.nl; number NL6783).

Patients

Patients who had a confirmed diagnosis of advanced hepatocellular carcinoma (HCC) or differentiated thyroid carcinoma with an indication for sorafenib treatment, and who were at least 18 years of age, were included in this study. Furthermore, patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of ≤2 and an adequate hematological, renal, and liver function defined as a Common Terminology Criteria for Adverse Events (CTCAE) grade of ≤2 at baseline. Besides, patients with known contraindications for probenecid use (e.g., history of uric acid kidney stones, an acute gouty attack, or blood dyscrasias) and/or the use of drugs that are strong CYP3A4 or UGT1A9 inducers or inhibitors were excluded. All included patients gave written informed consent.

Study procedures

Patients received sorafenib for at least two weeks to ensure steady-state pharmacokinetics of sorafenib. Since sorafenib has linear pharmacokinetics,[17] dose reductions were allowed after the start of the study. Sorafenib was administered at a 200–800-mg daily dose during the 15-day study period and was given concomitantly with probenecid (500 mg b.i.d.) from day 2 to day 15 of the study. Both sorafenib and probenecid were ingested at predefined timepoints at 10:00 a.m. and 10:00 p.m.

Pharmacokinetic sampling

Patients were admitted to the hospital on days 1 and 15 of the study for pharmacokinetic blood sampling. A total of nine blood samples for the determination of sorafenib, sorafenib *N*-oxide, and sorafenib glucuronide were obtained at predefined time points (T = pre, T = 0.5 h, T = 1 h, T = 2 h, T = 4 h, T = 6 h, T = 8 h, T = 10 h, and T = 12 h). Blood

samples were processed into plasma within 30 min, by vortex mixing and centrifugation for 10 min at 2500 g at 4 °C. Plasma concentrations were determined using a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method[18], at both the laboratory of Translational Pharmacology in the Erasmus MC, Rotterdam, and the laboratory of Pharmaceutics and Pharmaceutical Chemistry, Ohio State University, OH. Predefined pharmacokinetic endpoints were the dose-corrected area under the curve from pre-administration time point until 12 h after sorafenib intake ($AUC_{0-12\,h}$), maximum concentration (C_{max}), time until maximum concentration (T_{max}), and lowest plasma concentration (C_{trough}) and were determined using WinNonlin v. 7.0 (Phoenix, Certara, 5349 AB, Oss, The Netherlands) for sorafenib, sorafenib-*N*-oxide, and sorafenib glucuronide.

Skin biopsies

A 3-mm skin biopsy was obtained at days 1 and 15 of the study during PK sampling days for pharmacokinetic analysis. Skin biopsies were taken from either the forearm or the shoulder region, but always from the same region at the same timepoint in an individual patient during the two consecutive PK sampling days. If patients had HFSR lesions at the hand at the first PK day an additional skin biopsy was performed from the thenar eminence region of the hand for pathologic analysis on days 1 and 15. The biopsies were graded according to the scoring for interface dermatitis as used for graft-versus-host disease by an experienced pathologist (J.D.). There is no other pathologic grading scale for HFSR and our grading scale shows the most overlapping features from a pathologic perspective.[19] Furthermore, concentrations of sorafenib were determined from the skin biopsies after dilution in human plasma and homogenization using the validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method described earlier.[18]

In vitro transport assay

Transport assays assessing probenecid's inhibition of OATP1B1 were conducted as previously described.[20] The [3H]estradiol-17b-d-glucuronide, a positive control substrate for OATP1B1,[21] was obtained from American Radiolabeled Chemicals. Water-soluble probenecid was obtained from Invitrogen (Molecular Probes). The generation and characterization of Flp-In T-Rex293 cells expressing inducible OATP1B1 have been reported previously.[22, 23] Cells expressing OATP1B1 or vector control (VC) were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen) supplemented with 10% Fetal Bovine Serum (FBS), hygromycin B (25 mg/mL; Invitrogen), and blasticidin (37.5 mg/mL; Biovision, California, United States of America). Cells were seeded in 24-well plates in phenol red-free DMEM containing 10% FBS, hygromycin (25 mg/mL), blasticidin (37.5 mg/mL), and doxycycline (1 µg/mL) and were incubated at 37 °C for 24 h. Cells were then washed with warm PBS and pre-incubated with the indicated concentration of probenecid in phenol red-free DMEM (without FBS and supplements) at 37 °C for 15 min. Cells were

then incubated with phenol red-free DMEM containing the indicated concentration of probenecid and 0.2 μM [3H]estradiol-17b-D-glucuronide for an additional 15 min. The experiment was terminated by washing three times with ice-cold PBS. Cells were lysed in 1 N NaOH at 4 °C overnight, and then the solution was neutralized with 2 mol/L HCl. Total protein was measured using a Pierce BCA Protein Assay Kit (Thermo Scientific), and total protein content was quantified using a microplate spectrophotometer. Drug concentrations were determined in the remaining cell lysate by liquid scintillation counting using a scintillation counter. OATP1B1-mediated uptake was calculated by dividing the disintegrations per minute (dpm) from each replicate by the amount of protein (mg) and subtracting the dpm/mg protein in VC cell line from the dpm/mg protein in OATP1B1 overexpressing cells at each concentration of probenecid. OATP1B1-mediated uptake at each concentration of probenecid was then compared with OATP1B1-mediated uptake when only an equal volume of vehicle was added without probenecid (i.e., % control). The half maximal inhibitory concentration (IC_{50}) was calculated using a nonlinear fit comparing concentration of probenecid versus response.

Toxicity

Toxicity rates were determined at baseline, days 1 and day 15 of the study using Common Terminology Criteria for Adverse Events (CTCAE version 4.0, National Cancer Institute, Bethesda, Maryland, United States), and by evaluating the patient diaries during the sorafenib monotherapy phase and sorafenib concomitant with probenecid phase.

Statistical analysis

The primary objective of this study was to determine bioequivalence between sorafenib monotherapy and sorafenib concomitantly with probenecid to determine whether it is a safe option in clinical practice. Bioequivalence can be concluded when the 90% confidence interval (CI) of the ratio of geometric means is within 80% and 125%. Assuming a standard deviation of the difference of 0.25 for log ($AUC_{sorafenib}$), using a 90% power and two-sided alpha of 5%, the required number of evaluable patients was 16. Analyses of $AUC_{0\text{-}12h}$, $C_{trough,}$ and C_{max} were performed on log-transformed observations by means of the paired t-test. The point estimates and CIs were transformed back to the original scale in order to give the point estimates for the ratio of the geometric means and the CIs. T_{max} was analyzed by means of the Wilcoxon signed rank test and described with medians and interquartile ranges. Toxicity was described as the incidence of toxicity per phase. This was corrected for baseline toxicity and was only taken into account in case of an increase in CTCAE grade per PK sampling day. Since the design of this study was not appropriate to detect a significant difference in toxicity, these results had a descriptive character.

RESULTS

Patient Characteristics

Seventeen patients were included, of whom 16 patients were evaluable due to withdrawal of one patient. Most patients (n = 14) were male and had an HCC (n = 12). Eight patients with HCC had underlying liver cirrhosis due to alcohol abuse (n = 3) or chronic viral hepatitis (n = 5). Other patient characteristics can be found in **Table 1.**

Table 1. Patient characteristics

Characteristic	Evaluable patients (n = 16)
Sex	
Male	14 (88%)
Female	2 (12%)
Age (years) median [IQR]	66.5 [58-75]
Performance	
ECOG 0	1 (6%)
ECOG 1	13 (82%)
ECOG 2	2 (12%)
Tumor type	
HCC	12 (72%)
- Liver cirrhosis	*8 (66%)*
- Pre-existent hepatitis	*5 (42%)*
Thyroid carcinoma	4 (28%)
BMI (kg/m^2) median [IQR]	25.2 [22-30]
Race	
Caucasian	11 (70%)
African	1 (6%)
Arabic	3 (18%)
Asian	1 (6%)
Sorafenib daily dose at start of study	
200 milligrams	1 (6%)
400 milligrams	10 (63%)
600 milligrams	2 (12%)
800 milligrams	3 (19%)

Abbreviations: BMI = body mass index, ECOG = Eastern Cooperative Oncology Group; HCC = hepatocellular carcinoma, n = number of patients; IQR = interquartile range.

Pharmacokinetics

When sorafenib was administered with concomitant probenecid, the geometric mean sorafenib $AUC_{0-12\ h}$ was 26.8% (90% CI, −37.7% to −14.1%) lower than when sorafenib was administered alone. Similarly, sorafenib plasma C_{max} and C_{trough} decreased significantly by 25.1% (90% CI, −44.3% to −19.7%) and 26.0% (90% CI, −43.4% to −3.4%), respectively (**Table 2 and Figure 1**). Sorafenib metabolites showed a similar decrease in plasma concentration, although there was a substantial interpatient variability. The

Table 2. Pharmacokinetic results

Pharmacokinetic parameters	Sorafenib monotherapy	Metabolic ratio (metabolite/ sorafenib)	Sorafenib with probenecid	Metabolic ratio (metabolite/ sorafenib)	RD sorafenib monotherapy vs sorafenib + probenecid (90%CI)	P-value
Sorafenib						
$AUC_{0\text{-}12h}$ (CV %) geomean µg*h/mL	33,457.8 (42)		24,476.8 (57)		-26.8% (-37.7% to -14.1%)	<0.01
C_{max} (CV %) geomean µg/mL	4457.8 (52)		3337.2 (63)		-25.1% (-44.3% to -19.7%)	<0.01
C_{trough} (CV %) geomean µg/mL	2125.5 (60)		1571.9 (61)		-26.0% (-43.4% to -3.4%)	0.07
T_{max} (IQR) median hours	3.7 (1.5-4.15)		2.2 (1.0-2.01)		NA	0.53
Sorafenib-N-oxide						
$AUC_{0\text{-}12h}$ (CV %) geomean µg*h/mL	3442.8 (78)	0.10	2192.3 (77)	0.09	-36.3% (-52.8% to -14.1%)	0.02
C_{max} (CV %) geomean µg/mL	467.3 (77)		283.9 (74)		-39.2% (-54.6% to 18.7%)	<0.01
C_{trough} (CV %) geomean µg/mL	271.1 (282)		(71)		-35.2% (-59.7% to 4.3%)	0.13
Sorafenib-glucuronide						
$AUC_{0\text{-}12h}$ (CV %) geomean µg*h/mL	120,660 (55)	3.61	113,995 (59)	4.66	-5.5% (-18.0% to 8.9%)	0.49
C_{max} (CV %) geomean µg/mL	12,704 (51)		11,931 (64)		-6.1% (-21.7% to 12.7%)	0.56
C_{trough} (CV %) geomean µg/mL	9159 (65)		8400 (67)		-8.3% (-21.3% to 6.9%)	0.34
Tissue						
Sorafenib concentration in keratinocytes Geomean ng/mL (CV %)	50.0 (61)	$1.49 * 10^{-3}$ *	36.0 (63)	$1.47* 10^{-3}$	-28.1% (-46.3% to -3.7%)	0.07

Abbreviations: $AUC_{0\text{-}12h}$ = area under the curve, time point 0h to 12h; CI = confidence Interval; RD = relative difference; C_{max} = maximum concentration; C_{trough} = minimum concentration; CV = coefficient of variation; h = hours; n = number of patients; T_{max} = time until maximum concentration, IQR = interquartile range; NA = not applicable. * = ratio of plasma to skin sorafenib.

sorafenib-*N*-Oxide $AUC_{0-12\ h}$ decreased by 36.3% (90% CI, −52.8% to −14.1%) and C_{max} showed a similar significant decrease of 39.2% (90% CI, −54.6% to −18.7%). Interestingly, cotreatment with probenecid did not decrease the sorafenib-glucuronide $AUC_{0-12\ h}$ to a similar extent (5.5%; 90% CI, −18.0% to 8.9%), did not significantly influence C_{max} (6.1%; 90% CI, −21.7% to 12.7%), and, thus, shows bioequivalence (**Table 2 and Figure 1**). The ratio of sorafenib-glucuronide to sorafenib increased by 29% when sorafenib was co-administered with probenecid, whereas other metabolic ratios did not change significantly. Sorafenib concomitant with probenecid is not bioequivalent to sorafenib monotherapy.

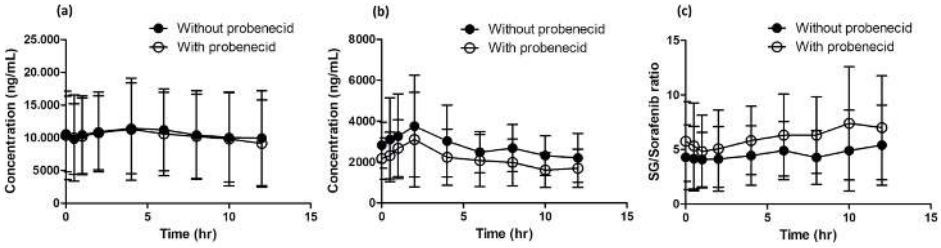

Figure 1. Pharmacokinetic results are displayed for (**a**) sorafenib-glucuronide concentration, (**b**) sorafenib concentration,(**c**) sorafenib-glucuronide (SG) to sorafenib ratio.

Sorafenib concentration in skin decreased in the presence of probenecid by 28.1% (90% CI, −46.3% to −3.7%), with a similar plasma sorafenib/sorafenib in skin ratio (**Table 2**). Furthermore, there was no difference between patients with or without liver cirrhosis in sorafenib plasma AUC (−6.3%; 90% CI, −32.9% to 30.7%; $P = 0.73$) and C_{trough} (−7.4%; 90% CL, −46.8% to 61.3%; $P = 0.81$).

In vitro transport assay

Subsequently, we hypothesized that probenecid interferes with enterohepatic sorafenib circulation via OATP1B1 inhibition and, therefore, measured the impact of probenecid on the cellular uptake of a probe OATP1B1 substrate, [3H] estradiol-17b-d-glucuronide, in a cell line overexpressing OATP1B1. Probenecid inhibited OATP1B1 function with an IC_{50} of 182 µM (**Figure 2**)). Given that probenecid achieves plasma concentrations higher than 200 µM at clinically relevant doses,[24] the results of this experiment support our hypothesis that OATP1B1 contributes to the observed drug–drug interaction between probenecid and sorafenib.

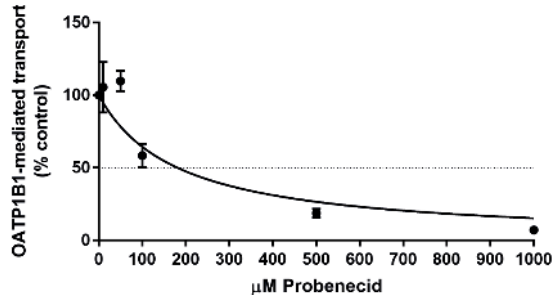

Figure 2. Inhibition of OATP1B1 function by probenecid in vitro. HEK293 cells expressing OATP1B1 or VC were pre-incubated with probenecid at the indicated concentrations for 15 minutes before incubation with probenecid and [3H] estradiol-17b-D-glucuronide for 15 minutes. Data represent uptake of OATP1B1-expressing cells at each concentration compared against vehicle after subtracting uptake by VC cells (mean ± SEM). Each concentration consists of 3-9 technical replicates across 1-3 biological replicates.

Toxicity

There were three serious adverse events, which were assumed to be not related to any of the study drugs (gastroenteritis with dehydration and dyspnea grade 3 during the probenecid part and atrial fibrillation *de novo* in the monotherapy part, all complicated with unplanned hospitalization). HFSR, rash, anorexia, and fatigue occurred more frequently during probenecid administration (65%) than during sorafenib monotherapy (43%) (**Table 3, Supplementary Table 1**). HFSR occurred in 10 of 16 patients with five patients experiencing HFSR at the first day of PK sampling. Three of these patients experienced

Table 3. Patient reported adverse events during study period.

	Sorafenib mono (N = 16)		Sorafenib concomitantly with probenecid (N = 16)	
Adverse event	Grade 1-2	Grade 3	Grade 1-2	Grade 3
HFSR	3	-	6	1
Rash	1	-	3	1
Nausea	1	-	2	-
Vomiting	0	-	1	-
Oral mucositis	1	-	1	-
Diarrhea	1	-	2	-
Constipation	2	-	3	-
Anorexia	4	-	7	-
Dyspnea	-	-	-	1
Edema	-	-	1	-
Fatigue	2	-	6	1
Fever	1	-	-	-
Pain	1	1	2	1
Serious adverse events (SAE)	1		2	

There were three serious adverse events (Atrial fibrillation de novo, dyspnea grade 3 and severe gastroenteritis with dehydration) during sorafenib therapy for which hospitalization was necessary.

progression of HFSR symptoms during the study. Most toxicity occurred after 3–6 weeks of treatment. Most grades 2 and 3 adverse events were seen when sorafenib was administered with probenecid. A total of five patients experienced HFSR at PK sampling day 1 and a biopsy of the thenar eminence region was taken in these patients on days 1 and 15 of the study. There was no difference in the grading of the HFSR between the first and second PK sampling day in these patients.

DISCUSSION

In this study, we investigated the influence of the OAT6 and OATP1B1 inhibitor probenecid on sorafenib pharmacokinetics and toxicity in patients, and found a significant decrease in the geometric mean of sorafenib plasma exposure and a nearly significant decrease in intracutaneous sorafenib exposure during concomitant probenecid administration making sorafenib concomitantly with probenecid not bioequivalent to sorafenib monotherapy. Of the metabolites, systemic sorafenib-*N*-oxide concentrations decreased proportionally with the parent drug, but the sorafenib-glucuronide to sorafenib ratio increased after probenecid administration, which does not support the hypothesis of UGT inhibition. This is in line with our previous findings on enterohepatic circulation of sorafenib-glucuronide, which demonstrated that OATP1B inhibition leads to an increase in plasma sorafenib glucuronide levels.[13, 25] Next to the relative increase in systemic sorafenib-glucuronide exposure, its reduced hepatocellular secretion would also explain the decrease in systemic sorafenib concentrations after probenecid administration, as these concentrations are less maintained via enterohepatic circulation of deconjugated sorafenib glucuronide. Moreover, as we found probenecid to inhibit OATP1B1-mediated transport in vitro at clinically relevant concentrations and as we previously showed that OATP1B1 contributes to enterohepatic sorafenib cycling,[13] it is plausible that reduced enterohepatic circulation of sorafenib led to its significant decrease in systemic exposure after probenecid. Alternatively, the relative systemic accumulation of sorafenib-glucuronide compared with sorafenib and sorafenib-*N*-oxide might be caused by decreased tubular secretion in the kidney, where probenecid is known to inhibit prominent drug transporters as OAT1 and OAT3.[16] However, data regarding this potential interaction are lacking.

This study was not designed to quantify these mechanisms and it should be noted that all patients followed the same sequence of treatment, i.e., sorafenib monotherapy followed by concomitant probenecid, which complicates the differentiation between effects of probenecid and time. Regardless of its etiology, the decrease in systemic sorafenib exposure rather than inhibited OAT6-mediated transport seemed to determine cutaneous sorafenib concentrations, as systemic and cutaneous sorafenib con-

centrations decreased proportionally and protective effect of probenecid on cutaneous exposure could not be demonstrated. This follows a recent population PK analysis in which systemic sorafenib and sorafenib-*N*-oxide were associated with earlier occurrence of HFSR.[26]

Despite lower sorafenib exposure, adverse events occurred more frequently during probenecid cotreatment. It is known that the prevalence of adverse events increases during the first weeks of sorafenib treatment.[27] The difference in adverse events is, therefore, unlikely a result of the drug interaction observed in this study. Patients participated in the study at a relatively early stage of the TKI treatment (i.e., maximal six weeks after start of sorafenib treatment). Usually, sorafenib adverse events such as hypertension occur early during TKI treatment [27] and HFSR usually develops 2–4 weeks after initiation of sorafenib. Hence, it is not likely that we missed this adverse event in our study population.[19,27] In the five patients who experienced HFSR at the first day of study, pathologic characteristics of skin biopsies from the thenar eminence region did not change during the study, potentially due to the non-specificity for HFSR of the used grading scale, i.e., the interface dermatitis score, or due to the absence of high-grade HFSR in our study population. Therefore, subtle HFSR specific changes could have been missed.

CONCLUSIONS

In conclusion, both systemic and cutaneous sorafenib exposure decreased proportionally during concomitant probenecid administration, which may have been caused by interruption of enterohepatic cycling via OATP1B1 inhibition.

REFERENCES

1. Hartmann JT, Haap M, Kopp HG, Lipp HP. Tyrosine kinase inhibitors-a review on pharmacology, metabolism and side effects. *Curr Drug Metab*. 2009;10:470–481.
2. Anderson RT, Keating KN, Doll HA, Camacho F. The Hand-Foot Skin Reaction and Quality of Life Questionnaire: An Assessment Tool for Oncology. *Oncologist*. 2015;20:831–838.
3. Llovet JM, Ricci, S, Mazzaferro V. et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med*. 2008;359:378–390.
4. Hsu C, Shen YC, Cheng AL. Sorafenib for the treatment of hepatocellular carcinoma across geographic regions. *Expert Rev Clin Pharmacol.* 2009;2: 129–136.
5. Li J, Gu J. Hand-foot skin reaction with vascular endothelial growth factor receptor tyrosine kinase inhibitors in cancer patients: A systematic review and meta-analysis. *Crit Rev Oncol Hematol*. 2017;119, 50–58.
6. Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. *Mol Cancer Ther*. 2008;7:3129–3140.
7. Escudier B, Eisen T, Stadler WM, et al. Sorafenib for treatment of renal cell carcinoma: Final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial. *J Clin Oncol*. 2009;27:3312–3318.
8. Rollig C, Serve H, Hüttmann A, et al. Addition of sorafenib versus placebo to standard therapy in patients aged 60 years or younger with newly diagnosed acute myeloid leukaemia (SORAML): a multicentre, phase 2, randomised controlled trial. *Lancet Oncol*. 2015;16:1691–1699.
9. Santos FPS, Kantarjian H, Fava C, et al. J. Clinical impact of dose reductions and interruptions of second-generation tyrosine kinase inhibitors in patients with chronic myeloid leukaemia. *Br J Haematol*. 2010;150:303–312.
10. Thomas L, Lai SY, Dong W, et al. Sorafenib in metastatic thyroid cancer: a systematic review. *Oncologist* 2014;19:251–258.
11. Zimmerman E.I, Gibson AA, Hu S, et al. Multikinase Inhibitors Induce Cutaneous Toxicity through OAT6-Mediated Uptake and MAP3K7-Driven Cell Death. *Cancer Res*. 2016;76:117–126.
12. Overbosch D, Van Gulpen C, Hermans J, Mattie, H. The effect of probenecid on the renal tubular excretion of benzylpenicillin. *Br. J Clin Pharmacol.* 1988;25:51–58.
13. Bins S, van Doorn L, Phelps MA, et al. Influence of OATP1B1 Function on the Disposition of Sorafenib-beta-D-Glucuronide. *Clin Transl Sci*. 2017;10:271–279.
14. Mendonza A, Hanna I, Meyers D, et al. Assessment of pharmacokinetic drug-drug interaction between pradigastat and atazanavir or probenecid. *J Clin Pharmacol.* 2016;56:355–364.
15. Uchaipichat V, Mackenzie PI, Guo XH, et al. Human udp-glucuronosyltransferases: isoform selectivity and kinetics of 4-methylumbelliferone and 1-naphthol glucuronidation, effects of organic solvents, and inhibition by diclofenac and probenecid. *Drug Metab Dispos.* 2004;32:413–423.
16. Hagos FT, Daood MJ, Ocque JA, et al. Probenecid, an organic anion transporter 1 and 3 inhibitor, increases plasma and brain exposure of N-acetylcysteine. *Xenobiotica* 2017;47:346–353.
17. Minami H, Kawada K, Ebi H et al. Phase I and pharmacokinetic study of sorafenib, an oral multikinase inhibitor, in Japanese patients with advanced refractory solid tumors. *Cancer Sci*. 2008;99:1492–1498.
18. Li L, Zhao M, Navid F, et al. Quantitation of sorafenib and its active metabolite sorafenib N-oxide in human plasma by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2010;*878*;3033–3038.

19. Lipworth AD, Robert C, Zhu AX. Hand-foot syndrome (hand-foot skin reaction, palmar-plantar erythrodysesthesia): focus on sorafenib and sunitinib. *Oncology.* 2009;*77*:257–271.
20. Zimmerman E.I, Hu S, Roberts JL, et al. Contribution of OATP1B1 and OATP1B3 to the disposition of sorafenib and sorafenib-glucuronide. *Clin Cancer Res.* 2013;19:1458–1466.
21. König J, Cui Y, Nies AT, Keppler D. Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide. *J Biol Chem*. 2000;275:23161–23168.
22. Ramsey LB, Bruun GH, Yang W, et al. Rare versus common variants in pharmacogenetics: SLCO1B1 variation and methotrexate disposition. *Genome Res.* 2012;*22*:1–8.
23. Lancaster CS, Bruun GH, Peer C.J, et al. A. OATP1B1 polymorphism as a determinant of erythromycin disposition. *Clin Pharmacol Ther.* 2012;*92*:642–650.
24. Selen A, Amidon GL, Welling PG. Pharmacokinetics of probenecid following oral doses to human volunteers. *J Pharm Sci*. 1982;71:1238–1242.
25. Vasilyeva A, Durmus S, Li L, et al. Hepatocellular Shuttling and Recirculation of Sorafenib-Glucuronide Is Dependent on Abcc2, Abcc3, and Oatp1a/1b. *Cancer Res*. 2015;75:2729–2736.
26. Inaba H, Panetta JC, Pounds SB, et al. Sorafenib Population Pharmacokinetics and Skin Toxicities in Children and Adolescents with Refractory/Relapsed Leukemia or Solid Tumor Malignancies. *Clin Cancer Res.* 2019;25:7320–7330.
27. Brose MS, Frenette CT, Keefe SM, Stein SM. Management of sorafenib-related adverse events: a clinician's perspective. *Semin Oncol*. 2014;41:S1–S16.

SUPPLEMENTARY Table 1 Toxicity per patient

Patient	Duration of sorafenib at baseline (days)	Sorafenib daily dose (mg)	Study phase	HFSR	Rash	Nausea	Diarrhea	Constipation	Anorexia	Fatigue	Pain	Hoarseness
			Baseline					1	1	1	1	
1	14	800	PK day 1		2			1		1	1	
		800	PK day 2					1	1	1	1	
			Baseline			1			1		1	
2	28	400	PK day 1							1	1	
		400	PK day 2			1			1	1	1	
			Baseline						1	1	1	
3	9	400	PK day 1		1				2	2	1	
		400	PK day 2						1	1	1	
			Baseline							1	1	
4	27	600	PK day 1				1		1	1	1	
		600	PK day 2	1					2	2	1	
			Baseline					1	1	1		
5	23	800	PK day 1	1				1	1	1	1	1
		800	PK day 2					2	1	1	1	
			Baseline									
6	35	800	PK day 1			1				1	1	
		800	PK day 2	3	2					1	2	
			Baseline	1				1		1	1	1
7	22	400	PK day 1	1				1		1	1	
		400	PK day 2	2				1		1	1	
			Baseline							1	1	1
8	8	400	PK day 1							1	1	
		600	PK day 2	1					1	1	1	
			Baseline						1	1		

SUPPLEMENTARY Table 1 Toxicity per patient (*continued*)

Patient	Duration of sorafenib at baseline (days)	Sorafenib daily dose (mg)	Study phase	HFSR	Rash	Nausea	Diarrhea	Constipation	Anorexia	Fatigue	Pain	Hoarseness
9	11	400	PK day 1					1	1	1		
		400	PK day 2	1	1			1	1	1		
			Baseline						1	1	1	1
10	23	200	PK day 1	1					1	1	1	
		200	PK day 2			1			1	2	1	1
			Baseline		1						1	1
11	42	400	PK day 1							2	1	
		400	PK day 2				1			2		
			Baseline		1							1
12	12	400	PK day 1									
		400	PK day 2	2	1					1		
			Baseline	1						1	1	1
13	41	400	PK day 1	1					1	1	3	
		400	PK day 2	2				1	1	1	3	1
			Baseline	1						2		1
14	29	400	PK day 1	1					1	2		1
		400	PK day 2		3				2	3	1	1
			Baseline		1		1		1			
15	24	400	PK day 1				1		1	1		
		400	PK day 2				1		1	1		
			Baseline						1	2		
16	23	400	PK day 1						1	2		
		400	PK day 2				2		2	2		

Legend: Toxicity per phase per patient according to the CTCAE grading. In general, there is an increase in adverse events during the study. Fields are left blank if toxicity is scored 0.

CHAPTER 9

SORAFENIB-INDUCED CHANGES IN THYROID HORMONE LEVELS IN PATIENTS TREATED FOR HEPATOCELLUAR CARCINOMA

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ABSTRACT

Background

The pathogenesis of tyrosine kinase inhibitor-induced thyroid hormone (TH) alterations The objective of this study was to determine the effects of sorafenib on TH levels in patients with hepatocellular carcinoma (HCC) and to evaluate possible mechanisms.

Methods

We performed a prospective cohort study between 2009 and 2016, in 57 patients with HCC who were treated with sorafenib in a tertiary referral center. Thyroid-stimulating hormone (TSH) and free thyroxine (FT4) levels were measured every 6 weeks, and extensive thyroid function tests (TFTs) were measured before treatment (t0), after 6 weeks (t6), and at the end of therapy. The effect of sorafenib on TH transport by monocarboxylate transporter (MCT)8 or MCT10 was tested in transfected COS1 cells.

Results

Four patients (7%) developed thyroiditis. Among the other patients, 30% had elevation of TSH or FT4 above the normal range. Overall, between t0 and t6, mean TSH increased from 1.28 to 1.57 mU/L ($P < 0.001$) and mean FT4 from 18.4 to 21.2 pmol/L ($P < 0.001$). Simultaneously, the serum triiodothyronine (T3)/reverse triiodothyronine ratio and the (T3/thyroxine) x 100 ratio decreased. Sorafenib decreased cellular T3 uptake by MCT8 and to a lesser extent by MCT10.

Conclusion

These *in vivo* data suggest that sorafenib affects TFT on multiple levels. Our *in vitro* experiments suggest a possible role of sorafenib-induced inhibition of T3 transport into the cell by MCT8 and MCT10.

INTRODUCTION

Several tyrosine kinase inhibitors (TKIs) are reported to be associated with changes in thyroid function tests (TFTs), of which sunitinib is the best studied.[1] Sorafenib is used in the treatment of advanced hepatocellular carcinoma (HCC), renal cell carcinoma, and radioactive iodine resistant differentiated thyroid carcinoma (DTC).[2-4] It has antiangiogenic, antiproliferative, and proapoptotic effects via multiple effector mechanisms, such as inhibition of the vascular endothelial growth factor receptor.[5] In 2011, we reported on two patients with sorafenib-induced thyroiditis;[6] since than other cases of sorafenib-induced thyroiditis have been described since.[7] Hypothyroidism and adverse effects, such as hypertension, are described to be associated with better prognosis among patients undergoing treatment with TKIs.[8,9]

The incidence of sorafenib-induced hypothyroidism varies from 18% to 50%. In previous studies[10-15] the diagnosis of sorafenib-induced hypothyroidism was only based on increased thyroid stimulating hormone (TSH) levels. Patients with preceding thyroiditis, a different entity contributing to TSH increase, were not excluded. The pathogenesis of the rise in TSH in non-thyroiditis cases has not been elucidated.

A study in athyroid patients treated with sorafenib suggests that there may be an enhanced peripheral degradation of thyroid hormone (TH) by deiodinase type 3 (D3).[16] D3 expression has been described in other tumors.[17] Inhibition of TH uptake via the cellular TH transporter (monocarboxylate transporter 8 (MCT8)) has been shown for sunitinib, imatinib, dasatinib and bosutinib, but this has not been studied for sorafenib.[18] The effects of TKIs on MCT10-mediated TH uptake into cells are, to the best of our knowledge, unknown.

The aim of the present study was to explore the effects of sorafenib on the hypothalamic-pituitary-thyroid axis (HPT) axis and peripheral TH metabolism by assessing detailed TFT in patients without known thyroid disease. By studying patients with intact thyroid glands, we were able to study effects on the HPT-axis at different levels. In addition, we studied the consequences of sorafenib on cellular triiodothyronine (T3) uptake *in vitro*.

PATIENTS AND METHODS

Patient Characteristics

We prospectively studied consecutive patients with progressive metastatic HCC and Child-Pugh A status who were treated with sorafenib between January 2007 and February 2016 in the Erasmus MC Cancer Institute, Rotterdam, Netherlands. Patients routinely started sorafenib at a dose of 400 mg, which was increased in 1 month to 800 mg if deemed safe by the treating physician.

Routine TFT (TSH and free thyroxine (FT4)) were determined every 6 weeks. In patients with enough material available, more extensive TFT were measured at baseline (t0), after 6 weeks (t6) and at the end of sorafenib therapy (Supplemental Figure.1). Patients with at least two laboratory evaluations were included in the study. The study was reviewed by the medical ethical committee of the Erasmus Medical Center (MEC 2015-755); requirements to obtain informed consent were waved.

Clinical outcome

Baseline World Health Organization (WHO) performance status was assessed by the treating physician. Progression-free survival (PFS) was computed as the time from treatment initiation to disease progression according to the treating physician or death.[19] Overall survival was computed as the time from treatment initiation to death. Adverse events were scored following Common Terminology Criteria for Adverse Events v4.0.[20] The incidence of all- grade hand foot skin reaction, hypertension, gastrointestinal complaints and thrombocytopenia was scored. Severe liver toxicity criteria were grade 3 or 4 liver test disturbances in liver transaminases,γ-glutamyl transferase, and alkaline phosphatase or grade 2 bilirubin disturbances.

TFTs

Serum was centrifuged and stored at −20°C immediately after withdrawal. TSH (reference value, 0.4 to 4.3 mU/L) was measured using the Immulite 2000 platform (Siemens, Erlangen, Germany). FT4 (reference value, 11 to 25 pmol/L), thyroxine (T4) (reference value, 58 to 128 nmol/L), and T3 (reference value, 1.4 to 2.5 nmol/L) were measured using the Vitros ECi immunoanalyzer (Ortho-Clinical Diagnostics, Raritan, NJ). Reverse triiodothyronine (rT3) (reference value, 0.21 to 0.54 nmol/L) was measured by in-house radioimmunoassay.[21] Intra- and interassay variability coefficients of all assays were <11%.[22] T3/rT3 (reference value, 2.65 to 7.65) and rT3/T4 x100 (reference value, 1.4-to 3.1) ratios were calculated as a proxy for peripheral deiodinase activity. Thyroid peroxidase antibodies (TPO-Abs) (reference value, <100 IU/mL) were measured using ImmunoCAP method (Phadia 250, Uppsala, Sweden). TSH receptor antibodies (TSHR-Abs) (reference value, <0.9 IU/L) were measured using 2009-2012 TRAK LIA test (Brahms, Hennigsdorf, Germany) and 2012-2015 TRAK Kryptor test (Brahms, Hennigsdorf, Germany), both WHO calibrated.

IN VITRO EXPERIMENTS

Effects of sorafenib on cellular uptake of T3 by human MCT8 and MCT10

Materials. Dulbecco's phosphate-buffered saline with calcium and magnesium (D-PBS) and GlutaMAX medium was obtained from Life Technologies (Bleiswijk, Netherlands),

culture dishes from Corning (Schiphol, Netherlands), COS1 cells from ATCC, bovine serum albumin (BSA), D-glucose, T3 from Sigma-Aldrich (Zwijndrecht, the Netherlands), transfection reagent X-tremeGENE9 from Roche (Almere, Netherlands), and $Na^{125}I$ from Perkin-Elmer (Groningen, Netherlands). [^{125}I] T3 was prepared in our laboratory as described previously.[23] The human MCT8 plasmid pcDNA3-hMCT8 and the human pcDNA3-hMCT10 were obtained as described elsewhere.[23]

Cellular T3 uptake assays. COS1 cells were cultured in 24-well dishes with 0.5mL Dulbecco's modified Eagle medium/F12 + GlutaMAX medium containing 9% heat-inactivated fetal bovine serum, 2% penicillin/streptomycin and 100 nM Na_2SeO_3. The cells were transfected with 100 ng empty pcDNA3, pcDNA3- hs MCT8 or pcDNA3-MCT10 as described.[24] T3 uptake was tested 48 hours after transfection. The cells were washed with the assay buffer (Dulbecco's phosphate-buffered saline with calcium and magnesium + $CaCl_2$ + $MgCl_2$ + 0.1% bovine serum albumin + 0.1% glucose) and incubated for 5 minutes at 37°C with 1 nM (10^5 cpm) [^{125}I] T3 and 0, 1, 10 or 100 µM sorafenib or 0, 1, 10 or 100 µM sunitinib in 0.5 mL assay buffer. After incubation, cells were washed with the assay buffer, lysed with 0.1 M NaOH, and counted in a gamma counter. Data were obtained in three independent experiments, each performed in duplicate.

D3 activity in HCC samples

To exclude a major contribution of D3 expression in HCC to the altered TFTs, we measured D3 in six random patients from whom presorafenib HCC biopsies were available. Tumor tissue was fresh frozen and stored at -80°C, until use. Thawed tissue samples were homogenized on ice in 10 volumes of PED10 buffer [0.05 M phosphate, 1 mM EDTA (pH 7.2), 10 mM DTT] using a Polytron (Kinematica AG, Lucerne, Switzerland). Liver D3 activities were measured in duplicate by incubation of tissue homogenate (250 µg protein) for 120 minutes at 37°C with 1 nM [3'-^{125}I]T3 (200,000 cpm) in 0.1 mL PED10 as described elsewere.[25]

Statistical analysis

Normal distribution was evaluated using the Kolmogorov-Smirnov test. Residuals that were not normally distributed underwent natural logarithmic transformation, and if still skewed *P* values were obtained via bootstrapping. Changes in TFTs were analyzed using a paired sample *t* test and if not normally distributed via a paired Wilcoxon signed-rank test. False discovery rate correction for multiple comparisons proposed by Benjamini [26] was applied. Logistic regression analysis was used to determine association between change in TFTs and adverse effects. Median survival time was calculated using Kaplan Meier. A Cox proportional-hazard regression model adjusted for age, sex, WHO performance status and average dose of sorafenib was used for the survival analysis. Proportional hazard and linearity assumptions were met. Two-way ANOVA with correction for

repeated testing and post hoc analysis with paired *t* test was used for sorafenib-induced inhibition of MCT8 and MCT10 transport of T3 into cells. Analyses were performed using SPSS version 23 (SPSS Inc., Chicago, IL).

RESULTS

Patient characteristics

Blood samples were collected from 57 patients with HCC. One patient had a TSH ≥10 mU/L before therapy and was therefore excluded from the analyses. None of the patients had pre-existent hyperthyroidism or hypothyroidism or used drugs interacting with TFTs, such as amiodarone and corticosteroids. This resulted in a final population of 56 patients, 44 (79%) of whom were male. Median age was 67 years [inter quartile range (IQR), 57 to 71 years] and median WHO performance status was 1 (IQR,1 to 2).

Thyroiditis

Four patients (7%) developed thyroid disease, with a pattern consistent with thyroiditis. Two of these patients have been reported in detail,[6] with clearly elevated levels of TPO-Ab (866 IU/mL) or TSHR-Ab (368 IU/L) at the time of thyroiditis. The other 2 patients also showed markedly increased TPO-Ab (1302 and 439 IU/mL) and TSHR-Ab's (19 IU/L) at the time of thyroiditis. Prospectively, both patients had elevated TPO-Ab before initiation of sorafenib treatment (140 and 343 IU/mL). In comparison, none of the 52 patients without thyroiditis had TPO-Ab's and only six (12%) showed mildly elevated TSHR-Ab, (median, 1.7 IU/L; (IQR 1.1 to 1.9). Ultrasound was not routinely performed prospectively. Patients with thyroiditis had a median PFS of 16.3 months [95% confidence interval (CI), 6.1 to 26.5], and patients without thyroiditis 4.9 months (95% CI, 2.3 to 7.5). Median overall survival was 18.5 months (95% CI, 0.1 to 43.5) vs 10.8 months (95% CI, 8.6 to 13.0), respectively. We refrained from statistical analysis due to small patient number.

TFTs

Patients with thyroiditis were excluded from subsequent analyses of TFTs. Five out of the remaining 52 patients had mild subclinical baseline thyroid dysfunction: thee patients had an isolated TSH elevation (5.14, 5.15 and 6.59 mU/L), and two patients had a low TSH (0.25 and 0.39 mU/L).

In 14 of the other 47 patients (30%), TSH or FT4 became elevated above the upper limit of normal during treatment. Overall, TSH and FT4 levels rose significantly after start of treatment (**Figure 1**). Similarly, rT3 and T4 levels increased significantly (**Table 1**) whereas the serum T3/rT3 and T3/T4 ratio significantly decreased (**Figure 1**). These

changes in TFT occurred within 6 weeks after start of treatment and persisted until the end of treatment.

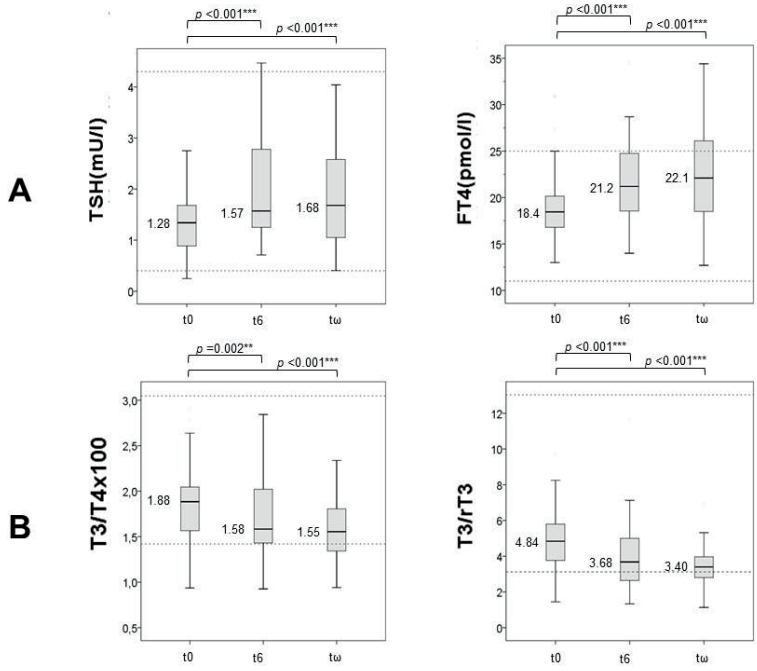


Figure 1. Change in TFTs at t0, t6, and end of therapy (tω). Changes in TFT were analyzed using a paired sample *t* test and if not normally distributed via a paired Wilcoxon signed-rank test. False discovery rate correction for multiple comparisons proposed by Benjamini[26] was applied. **, $P < 0.01$; ***, $P < 0.001$.

Table 1. Change in TFTs

Variable	*Reference values*	*t0, median [IQR]*	*t6, median [IQR]*	*P Value, t0 vs t6*	*tω, Median [IQR]*	*P Value, t0 vs tω*
Parameter						
TSH, mU/L	0.4-4.3	1.28 [0.88- 1.66]	1.57 [1.23-2.78]	<0.001[a]	1.68[1.03-2.65]	<0.001[a]
FT4, pmol/L	11-25	18.4 [16.9-19.9]	21.2 [18.5-24.8]	<0.001[a]	22.1[18.4-26.6]	<0.001[a]
T4, nmol/L	58-128	115 [101-138]	121 [98-152]	0.01[b]	123[108-144]	0.02[b]
T3, nmol/L	1.43-2.51	2.12 [1.87-2.39]	2.06 [1.73-2.31]	0.12	1.95[1.75-2.17]	0.001[c]
rT3, nmol/L	0.21-0.54	0.41 [0.36-0.59]	0.57 [0.41-0.73]	<0.001[a]	0.58[0.46-0.76]	<0.001[a]
Ratio						
T3/rT3	2.65-7.65	4.84 [3.74-5.84]	3.68 [2.63-5.01]	<0.001[a]	3.40[2.73-3.99]	<0.001[a]
T3/T4 x 100	1.42-3.05	1.88 [1.56-2.05]	1.58 [1.41-2.03]	0.003[c]	1.55[1.29-1.81]	<0.001[a]

Changes in TFT were analyzed using a paired sample *t* test and if not normally distributed via a paired Wilcoxon signed-rank test. False discovery rate correction for multiple comparisons proposed by Benjamini [26] was applied. [a]$P < 0.001$; [b]$P < 0.05$; [c]$P < 0.01$.

In vitro experiments

Cellular T3 uptake mediated by MCT8 was significantly and dose-dependently inhibited by sorafenib and very similar effects were observed with sunitinib (**Figure 2**). Both sorafenib and sunitinib had marginal effects on T3 uptake by control cells transfected with empty vector and on T3 uptake induced by transfection of cells with MCT10. The

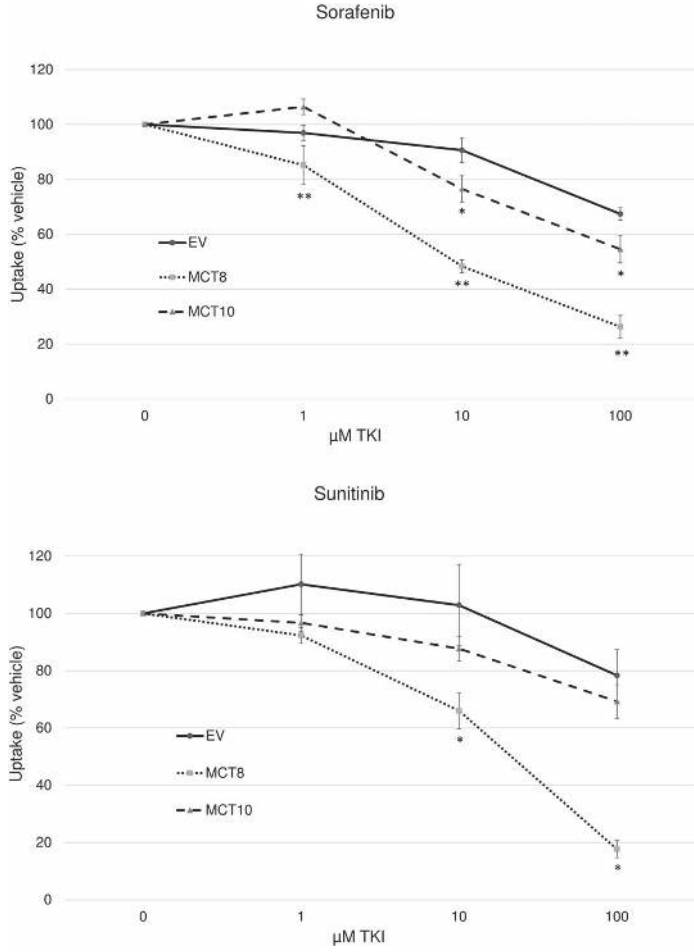


Figure 2. Effects of sorafenib and sunitinib on T3 uptake by MCT8 or MCT10. Cells were incubated for 5 minutes with 1 nM [^{125}I]T3 in the absence or presence of 1, 10 or 100 µM sorafenib or sunitinib as described in Materials and Methods. T3 uptake by cells transfected with MCT8 or MCT10 is T3 uptake by control cells transfected with empty vector (EV). T3 uptake is expressed as mean (standard error of mean) percentage of that in the absence of sorafenib or sunitinib of 3 independent experiments each performed in duplicate. Two-way ANOVA with correction for repeated testing showed significant change in T3 uptake after for sorafenib ($P < 0.02$) and sunitinib $P < 0.05$). *Post-hoc*, the significance of the difference between the effects of sorafenib or sunitinib on T3 uptake by MCT8 or MCT10 expressing cells vs control cells is shown in the figure. This was tested by paired *t* test. * $P < 0.05$; ** $P < 0.01$.

short exposure of the cells to sorafenib and sunitinib (5 minutes) and the differential effects of the inhibitors on MCT8-mediated vs MCT10-mediated and background T3 uptake argue against an important contribution of possible cytotoxic effects of sorafenib and sunitinib in these experiments. The average D3 activity in the HCC tissue before sorafenib treatment was 1.17 (IQR, <0.1 to 1.63) fmol/min/mg protein. This was not increased compared to the D3 activities in historical control livers (liver biopsies of patients that died of severe brain damage, taken within minutes after death) that we studied previously.[27]

Clinical outcome

An increase in TSH level was associated with a deterioration of PFS (Supplemental Table 1). This negative effect of TSH on PFS persisted in a multiple Cox-regression analysis with correction for age, sex, WHO performance status, and average dose of sorafenib (**Figure 3**). Adding response evaluation criteria in solid tumors [28] to the model did not influence results. There was no association of FT4 with PFS. TSH showed the same trend for overall survival but did not reach statistical significance (Supplemental Table 1). Changes in TFT were not correlated with adverse events (Supplemental Table 1).

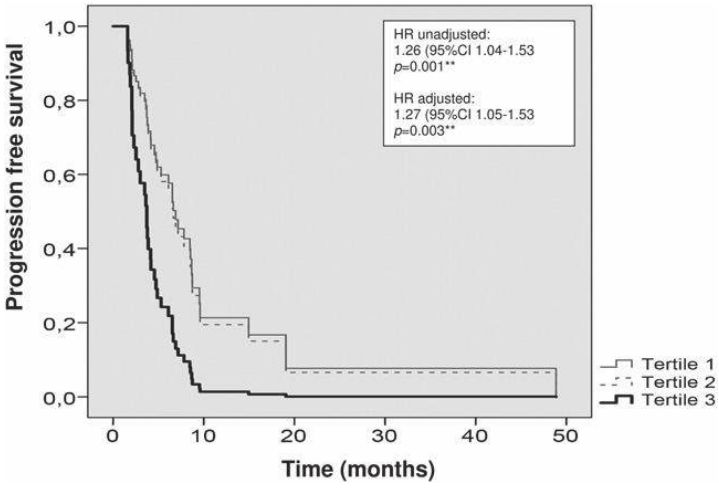


Figure 3. The proportion of progression free survival by Δ TSH tertiles. Adjusted hazard ratios (HR) were calculated in a Cox proportional-hazard regression model adjusted for age, sex, WHO performance status, and average dose of sorafenib.

DISCUSSION

In this cohort of sorafenib-treated patients with HCC, we demonstrate that sorafenib affects TFTs via multiple mechanisms. Thyroiditis occurred in a small percentage of pa-

tients, whereas there was a combined increase in TSH and FT4 in the remaining patients, which suggests a central effect on the HPT-axis. Finally, there was a decrease in the T3/rT3 and T3/T4 ratios, suggesting additional effects on the peripheral metabolism of TH.

Thyroiditis

The incidence of thyroiditis was 7%, compared with 23% [14] and 5% [11] in other sorafenib cohorts and 40% [29] in patients treated with sunitinib. The incidence of subacute thyroiditis in the general population is much lower around 12.1 cases per 100.000/y.[30] However, we cannot draw conclusions on our observations since only four patients developed a thyroiditis in this cohort.

Altered sensitivity of the HPT axis

Thirty percent of patients developed TSH or FT4 levels above the normal range during treatment. Median TSH and FT4 levels rose significantly. This simultaneous increase of TSH and FT4 suggests an altered set-point of the HPT axis, since an increase in FT4 would normally be accompanied by a decrease in TSH. For other TKIs, such as axitinib, an isolated increase in TSH accompanied by TH levels within the normal range has also been described.[31] This altered set-point could be explained at several levels, since not only serum levels of FT3 and FT4, but also TH transport into the cell and intracellular deiodinase activity are determinants of TH action and the HPT axis set-point.[32]

Our *in vitro* experiments showed inhibition of MCT8-mediated T3 uptake by sorafenib. MCT8 is one of the transporters that is highly expressed in brain and pituitary,[33] suggesting that sorafenib-induced interference with TH uptake by the hypothalamus or pituitary may be one of the mechanisms.

Alternatively, a sorafenib-induced decrease in deiodinase type 2 activity in the hypothalamus or pituitary (described later) would have similar consequences on the HPT axis setpoint. Alternative explanations could be interference with binding of T3 to its nuclear receptor, a diminished pituitary blood flow due to the antiangiogenic effects of sorafenib [34] or a reduced clearance of TSH.[35] Future studies should investigate which mechanisms contribute to this altered sensitivity of the HPT axis.

Peripheral TH metabolism

There was a marked decrease in the serum T3/rT3 and T3/T4 ratios, suggesting additional changes in the peripheral metabolism of TH, since an isolated change in the HPT axis set-point would not necessarily affect these ratios.

In previous experiments, we showed sunitinib-induced D3 activity in normal rat liver.[34] The decreased T3/rT3 ratio observed in the current study fits with an induction of TH inactivation by D3, as has been described previously by Abdulrahman et al.[16] In the case of constant T4 production, increased D3 activity would not only lead to increased rT3

production but also to a decrease in T4 levels. However, in contrast to the study of Abdulrahman et al.,[16] which analyzed thyroidectomized patients on TSH suppressive doses of levothyroxine treatment, our study was performed in patients with intact thyroid glands. We did not only see effects on peripheral metabolism of TH but also a central effect on the pituitary, with an increase in TSH that would increase T4 production. This likely explains why T4 increases despite the decreased T3/rT3 ratio, which we assume to be caused by an increase in D3 activity. Similarly, the decreased T3/T4 ratio fits with a lower T4 to T3 conversion. The higher T4 production may explain why T3 levels remain stable despite this lower T4 to T3 conversion.

The increase in FT4 and the subsequent decrease in T3/T4 might suggest that other peripheral TH metabolizing enzymes are also affected, such as a decrease in deiodinase type 1 or deiodinase type 2. In addition, uptake of TH into cells is rate limiting for subsequent metabolism. Becauce MCT8 is not only expressed in the brain but also in multiple other tissues,[36] and MCT10 is highly expressed in skeletal muscle, kidney, pancreas and intestine,[33] sorafenib-induced inhibition of T3 uptake by MCT8 and MCT10 may also have contributed to the alterations in peripheral metabolism of TH. Further experiments are needed to investigate the contribution of these different mechanisms in more detail.

Nonthyroidal illness (NTI) is not likely to be a major contributor because (1) changes in T3/rT3 and T3/T4 were most evident in the first weeks after start of treatment and not at the end of study (when the cancer was progressive) and (2) TSH and FT4 may remain normal in mild NTI, but the persistent and progressive increase in TSH and FT4 does not fit the pattern of NTI.[37]

The changes in TH metabolism are independent of changes in binding proteins. A decrease in TH binding proteins would have led to a similar decrease in all iodothyronines, whereas we did see marked changes in the total T3/rT3 and rT3/T4 ratios, in which the binding proteins are both in the numerator and the denominator.[38] Furthermore, measurements of FT4 levels and total T4 levels changed in the same direction.

Clinical outcome

In this study, we found that an increase in TSH is an independent negative prognostic marker for PFS. There was no association between FT4 and survival. *In vivo*, local hypothyroidism is known to be associated with HCC progression.[39] In patients with other solid tumors, especially basal cell carcinoma, induction of D3 is associated with hyperproliferative state and carcinogenesis.[40] However, our results seem to be in contrast with two other studies in patients treated with sorafenib or sunitinib, where an increased TSH was negatively associated with tumor progression.[10,13] It is not yet clear how to explain this inconsistency between these studies. However, there are a few differences between the studies: (1) in the previous studies, only patients with TSH levels above the normal range were investigated whereas we assessed the absolute change of TSH on PFS and

survival in all patients (including patients within the reference range); (2) In the previous studies, patients received levothyroxine, which may have affected tumor progression and makes it difficult to compare the results; and (3) in the other studies, patients with thyroiditis, who might have different prognostic profile, were not excluded. Future studies are therefore needed to unravel if and how the effects of sorafenib on thyroid function can be regarded as a prognostic factor.

CONCLUSIONS

Our clinical data demonstrate that sorafenib-induced changes in TFTs are mediated at several levels, with *in-vitro* experiments showing sorafenib-induced inhibition of T3 transport into the cell by MCT8 and MCT10.

ACKNOWLEDGMENTS

We thank Esther Oomen-de Hoop for her statistical advise.

ABBREVIATIONS

CI, confidence interval; D3, deiodinase type 3; FT4, free thyroxine; HCC, hepatocellular carcinoma; HPT, hypothalamic-pituitary-thyroid; IQR, interquartile range; MCT, monocarboxylate transporter; NTI, nonthyroidal illness; PFS, progression free survival; rT3, triiodothyronine; trt0, before treatment; t6, after 6 weeks of treatment; T3, triiodothyronine; T4, thyroxine; TH, thyroid hormone; TFT, thyroid function tests; TKI, tyrosine kinase inhibitor; TPO-Ab, thyroid peroxidase antibody; TSH, thyroid stimulating hormone; TSHR-Ab, thyroid stimulating hormone receptor antibody; TSH receptor antibody; rT3, reverse triiodothyronine; vs, versus; WHO, World Health Organization.

REFERENCES

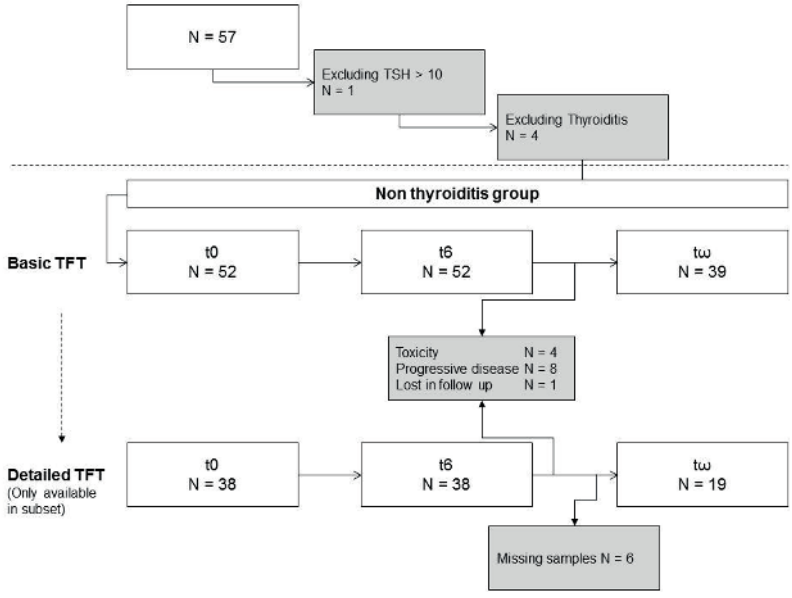
1. Makita N, Iiri T. Tyrosine kinase inhibitor-induced thyroid disorders: a review and hypothesis. *Thyroid.* 2013;23(2):151-159.
2. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. *The New England journal of medicine.* 2008;359(4):378-390.
3. Escudier B, Eisen T, Stadler WM, et al. Sorafenib in advanced clear-cell renal-cell carcinoma. *The New England journal of medicine.* 2007;356(2):125-134.
4. Brose MS, Nutting CM, Jarzab B, et al. Sorafenib in radioactive iodine-refractory, locally advanced or metastatic differentiated thyroid cancer: a randomised, double-blind, phase 3 trial. *Lancet.* 2014;384(9940):319-328.
5. Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. *Mol Cancer Ther.* 2008;7(10):3129-3140.
6. van Doorn L, Eskens FA, Visser TJ, van der Lugt A, Mathijssen RH, Peeters RP. Sorafenib induced thyroiditis in two patients with hepatocellular carcinoma. *Thyroid.* 2011;21(2):197-202.
7. Haraldsdottir S, Li Q, Villalona-Calero MA, Olencki TE, Kendra K, Ing SW. Case of sorafenib-induced thyroid storm. *J Clin Oncol.* 2013;31(16):e262-264.
8. Granito A, Marinelli S, Negrini G, Menetti S, Benevento F, Bolondi L. Prognostic significance of adverse events in patients with hepatocellular carcinoma treated with sorafenib. *Therap Adv Gastroenterol.* 2016;9(2):240-249.
9. Rini BI, Cohen DP, Lu DR, et al. Hypertension as a biomarker of efficacy in patients with metastatic renal cell carcinoma treated with sunitinib. *J Natl Cancer Inst.* 2011;103(9):763-773.
10. Schmidinger M, Vogl UM, Bojic M, et al. Hypothyroidism in patients with renal cell carcinoma: blessing or curse? *Cancer.* 2011;117(3):534-544.
11. Tamaskar I, Bukowski R, Elson P, et al. Thyroid function test abnormalities in patients with metastatic renal cell carcinoma treated with sorafenib. *Ann Oncol.* 2008;19(2):265-268.
12. Feldt S, Schussel K, Quinzler R, et al. Incidence of thyroid hormone therapy in patients treated with sunitinib or sorafenib: a cohort study. *Eur J Cancer.* 2012;48(7):974-981.
13. Riesenbeck LM, Bierer S, Hoffmeister I, et al. Hypothyroidism correlates with a better prognosis in metastatic renal cancer patients treated with sorafenib or sunitinib. *World J Urol.* 2011;29(6):807-813.
14. Miyake H, Kurahashi T, Yamanaka K, et al. Abnormalities of thyroid function in Japanese patients with metastatic renal cell carcinoma treated with sorafenib: a prospective evaluation. *Urol Oncol.* 2010;28(5):515-519.
15. Daimon M, Kato T, Kaino W, et al. Thyroid dysfunction in patients treated with tyrosine kinase inhibitors, sunitinib, sorafenib and axitinib, for metastatic renal cell carcinoma. *Jpn J Clin Oncol.* 2012;42(8):742-747.
16. Abdulrahman RM, Verloop H, Hoftijzer H, et al. Sorafenib-induced hypothyroidism is associated with increased type 3 deiodination. *J Clin Endocrinol Metab.* 2010;95(8):3758-3762.
17. Maynard MA, Huang SA. Thyroid hormone inactivation in gastrointestinal stromal tumors. *The New England journal of medicine.* 2014;371(1):86-87.
18. Braun D, Kim TD, le Coutre P, Kohrle J, Hershman JM, Schweizer U. Tyrosine kinase inhibitors noncompetitively inhibit MCT8-mediated iodothyronine transport. *The Journal of clinical endocrinology and metabolism*. 2012;97(1):E100-105.

19. Raoul JL, Adhoute X, Gilabert M, Edeline J. How to assess the efficacy or failure of targeted therapy: Deciding when to stop sorafenib in hepatocellular carcinoma. *World J Hepatol.* 2016;8(35):1541-1546.
20. Institute NC. Common Terminology Criteria for Adverse Events v4.0 *NIH publication.* Vol 09-74732009.
21. Eelkman Rooda SJ, Kaptein E, Visser TJ. Serum triiodothyronine sulfate in man measured by radioimmunoassay. *The Journal of clinical endocrinology and metabolism.* 1989;69(3):552-556.
22. van den Beld AW, Visser TJ, Feelders RA, Grobbee DE, Lamberts SW. Thyroid hormone concentrations, disease, physical function, and mortality in elderly men. *The Journal of clinical endocrinology and metabolism.* 2005;90(12):6403-6409.
23. Anik A, Kersseboom S, Demir K, et al. Psychomotor retardation caused by a defective thyroid hormone transporter: report of two families with different MCT8 mutations. *Horm Res Paediatr.* 2014;82(4):261-271.
24. Groeneweg S, Friesema EC, Kersseboom S, et al. The role of Arg445 and Asp498 in the human thyroid hormone transporter MCT8. *Endocrinology.* 2014;155(2):618-626.
25. Peeters RP, Wouters PJ, van Toor H, Kaptein E, Visser TJ, Van den Berghe G. Serum 3,3',5'-triiodothyronine (rT3) and 3,5,3'-triiodothyronine/rT3 are prognostic markers in critically ill patients and are associated with postmortem tissue deiodinase activities. *The Journal of clinical endocrinology and metabolism.* 2005;90(8):4559-4565.
26. Benjamini Y HY. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society.* 1995;57(1):289–300.
27. Peeters RP, Wouters PJ, Kaptein E, van Toor H, Visser TJ, Van den Berghe G. Reduced activation and increased inactivation of thyroid hormone in tissues of critically ill patients. *The Journal of clinical endocrinology and metabolism.* 2003;88(7):3202-3211.
28. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst.* 2000;92(3):205-216.
29. Desai J, Yassa L, Marqusee E, et al. Hypothyroidism after sunitinib treatment for patients with gastrointestinal stromal tumors. *Annals of internal medicine.* 2006;145(9):660-664.
30. Furszyfer J, McConahey WM, Wahner HW, Kurland LT. Subacute (granulomatous) thyroiditis in Olmsted County, Minnesota. *Mayo Clin Proc.* 1970;45(5):396-404.
31. Ohba K, Takayama T, Matsunaga H, et al. Inappropriate elevation of serum thyrotropin levels in patients treated with axitinib. *Thyroid : official journal of the American Thyroid Association.* 2013;23(4):443-448.
32. Larsen PR, Silva JE, Kaplan MM. Relationships between circulating and intracellular thyroid hormones: physiological and clinical implications. *Endocrine reviews.* 1981;2(1):87-102.
33. Price NT, Jackson VN, Halestrap AP. Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. *The Biochemical journal.* 1998;329 (Pt 2):321-328.
34. Kappers MH, van Esch JH, Smedts FM, et al. Sunitinib-induced hypothyroidism is due to induction of type 3 deiodinase activity and thyroidal capillary regression. *The Journal of clinical endocrinology and metabolism.* 2011;96(10):3087-3094.
35. Verloop H, Smit JW, Dekkers OM. Sorafenib therapy decreases the clearance of thyrotropin. *European journal of endocrinology / European Federation of Endocrine Societies.* 2013;168(2):163-167.

36. Kersseboom S, Kremers GJ, Friesema EC, et al. Mutations in MCT8 in patients with Allan-Herndon-Dudley-syndrome affecting its cellular distribution. *Mol Endocrinol.* 2013;27(5):801-813.
37. de Vries EM, Fliers E, Boelen A. The molecular basis of the non-thyroidal illness syndrome. *J Endocrinol.* 2015;225(3):R67-81.
38. Peeters RP, van Toor H, Klootwijk W, et al. Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. *The Journal of clinical endocrinology and metabolism.* 2003;88(6):2880-2888.
39. Frau C, Loi R, Petrelli A, et al. Local hypothyroidism favors the progression of preneoplastic lesions to hepatocellular carcinoma in rats. *Hepatology.* 2015;61(1):249-259.
40. Dentice M, Antonini D, Salvatore D. Type 3 deiodinase and solid tumors: an intriguing pair. *Expert Opin Ther Targets.* 2013;17(11):1369-1379.

SUPPLEMENTARY INFORMATION

Supplemental Figure 1. Flowchart



SUPPLEMENTAL TABLE 1

Table 1. Change in thyroid function tests and clinical response.

	Delta TSH	*P value*	Delta FT4	*P* value
Prognosis HR (CI 95%)				
Death	1.16(0.98-1.37)	0.09	1.02(0.94-1.10)	0.79
Progression	1.26(1.04-1.53)	0.001**	0.96(0.87-1.04)	0.31
Adverse events OR (CI 95%)				
HFSR	1.14(0.76-1.70)	0.53	0.92(0.80-1.06)	0.26
Hypertension	1.00(0.56-1.76)	0.99	1.00(0.81-1.24)	0.97
Severe liver toxicity	0.74(0.49-1.12)	0.15	1.05(0.89-1.23)	0.59
Gastrointestinal	1.40(0.75-2.62)	0.29	0.92(0.78-1.08)	0.28
Thrombocytopenia	0.82(0.53-1.27)	0.37	0.97(0.85-1.12)	0.69

Adjusted hazard ratios (HR) were calculated in de cox proportional- hazard regression model adjusted for age, sex, WHO performance status and average dose of sorafenib.

CI 95% confidence interval; delta FT4, FT4 t6-FT4 t0; delta TSH, TSH t6-TSH t0; HFSR, hand foot skin reaction; OR, odds ratio; t0, before treatment; t6, after six weeks of treatment; *P < 0.05; ** *P* < 0.01.

CHAPTER 10

COMBINING SORAFENIB AND IMMUNOSUPPRESION IN LIVER TRANSPLANT RECIPIENTS WITH HEPATOCELLULAR CARCINOMA

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ABSTRACT

Hepatocellular carcinoma (HCC) recurrence after liver transplantation occurs in approximately 20% of patients. Most of these patients use immunosuppressant drugs. Meanwhile, patients with HCC recurrence are frequently treated with the small molecule kinase inhibitor (SMKI) sorafenib. However, sorafenib and many immunosuppressants are substrates of the same enzymatic pathways (e.g., CYP3A4), which may potentially result in altered SMKI or immunosuppressant plasma levels. Therefore, we investigated changes in drug exposure of both sorafenib and immunosuppressants over time in four patients with systemic immunosuppressant and sorafenib treatment after HCC recurrence. In this study, sorafenib exposure declined over time during combined treatment with immunosuppressants, while two patients also experienced declining tacrolimus plasma levels. Importantly, patients were unable to increase the sorafenib dose higher than 200 mg b.i.d. without experiencing significant toxicity.We recommend to treat patients using both sorafenib and immunosuppressants with a sorafenib starting dose of 200 mg b.i.d.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most frequently diagnosed cancer type worldwide and the fourth leading cause of cancer death in the world.[1] Liver transplantation[2] is indicated in patients with localized HCC, with a 5-year survival rate of approximately 70%.[3] Still, HCC recurrence in the transplanted liver occurs in about 20% of patients.[3] After HCC recurrence, one of the most applied therapies is sorafenib, an orally active multi-kinase inhibitor approved for the treatment of HCC, resulting in a median overall survival benefit of 7.4 months.[4-7] Usually sorafenib is started in a 200mg b.i.d. dose in this patient group due to expected sorafenib side-effects in patients after liver transplantation and is gradually increased based on toxicity. Patients with HCC recurrence after liver transplantation seems to be more susceptible to sorafenib related side effects. Sorafenib side effects include --among others—gastro-intestinal related side effects (e.g., diarrhea) and cutaneous side effects (e.g., hand-foot skin reaction). These side effects lead to dose reduction or even cessation of sorafenib therapy in 15%-77% of the treated patients after liver transplantation.[8] The higher incidence of side effects in patients with a liver transplantation may be due to a pharmacokinetic and/or pharmacodynamic drug-drug interaction with immunosuppressants.[9-11] Sorafenib and immunosuppressants have overlapping metabolic pathways, which increases the risk of a drug-drug interaction. Sorafenib is metabolized by CYP3A4 and by UGT1A9, while CYP3A4 is also the most important enzyme in the metabolism of several immunosuppressive drugs (e.g., tacrolimus, MTOR inhibitors).[11,12] Here, we present a case series of four patients with HCC recurrence after liver transplant using tacrolimus concomitantly with sorafenib which allowed to study a possible drug-drug interaction.

METHODS

In all four patients serial blood samples for the determination of both sorafenib and tacrolimus have been taken as part of usual clinical care, for patient safety reasons. None of these patients used additional interacting comedication. Blood samples were taken at day 7 and 14 after the start of sorafenib for the determination of sorafenib area under the curve ($AUC_{0\text{-}7.5}$) and C_{max}, at time point t = 0h (before intake of sorafenib) as well as 2, 4, and 7.5 hour after intake of sorafenib. At timepoint t = 0h, blood was also taken for the determination of tacrolimus $C_{trough.}$ Next, both tacrolimus and sorafenib C_{trough} were determined on a regular basis at the outpatient clinic. All patients gave written consent for the use of these samples and clinical data for scientific purposes, including this publication.

RESULTS

Case 1

A 62-year old male patient was referred to the department of Medical Oncology for systemic treatment with sorafenib. He had been diagnosed with chronic hepatitis C virus-induced liver cirrhosis before and underwent a liver transplantation for HCC in 2015, followed by tacrolimus monotherapy without previous systemic or local therapy. He had one lesion <5 cm with adequate liver function and no vascular invasion (Barcelona Clinic Liver Cancer (BCLC) score A and Model for End-Stage Liver Disease (MELD)-score was 18). His hepatitis C was treated with ledipasvir, daclatasvir and ribavirin. At start of the study and during hospital admissions patient used loperamide, losartan, metformin and metoprolol as concomitant medication. In June 2017, sorafenib 200 mg b.i.d. was started after HCC recurrence with pulmonary metastases, at which time tacrolimus was dosed at 3 mg once daily providing a tacrolimus trough concentration (C_{trough}) of 5.9 µg/L (reference: 4-8 ug/L). The $AUC_{0\text{-}7.5h}$ of sorafenib was 2.1% higher at day 14 compared to day 7, while the sorafenib C_{max} was 24% lower (**Table 1**). In general, both sorafenib and tacrolimus trough levels showed a relevant decrease in the first months of treatment, up to a 90% decrease for sorafenib plasma trough levels compared to the baseline trough level and up to 64% for tacrolimus (**Figure 1**). The tacrolimus dose was increased to 4 mg once daily (q.d.) in August 2017, in an attempt to maintain adequate tacrolimus concentrations. As a result, tacrolimus levels increased, while sorafenib levels further decreased. Therefore, also the sorafenib dose was increased with 50% to 200 mg in the morning and 400 mg in the evening in December 2017, after which also the sorafenib C_{trough} increased. Due to CTCAE grade 3 liver toxicity the sorafenib dose had to be reduced again to 200 mg b.i.d. at first and to 300 mg q.d. (400mg one day and 200mg the other) in February 2018. Subsequently, sorafenib concentrations decreased and tacrolimus concentrations further increased. Sorafenib was stopped in May 2018 after progressive disease was noticed at the CT scan.

Table 1. $AUC_{0\text{-}7.5h}$ and C_{max} of each individual case

	Day 7		Day 14			
Case	**$AUC_{0\text{-}7.5h}$ sorafenib (mg*h/L)**	**C_{max} sorafenib (mg/L)**	**$AUC_{0\text{-}7.5h}$ sorafenib (mg*h/L)**	**C_{max} sorafenib (mg/L)**	**RD $AUC_{0\text{-}7.5h}$ (%)**	**RD (%) C_{max}**
1	33.4	8.5	34.1	6.4	+2.1	-24.4
2	47.8	8.7	48.2	10.7	+0.9	+22.1
3	37.6	6.3	22.6	4.0	-37.3	-40.0
4	24.9	6.0	13.9	2.3	-62.1	-44.0

All patients used sorafenib 200 mg b.i.d. Abbreviations: AUC= area under the plasma curve, C_{max} = maximum concentration; RD = relative difference.

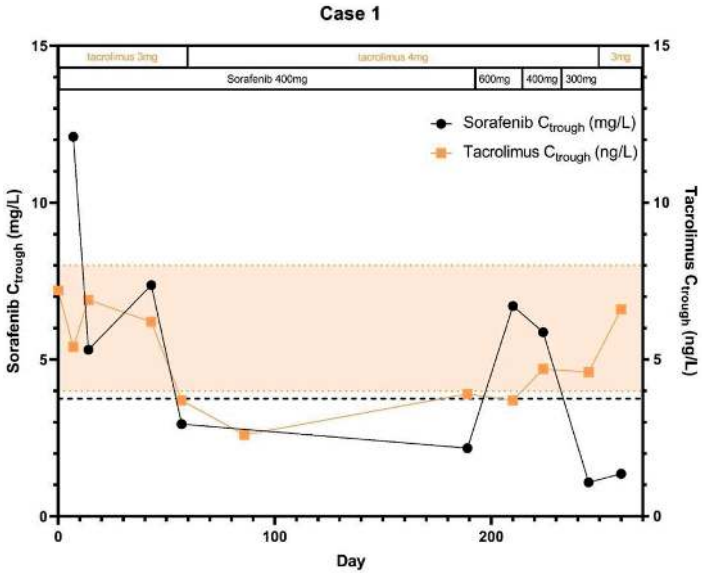

Figure 1. Sorafenib and tacrolimus C_{trough} concentrations over time for Subject 1: The C_{trough} levels are displayed over time after the start of sorafenib treatment. Furthermore, the optimal C_{trough} levels of both sorafenib and tacrolimus are provided.

Case 2

A 70-year old female with alcohol induced liver cirrhosis was diagnosed with HCC in 2009, which was at first successfully treated with trans-arterial chemo-embolization (TACE). She had one lesion <5cm with adequate liver function and no vascular invasion (MELD score: 6), for which she underwent a liver transplantation in January 2011. She developed disease recurrence with pulmonary metastases in 2018, after which she was referred to the department of Medical Oncology for systemic treatment with sorafenib, which was started at a 200 mg b.i.d. dose in July 2018. Patient had no signs of liver fibrosis and had a normal liver function when sorafenib was started. Next to tacrolimus and sorafenib patient used hydrochlorothiazide, losartan and oxazepam concomitantly during start of the study and the hospital admission days. Before start of sorafenib, the tacrolimus dose was 4 mg daily and tacrolimus C_{trough} was 5.2 µg/L. On day 14, $AUC_{0\text{-}7.5h}$ and C_{max} of sorafenib were respectively 0.9% and 22.1% higher than at day 7 (**Table 1**). Sorafenib C_{trough} remained stable during the first 2 weeks of concomitant treatment with tacrolimus but generally declined over time (**Figure 2**). Hereafter, in August 2018, immunosuppressant therapy was stopped completely by the treating gastroenterologist and sorafenib concentrations further decreased over time. In August 2019, this patient had proven progressive disease and sorafenib was stopped after 19 months of treatment in which there was already a slight progression of disease over time.

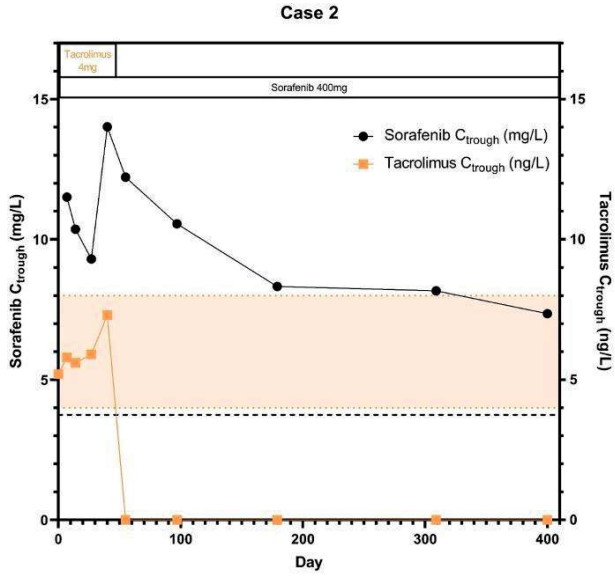

Figure 2. Sorafenib and tacrolimus C_{trough} concentrations over time for Subject 2: The C_{trough} levels over time after the start of sorafenib treatment. Furthermore, the optimal C_{trough} levels of both sorafenib and tacrolimus are provided.

Case 3

A 65-year old male patient with chronic hepatitis C virus-induced liver cirrhosis was diagnosed with HCC for which he received a liver transplantation in 2018. As transplantation indication he initially had one lesion <5cm with adequate liver function but with vascular invasion (tumor thrombus), which was first treated with transarterial radioembolization after which there was complete resolvement of the thrombus (BCLC-score C and MELD-score was 6 at time of transplantation). His hepatitis C was treated with peginterferon and ribavirin in 2003, after which there was complete remission. At start of the study and during hospital admissions patient used clopidogrel, temazepam, pravastatin, oxycodon, ursodeoxycholicacid and pantoprazole as concomitant medication. Immunosuppressive treatment consisted of mycophenolate mofetil (MMF) 1000 mg b.i.d. and tacrolimus (4 mg b.i.d., which was later reduced to 4 mg q.d.). Later, the patient switched from MMF to sirolimus (2 mg q.d.) due to livertoxicity. In April 2019, the patient had a recurrence of disease after which sorafenib was started in a dose of 200 mg b.i.d. Both tacrolimus and sirolimus concentrations were adequate at baseline (C_{trough} = 4.7 µg/L and C_{trough} = 8.0 µg/L, respectively). At day eight of sorafenib treatment, tacrolimus was stopped by the gastroenterologist according to physician's choice and the patient continued with sirolimus monotherapy. After cessation of tacrolimus, the sorafenib concentration initially decreased and remained relatively stable until disease progression, which was also the case for sirolimus concentration (**Figure 3**). $AUC_{0\text{-}7.5h}$ and

C_{max} of sorafenib decreased with 40.0% and 37.3% respectively at day 14 compared to day 7 (**Table 1**). After just 2 months of treatment, this patient had disease progression after which sorafenib treatment was stopped and best supportive care was started. After stopping sorafenib therapy, the sirolimus plasma levels further decreased with 42.6% compared to the latest C_{trough} with the combination therapy.

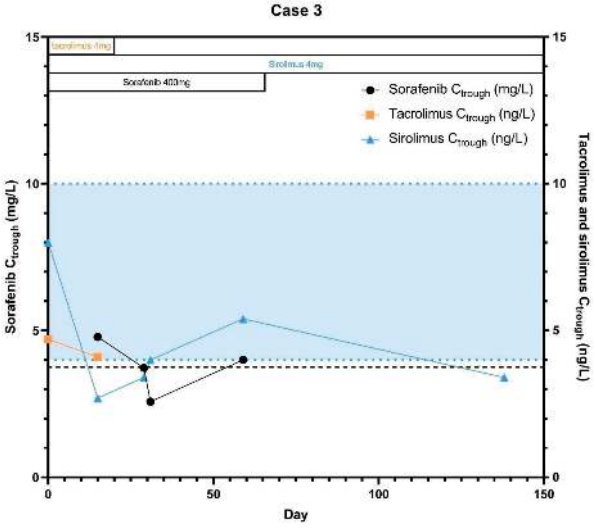

Figure 3. Sorafenib and tacrolimus C_{trough} concentrations over time for Subject 3: The C_{trough} levels are displayed over time after the start of sorafenib treatment. Furthermore, the optimal C_{trough} levels of both sorafenib and sirolimus are provided. Case 3 was initially treated with both sirolimus and tacrolimus but stopped tacrolimus short after start of sorafenib as was shown in this figure.

Case 4

A 69-year old male with alcohol-induced liver cirrhosis was diagnosed with HCC and underwent a liver transplantation in March 2019. He initially did not fit into the Milan criteria, because he had three lesions of which one lesion was more than 3 cm. This lesion was treated with transarterial chemoembolization after which he fell inside the Milan criteria (BCLC-score: A, MELD-score was 11). Due to rapid disease recurrence, this patient started with sorafenib in June 2019. At time of start of the study and during hospital admissions patient used tiotropium, perindopril, tamsulosin, insulin, oxazepam, pantoprazole, metformin, salbutamol, prednisolone and metoprolol as additional comedication. His dose of tacrolimus was 10 mg q.d., with a baseline tacrolimus C_{trough} of 3.9 µg/L. Sorafenib exposure was remarkably lower at day 14 than at day 7, as the AUC_{last} decreased with 44% and C_{max} with 62% respectively (**Table 1)**. During the further treatment, sorafenib showed a decrease in plasma trough levels over time despite a dose

increase to 200 mg once daily and 400 mg once daily (**Figure 4**). On the other hand, the tacrolimus plasma concentration remained relatively stable over time. In October 2019 sorafenib was stopped due to progression of disease.

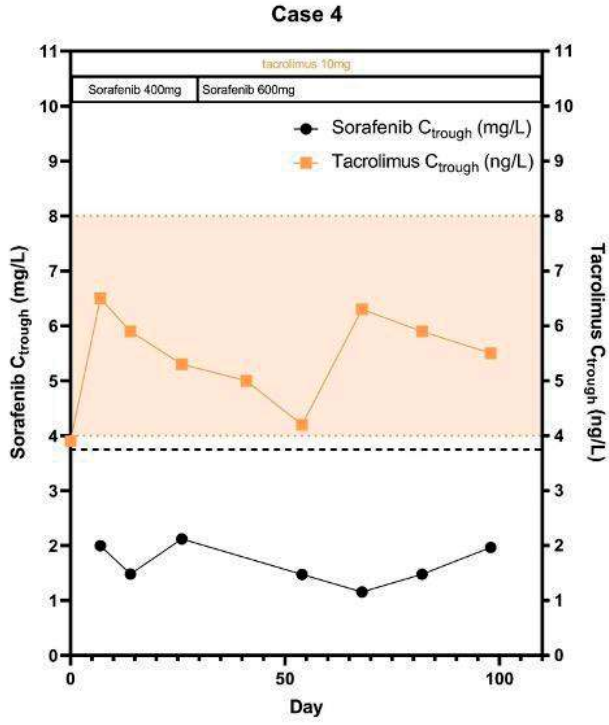

Figure 4. Sorafenib and tacrolimus C_{trough} concentrations over time for Subject 4: The C_{trough} levels are displayed over time after the start of sorafenib treatment. Furthermore, the optimal C_{trough} levels of both sorafenib and tacrolimus are provided.

DISCUSSION

In this study we present the first case series of patients treated with sorafenib for HCC recurrence after liver transplantation investigating both sorafenib and immunosuppressant plasma concentration over time. In all four patients the plasma pharmacokinetics of both immunosuppressants and sorafenib were longitudinally monitored until sorafenib discontinuation. Sorafenib plasma concentrations (C_{trough}) decreased over time in every case, even after discontinuation of tacrolimus in two of four cases. Long term decrease in TKI exposure is a recognized phenomenon and we cannot distill a consequent pharmacokinetic influence of immunosuppression on the gradually decreased sorafenib exposure from our results. This decline in sorafenib exposure may be induced by autoinduction of CYP3A4, which results in declining plasma levels over time as was

demonstrated for imatinib before.[13,14] However, variation in immunosuppression concentrations was not structural (two patients showed a decline in immunosuppression plasma exposure, while the other two patients showed opposite effects), which makes structural CYP3A4 induction less likely.[15] Potentially sorafenib non-adherence may have contributed to the decline in sorafenib concentrations over time, since patient adherence was only questioned when meeting the treating oncologist. Moreover, about 50% of patients on long term oral anticancer drug therapy tend to be non-adherent to their treatment resulting in a diminished therapy efficacy and (unexplained) decline in plasma levels.[16] Although a clear pharmacokinetic interaction of tacrolimus and sorafenib was not found, a sorafenib dose increment to 600 mg daily led to severe hepatotoxicity in case 1. Although sorafenib concentrations increased prior to occurrence of the adverse events, the absolute concentrations of sorafenib did not exceed those measured at start of therapy, which contradicts a sole pharmacokinetic explanation. Both laboratory and imaging findings did not show other causes of hepatotoxicity (e.g., viral hepatitis) and other side -effects in our patients. Therefore, it is likely that an additional pharmacodynamic mechanism is causing the high incidence of sorafenib-induced toxicity after liver transplantation. In this study there were no acute rejections, but patients experienced many side-effects with increasing sorafenib dose. As mentioned before, sorafenib toxicity rates are higher in patients treated with immunosuppression. In several studies, a high incidence of sorafenib dose reduction or discontinuation (15%-77%) has been reported in patients with HCC after liver transplantation when starting with a 400 mg b.i.d. dose.[17-19] However, the proportion of patients in need of dose reduction or discontinuation seemed to be lower in Asian population studies, suggesting a possible genetic difference.[4] Based on these observations, starting with a lower than regular sorafenib dose seems to be justified in most patients, since the majority of patients required a dose reduction and most patients did not experience significant toxicity at lower dosing levels.[19] Although it is currently no standard of care, this strategy may also improve patient adherence in patients without a previous liver transplantation, as a result of lower toxicity rates compared to the 400 mg starting dose. Unfortunately, none of these studies investigated sorafenib or immunosuppressant pharmacokinetics. Because sorafenib plasma trough concentrations showed a decrease in our patients, the underlying mechanism of this increase in side effects most likely is of pharmacodynamic origin. Moreover, the immunocompromised status of these patients may be related to an increased incidence of side effects in post liver transplantation patients. However, the exact mechanism remains unknown. Moreover, an important aspect in the immunosuppressant treatment of patients with HCC recurrence after liver transplantation is the class of immunosuppressants used. Latest evidence suggests survival benefit of treatment with mammalian target of rapamycin (mTOR) inhibitors compared to calcineurin inhibitors like tacrolimus especially when used with sorafenib.[6] However, general consensus

on this topic is not yet reached and alternative therapies, such as lowering immunosuppressant dosing as much as possible, are used in clinical practice. All the patients in this study are treated according to the national treatment guidelines in the Netherlands. From a pharmacokinetic point of view most CNIs have similar pharmacokinetic properties compared to mTOR inhibitors the effects seen in this case-series may also be applied for these classes of immunosuppressants. Moreover, additional treatment strategies for hepatocellular carcinoma patients are emerging, among which immunotherapy regimens. However, this is no option in patient with a liver transplantation, because of the major risk of transplant rejection.[20] Therefore TKI treatment remains the standard treatment in these patients despite this new development. Next to sorafenib, alternative dosing strategies for other TKIs such as cabozantinib, regorafenib, and imatinib, were suggested before. However, evidence in liver transplantation patients is lacking.[21-23] In transplanted patients with a malignancy in general, physicians attempt to lower the overall immunosuppressive load as much as possible, but it is very difficult to define the lower threshold of the target range for individual patients. Sometimes with trial and error, dosages are reduced stepwise, while liver function is monitored closely. In the second case of our series the immunosuppression was stopped completely, and patient and medical team were fortunate that this did not result in a rejection episode.

Several lessons can be learned from this case series. First of all, there is currently a lack of knowledge in the management of the combination of sorafenib and tacrolimus. Oncologists often determine the sorafenib starting dose on the basis of personal experience with this treatment combination. Overall, there is a decrease in sorafenib plasma levels over time, even when it is not combined with tacrolimus. Due to an increased risk of side effects in patients with a liver transplantation,[9] and based on the high incidence of side effects with higher sorafenib doses we would recommend to start treatment with a reduced daily dose of 200 mg b.i.d.[4] Based on tolerability, the dose can then gradually be escalated. Moreover, a daily sorafenib dose of 200mg b.i.d. has demonstrated to be an effective dosing strategy, which indicates a possible overdosing in most patients treated with sorafenib.[24]

CONCLUSIONS

In conclusion, the interaction between sorafenib and immunosuppressive drugs is clinically relevant in view of the high toxicity rates compared to patients without a liver transplantation. More research is needed to investigate the pharmacokinetic aspects of this drug-drug interaction.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians.* 2018;68(6):394-424.
2. Iavarone M, Invernizzi F, Czauderna C, et al. Preliminary experience on safety of regorafenib after sorafenib failure in recurrent hepatocellular carcinoma after liver transplantation. *Am J Transplant.* 2019.
3. Zimmerman MA, Ghobrial RM, Tong MJ, et al. Recurrence of Hepatocellular Carcinoma Following Liver Transplantation: A Review of Preoperative and Postoperative Prognostic Indicators. *Archives of Surgery.* 2008;143(2):182-188.
4. Kang SH, Cho H, Cho EJ, et al. Efficacy of Sorafenib for the Treatment of Post-Transplant Hepatocellular Carcinoma Recurrence. *J Korean Med Sci.* 2018;33(45):e283.
5. Sposito C, Mariani L, Germini A, et al. Comparative efficacy of sorafenib versus best supportive care in recurrent hepatocellular carcinoma after liver transplantation: a case-control study. *J Hepatol.* 2013;59(1):59-66.
6. Verna EC, Patel YA, Aggarwal A, et al. Liver transplantation for hepatocellular carcinoma: Management after the transplant. *American Journal of Transplantation.* 2020;20(2):333-347.
7. Berenguer M, Burra P, Ghobrial M, et al. Posttransplant management of recipients undergoing liver transplantation for hepatocellular carcinoma. Working Group Report from the ILTS Transplant Oncology Consensus Conference. *Transplantation.* 9000;Online First.
8. Li Y, Gao ZH, Qu XJ. The adverse effects of sorafenib in patients with advanced cancers. *Basic Clin Pharmacol Toxicol.* 2015;116(3):216-221.
9. Staufer K, Fischer L, Seegers B, Vettorazzi E, Nashan B, Sterneck M. High toxicity of sorafenib for recurrent hepatocellular carcinoma after liver transplantation. *Transpl Int.* 2012;25(11):1158-1164.
10. Kim R, El-Gazzaz G, Tan A, et al. Safety and feasibility of using sorafenib in recurrent hepatocellular carcinoma after orthotopic liver transplantation. *Oncology.* 2010;79(1-2):62-66.
11. Takahara T, Nitta H, Hasegawa Y, Itou N, Takahashi M, Wakabayashi G. Using sorafenib for recurrent hepatocellular carcinoma after liver transplantation--interactions between calcineurin inhibitor: two case reports. *Transplant Proc.* 2011;43(7):2800-2805.
12. Bins S, van Doorn L, Phelps MA, et al. Influence of OATP1B1 Function on the Disposition of Sorafenib-beta-D-Glucuronide. *Clin Transl Sci.* 2017;10(4):271-279.
13. Filppula AM, Laitila J, Neuvonen PJ, Backman JT. Potent mechanism-based inhibition of CYP3A4 by imatinib explains its liability to interact with CYP3A4 substrates. *Br J Pharmacol.* 2012;165(8):2787-2798.
14. Eechoute K, Fransson MN, Reyners AK, et al. A long-term prospective population pharmacokinetic study on imatinib plasma concentrations in GIST patients. *Clin Cancer Res.* 2012;18(20):5780-5787.
15. Hussaarts K, Veerman GDM, Jansman FGA, van Gelder T, Mathijssen RHJ, van Leeuwen RWF. Clinically relevant drug interactions with multikinase inhibitors: a review. *Ther Adv Med Oncol.* 2019;11:1758835918818347.
16. Timmers L, Boons CCLM, Verbrugghe M, van den Bemt BJF, Van Hecke A, Hugtenburg JG. Supporting adherence to oral anticancer agents: clinical practice and clues to improve care provided by physicians, nurse practitioners, nurses and pharmacists. *BMC Cancer.* 2017;17(1):122-122.
17. De Simone P, Crocetti L, Pezzati D, et al. Efficacy and Safety of Combination Therapy With Everolimus and Sorafenib for Recurrence of Hepatocellular Carcinoma After Liver Transplantation. *Transplantation Proceedings.* 2014;46(1):241-244.

18. Weinmann A, Niederle IM, Koch S, et al. Sorafenib for recurrence of hepatocellular carcinoma after liver transplantation. *Digestive and Liver Disease.* 2012;44(5):432-437.
19. Castelli G, Burra P, Giacomin A, et al. Sorafenib use in the transplant setting. *Liver Transpl.* 2014;20(9):1021-1028.
20. Pinter M, Scheiner B, Peck-Radosavljevic M. Immunotherapy for advanced hepatocellular carcinoma: a focus on special subgroups. *Gut.* 2020;70(1):204-214.
21. Ramadori G, Füzesi L, Grabbe E, Pieler T, Armbrust T. Successful treatment of hepatocellular carcinoma with the tyrosine kinase inhibitor imatinib in a patient with liver cirrhosis. *Anticancer Drugs.* 2004;15(4):405-409.
22. Lin AY, Fisher GA, So S, Tang C, Levitt L. Phase II study of imatinib in unresectable hepatocellular carcinoma. *Am J Clin Oncol.* 2008;31(1):84-88.
23. Delos Santos S, Udayakumar S, Nguyen A, et al. A systematic review and network meta-analysis of second-line therapy in hepatocellular carcinoma. *Curr Oncol.* 2020;27(6):300-306.
24. Siegel AB, El-Khoueiry AB, Finn RS, et al. Phase I trial of sorafenib following liver transplantation in patients with high-risk hepatocellular carcinoma. *Liver Cancer.* 2015;4(2):115-125.

CHAPTER 11

CARDIOVASCULAR TOXICITY OF ANGIOGENESIS INHIBITORS AND IMMUNE CHECKPOINT INHIBITORS: SYNERGISTIC ANTI-TUMOUR EFFECTS AT THE COST OF INCREASED CARDIOVASCULAIR RISK?

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ABSTRACT

In the past two decades, treatment outcomes for a wide range of malignancies have im proved remarkably due to the development of novel anti-cancer therapies, includ-ing vascular endothelial growth factor inhibitors (VEGFIs) and immune checkpoint inhibitors (ICIs). Despite their unprecedented anti-tumour effects, it is becoming increasingly clear that both types of agents are associated with specific cardiovascular toxicity, including hypertension, congestive heart failure, myocarditis and acceleration of atherosclerosis. Currently, VEGFI and ICI combination therapy is recommended for the treatment of advanced renal cell carcinoma (RCC) and has shown promising treatment efficacy in other tumour types as well. Consequently, VEGFI and ICI combination therapy will most likely become an important therapeutic strategy for various malignancies. However, this combinatory approach is expected to be accompanied by a substantial increase in cardiovascular risk, as both types of agents could act synergistically to induce cardiovascular sequelae. Therefore, a comprehensive baseline assessment and adequate monitoring by specialised cardio-oncology teams is essential in case these agents are used in combination, particularly in high-risk patients. This review summarises the mechanisms of action and treatment indications for currently registered VEGFIs and ICIs, and discusses their main vascular and cardiac toxicity. Subsequently, we provide the biological rationales for the observed promising synergistic anti-tumour effects of combined VEGFI/ICI administration. Lastly, we speculate on the in- creased risk for cardiovascular toxicity in case these agents are used in combination and its implications and future directions for the clinical situation.

INTRODUCTION

In the past two decades, the development of a multitude of targeted anti-cancer therapies has substantially increased survival outcomes for many types of cancer. Among these are vascular endothelial growth factor inhibitors (VEGFIs), which exert anti-tumour effects by exploiting the tumour's dependency on its vascular supply for its growth and metastatic spread.[1] More recently, immune checkpoint inhibitors (ICIs) have been introduced, which act by inducing a T-cell mediated anti-tumour response.[2] Although both types of treatment have revolutionized the therapeutic armamentarium for a wide variety of tumours and often lead to durable anti-cancer responses, serious concerns regarding their short- and long-term safety profiles have arisen.[3,4] For a while, it has been recognized that VEGFIs lead to cardiovascular toxicity in a substantial proportion of treated patients.[5] However, ICIs are best known for their immune-related side-effects such as pneumonitis, hypophysitis and thyroiditis,[6] their possible detrimental effects on the cardiovascular system have only recently gained attention.[3,7] Initial safety reports might have underestimated the true incidences of ICI-induced cardiovascular toxicity, including myocarditis and vasculitis,[8-10] and information on long-term cardiovascular effects is scarce.[11] Given the continuous expansion of indications for ICIs, including adjuvant therapy in melanoma to prevent recurrence and metastatic spread, the incidence of ICI-associated cardiovascular toxicity is expected to increase, which necessitates effective preventive strategies.[12]

A proportion of cancer patients do not respond to ICI therapy or acquire treatment resistance. Therefore, combinatory approaches of ICIs with alternative anti-cancer drugs are currently under investigation, which include a combination of ICIs and VEGFIs, which has shown clear synergistic anti-tumour effects in both preclinical and clinical studies.[13] In fact, their combined use is already recommended by the European Society of Medical Oncology (ESMO) as first-line therapy in advanced clear cell renal cell carcinoma (RCC) and ongoing clinical trials have provided promising results for other cancer types as well.[13-16] These favourable anti-tumour effects are likely to be mirrored by unfavourable effects on the cardiovascular system, given that both classes of agents are demonstrated to lead to cardiovascular side-effects via largely distinct mechanisms. As this combinatory approach is expected to lead to prolonged survival outcomes in cancer patients, the potential long-term cardiovascular effects will become increasingly relevant. Therefore, the adverse cardiovascular sequelae of their combined use should become a major focus of the scientific and clinical endeavour of cardio-oncology: a medical subspeciality that aims to understand and mitigate adverse cardiovascular effects associated with anti-cancer therapies to optimize cardiovascular health in the oncology patient population.[17,18]

This review focuses on the cardiovascular toxicity of VEGFIs and ICIs. After summarizing the working mechanisms and main indications, the incidence and pathogenesis of major cardiovascular events associated with both types of therapy are reviewed, including hypertension, direct cardiotoxicity and thrombotic events. Subsequently, the synergistic effects of combined VEGFI and ICI administration are discussed, and clinical considerations and future directions for their combined use are provided, particularly in terms of the prevention, the monitoring and the management of the expected cardiovascular toxicity.

Vascular Endothelial Growth Factor Inhibitors (VEGFIs)

Mechanism of action and therapeutic indications

Angiogenesis, the formation of new blood vessels from pre-existing vasculature, is critical for many physiological processes, including embryonal development, placental function and wound healing, but also for tumour growth. Tumours promote angiogenesis by secretion of proangiogenic factors to facilitate supply of nutrients for their expansion and metastatic spread. Vascular endothelial growth factor (VEGF) is the main proangiogenic factor and is secreted by a multitude of cell types, such as endothelial cells, fibroblasts and tumour cells. Upon binding to one of its three receptors (VEGFR-1, VEGFR-2, VEGFR-3), VEGF stimulates endothelial cell proliferation, migration and survival via several intracellular signaling pathways, including the phosphatidylinositol 3-kinase (PI3k)/Akt pathway, and increases vascular permeability to enable extracellular matrix production. Eventually, these processes lead to angiogenesis.[19,20] Next to angiogenesis, VEGF also plays a critical role in the maintenance of vascular homeostasis and in cardiac development and function.[21]

Targeting VEGF-induced angiogenesis to establish anti-neoplastic effects was first proposed by Folkman in 1971.[22] In 2004, the anti-VEGF monoclonal antibody (mAb) bevacizumab was the first VEGFI to obtain approval from the United States Food and Drug Administration (FDA) for the treatment of metastatic colorectal carcinoma in combination with conventional chemotherapy.[23] Since then, four main classes of VEGFIs have been developed that either target VEGF or its receptors and downstream signaling (**Figure 1**): (1) Anti-VEGF monoclonal antibodies, (2) VEGF soluble decoy receptors capturing free available VEGF (VEGF-trap), (3) Anti-VEGFR monoclonal antibodies and (4) Tyrosine kinase inhibitors (TKIs) with anti-VEGFR activity. Most of these TKIs are "multi-targeted" and do not selectively target the VEGFRs, but also other tyrosine kinases. For example, the TKI sunitinib inhibits various tyrosine kinases that are implicated in the growth and survival of tumour cells, including VEGFR, platelet-derived growth factor receptor (PDGFR), fms-like tyrosine kinase (Flt-3) and the stem cell factor receptor, c-Kit. Originally, it was hypothesised that inhibition of VEGF-induced angiogenesis exerted

anti-cancer effects by impairing the blood supply to the tumour, leading to starvation and subsequent death of tumour cells. If this holds true, the impairment of the tumour blood supply would induce hypoxia (a trigger for tumours aggressiveness) and decrease the delivery and effectivity of other co-administrated anti-neoplastic agents.[24]

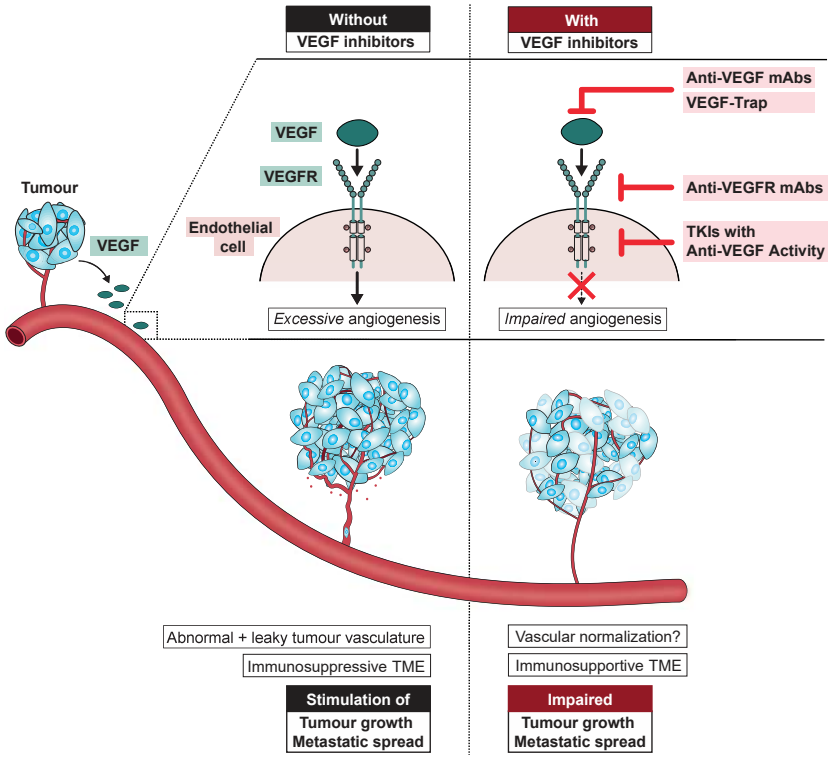

Figure 1. Mechanism of action of VEGFIs for the treatment of cancer

Tumours stimulate angiogenesis for their growth and metastatic spread by excessive secretion of VEGF. This results in the formation of an abnormal, leaky tumour vasculature, which could lead to tumour hypoxia and an immunosuppressive tumour microenvironment (TME). VEGFIs impair tumour angiogenesis and promote vascular normalization and an immunosupportive TME, which inhibits tumour expansion and metastatic spread. Clinically, four different classes of agents to inhibit VEGF or the VEGF receptor (VEGFR) can be distinguished: (1) monoclonal antibodies directed against circulating VEGF; (2) VEGF-traps; (3) mAb against the VEGFR; (4) VEGFR TKIs that act on the intracellular tyrosine kinase domains of VEGFRs to inhibit their activation.

However, the contrary seems to be the case as VEGFIs greatly potentiate the anti-tumour effects of concomitant cytotoxic therapies.[25] Although the exact mechanisms of action of VEGFIs remain subject of investigation, Jain et al. proposed that these agents act by normalizing the tumour vasculature.[25] This vascular normalization theory proposes that excessive VEGF secretion by tumour cells promotes the formation of an abnormal and leaky tumour vasculature, which results in suboptimal blood flow, tumour hypoxia and an immunosuppressive tumour microenvironment (**Figure 1**). Consequently, inhibition

of VEGF signaling normalises the tumour vasculature, which alleviates tumour hypoxia and facilitates the delivery of concomitant anti-cancer drugs and the infiltration of immune cells with anti-cancer activity.[15,25,26] This latter effect has been further reviewed recently by Huinen et al. who propose that VEGFIs stimulate immune cell infiltration via reversion of vascular endothelial cell anergy.[15] Together, these processes underlie the anti-tumour effects of VEGFIs. Currently, VEGFIs are in use for a wide range of malignancies, often combined with conventional chemotherapy. **Table 1** displays approved VEGFIs up to May 2021 with their cellular targets and main indications.

Table 1 Molecular targets and approved indications (as of May 2021) for VEGFIs

Drug class	Drug	Target	EMA and FDA-approved indications (*FDA approved indication only)
Anti-VEGF mAb	Bevacizumab	VEGF-A	Breast and ovarian cancer, CRC, GBM*, HCC, NSCLC, primary peritoneal cancer, RCC
VEGF-trap	Aflibercept	PlGF, VEGF-A, VEGF-B	CRC
Anti-VEGFR mAb	Ramucirumab	VEGFR2	CRC, gastric or gastro-esophageal junction adenocarcinoma, HCC, NSCLC
VEGF-TKI	Axitinib	VEGFR1-3	RCC
	Cabozantinib	AXL, c-Kit, FLT3, MER, MET, RET, ROS1, TIE-2, TRKB, TYRO3, VEGFR1-3	HCC, MTC, RCC
	Lenvatinib	c-Kit, FGFR1-4, PDGFR, RET, VEGFR1-3	HCC, RCC*, thyroid cancer
	Pazopanib	c-Kit, PDGFR, VEGFR1-3	RCC, soft-tissue sarcoma
	Ponatinib	BCR-ABL, c-Kit, FGFR, FLT3, PDGFR, RET, VEGFR	CML, Ph+ ALL
	Regorafenib	BRAF, $BRAF^{V600E}$, c-Kit, CSF1R, FGFR, PDGFR, RAF-1, RET, TIE-2, VEGFR1-3	CRC, GIST, HCC
	Sorafenib	BRAF, $BRAF^{V600E}$, c-Kit, CRAF, FLT3, PDGFR, VEGFR2-3	HCC, RCC, thyroid cancer
	Sunitinib	c-Kit, CSF-1R, FLT-3, PDGFR, RET, VEGFR1-3	GIST, pNET, RCC
	Vandetanib	EGFR, RET, VEGFR2	MTC

Table based on.[37] Abbreviations: BCR-ABL, breakpoint cluster region-Abelson; BRAF, v-raf murine sarcoma viral oncogene homolog B1; CML, chronic myeloid leukemia; CRC, colorectal carcinoma; CSF-1R, colony stimulating factor 1 receptor; EGFR, epidermal growth factor receptor; EMA, European Medicines Agency; FDA, United States Food and Drug Administration; FGFR, fibroblast growth factor receptor; FLT, fetal liver tyrosine kinase 3; GBM, glioblastoma multiforme; GIST, gastrointestinal stroma cell tumour; HCC, hepatocellular carcinoma; mAb, monoclonal antibody; MET, mesenchymal-epithelial transition factor; MTC, medullary thyroid cancer; NSCLC, non-small cell lung cancer; PDGFR, platelet-derived growth factor receptor; Ph+ALL, Philadelphia chromosome positive acute lymphatic leukemia; PlGF, placental growth factor; pNET, pancreatic neuroendocrine tumour; RCC, renal cell carcinoma; RET, rearranged during transfection; TRKB, tropomyosin receptor kinase B; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

Cardiovascular toxicities

VEGFIs are associated with multiple cardiovascular toxicities, including hypertension, thrombosis, left ventricular dysfunction and QTc interval prolongation. These adverse effects seem to be a consequence of inhibition of the critical role of VEGF for the function and maintenance of homeostasis within the cardiovascular system, but the exact pathophysiology underlying most of these side-effects remains to be elucidated.[27,28] Apart from the direct harmful effects, cardiovascular toxicity might require a reduction or (temporary) discontinuation of effective anti-cancer therapy, possibly impairing patient survival. Therefore, it is important to understand these side-effects to enable prevention and optimal treatment in the clinical situation.

Hypertension

Hypertension is the most frequent and best characterized cardiovascular toxicity associated with VEGFI therapy.[29] Most trials have observed an incidence of VEGFI-induced hypertension of approximately 20-40%.[30] However, percentages of up to 90% have also been reported, depending on the type and dosage of VEGFI administered.[31] Of note, virtually every patient experiences a rapid increase in blood pressure within days after initiation of therapy.[32,33] Depending on the absolute blood pressure levels at baseline this may lead to high-grade hypertension, although patients who are hypertensive at the start of VEGFI therapy do not necessarily demonstrate a more substantial increase in blood pressure than initial normotensive patients.[34] Usually, the prohypertensive effects of VEGFI therapy can be managed with conventional anti-hypertensive drugs, but acute hypertensive end-organ complications have been described, including acute hemorrhagic events and posterior reversible encephalopathy syndrome in severe cases.[4,35,36] Given that a rise in blood pressure is possible to occur with every type of VEGFI and is dose-dependent, this supports that VEGFI-induced hypertension is an on-target mechanism.

The mechanisms underlying VEGFI-induced hypertension remain the subject of investigation and have been reviewed extensively elsewhere.[29,33,37] In short, VEGF plays an important role in the maintenance of the vascular tone by establishing a balance between vasoconstrictor and vasodilator factors. In both the preclinical and clinical situation, inhibition of VEGF signaling leads to elevated concentrations of the potent vasoconstrictor endothelin-1 (ET-1) and decreased levels of the vasodilator nitric oxide (NO) via inhibition of endothelial nitric oxide synthase (eNOS). This imbalance in favour of vasoconstriction leads to elevated peripheral vascular resistance, contributing to hypertension.[38] Increases in oxidative stress and reactive oxygen species (ROS) has also been put forward in the etiology of VEGFI-induced hypertension [39,40] and a preclinical study further verified that redox-sensitive processes underlie VEGFI-induced vascular toxicity.[41] However, treatment with the ROS scavenger tempol did not lead to a relevant

attenuation of the rise in blood pressure during sunitinib therapy in rats.[42] Microvascular rarefaction, which is a reduction in microvessel density, has been proposed to contribute to VEGFI-induced hypertension by increasing peripheral vascular resistance.[43] Most likely, this is a functional rather than a structural phenomenon, given the rapidity of blood pressure normalization after treatment cessation and the high degree of structural microvascular rarefaction necessary to result in significant increases in blood pressure.[44] In addition, VEGFIs can lead to renal toxicities, including proteinuria in up to 21-63% of patients and, in severe cases, histological abnormalities such as thrombotic microangiopathy.[45] Although these nephrotoxic effects seem to occur largely independently from the prohypertensive effects, they have the potential to further increase blood pressure by leading to salt and water retention.[46] Indeed, sunitinib-induced hypertension was demonstrated to be salt-sensitive in a preclinical study, which implies that targeting this salt-sensitivity could be an effective measure to ameliorate VEGFI-induced hypertension.[37,47] This hypothesis is currently tested in a prospective clinical trial evaluating the effects of salt restriction in cancer patients who develop hypertension during VEGFI therapy (Netherlands Trial Register NL7340).

The vascular and renal effects of VEGFI largely resemble the hallmarks of the severe pregnancy complication preeclampsia and have consequently been termed a "preeclampsia-like syndrome".[37,42] Preeclampsia is characterized by hypertension, proteinuria and elevated plasma levels of soluble fms-like tyrosine kinase (sFlt-1); a soluble VEGFR that captures freely available VEGF. Consequently, VEGF bioavailability in preeclamptic women is largely diminished, which is thought to play a key role its pathogenesis, a situation comparable to cancer patients receiving VEGFIs.[48] Given the significant overlap in the pathogenesis of preeclampsia and VEGFI-induced cardiovascular toxicity, findings in one population may provide useful information for the other.[37] As aspirin, a cyclooxygenase (COX) inhibitor, is currently recommended for women at high risk for developing preeclampsia, aspirin treatment might also be useful for the prevention of VEGFI-induced hypertension and proteinuria.[49] Indeed, aspirin was able to ameliorate these toxicities in a preclinical study, which now warrants further clinical investigation.[50] An important question that remains is whether the preventive effects of aspirin are mediated via the COX-1 or COX-2 enzyme, or both.[50]

The occurrence of VEGFI-induced hypertension has been proposed to be a biomarker for anti-cancer treatment efficacy. Retrospective analyses demonstrated improved overall survival outcomes in patients that developed hypertension during sunitinib therapy.[51,52] However, this association was not verified by an analysis of 7 studies in patients that received bevacizumab therapy for metastatic cancers,[53] nor by a retrospective study in patients with soft-tissue sarcoma who received pazopanib.[54] Importantly, concomitant anti-hypertensive prophylaxis or treatment of VEGFI-induced hypertension did not affect anti-neoplastic efficacy of these agents.[51,55] Therefore, intensive control of blood

pressure before and throughout VEGFI treatment is recommended.[29] In general, weekly blood pressure monitoring during the first VEGFI treatment cycle is advised, and systolic blood pressure treatment thresholds of 130 mmHg and 140 mmHg have been proposed for the initiation of anti-hypertensive therapy.[4,29,56,57] The frequency of monitoring may be adjusted in subsequent treatment cycles, depending on blood pressure control.

Arterial and venous thrombosis

Patients with cancer are at increased risk of developing thrombosis, due to a generalised hypercoagulable state.[58] Therefore, it is important to be aware of possible additional prothrombotic effects of administered anti-cancer therapies. VEGFI therapy is consistently associated with an increased incidence of arterial thromboembolic events (ATEs). In most studies, the incidence of ATE was determined as a composite outcome of arterial thrombosis, myocardial infarction and cerebrovascular events. A pooled analysis of 1745 patients with a variety of malignancies, including colorectal cancer, breast cancer and non-small cell lung cancer, showed that the addition of bevacizumab to the chemotherapy regimen increased the risk of developing ATE from 1.7% to 3.8%.[59] These findings were verified by a large meta-analysis in approximately 10000 cancer patients, demonstrating that sunitinib and sorafenib therapy increased ATE incidence by three-fold to an absolute incidence of 1.4%.[60] Additionally, a meta-analysis in patients treated with bevacizumab found an absolute incidence of all- and high-grade ATE of 3.3% and 2.0% respectively, and fatal ATE occurred in 0.4% of patients. Notably, different versions of the Common Terminology Criteria for Adverse Events were used to grade ATE severity in the included studies, which could lead to inconsistencies in the reported events.[61] Generally, ATE seem to occur early during VEGFI therapy: in bevacizumab-treated patients, the median time to the first ATE was 2.6 months. Risk factors for the development of ATE in this patient population included age ≥ 65 years and a history of ATE. In patients with both risk factors, ATE occurred 7.6- times more frequently. Thus, despite the fact that the reported risk of ATE during VEGFI therapy is relatively low in the general oncology population, ranging from 1 to 4%, particular caution is warranted in case VEGFI therapy is administered in high-risk patient populations.[59]

Contrary to the increased incidence of ATE, previous studies have provided conflicting results on the potential of VEGFIs to elevate the risk of venous thromboembolic events (VTE).[59] A meta-analysis in almost 8000 patients with various cancer types demonstrated a modest increase in the incidence of VTE in bevacizumab-treated patients compared to patients receiving standard anti-neoplastic therapy (relative risk 1.33).[62] Additionally, total incidence of VTE increased from 7.3% to 9.3% in a randomised controlled trial investigating the survival benefits of addition of aflibercept to the FOLFIRI (fluorouracil, leucovorin, irinotecan) regimen in metastatic colorectal carcinoma patients.[63] However, a direct statistical comparison was not performed in this study and the increased incidence

of VTE was not confirmed by a retrospective analysis in approximately 6000 bevacizumab-treated patients with advanced solid tumours.[64] In line with this, two recent large meta-analyses investigated the associations between the incidence of VTE and treatment with TKIs with anti-VEGFR activity [65] and all four classes of VEGFI therapy.[66] In both studies, treatment with VEGFIs did not increase the risk to develop VTE. Although the underlying causes for the prothrombotic effects of inhibition of VEGF signaling remain largely unclear, these effects were not completely unexpected given that VEGF is crucial for the health of endothelial cells by promotion of endothelial cell survival and upregulation of anti-apoptotic cellular cascades.[33] Inhibition of the protective effects of VEGF on the endothelium could lead to impaired endothelial cell regeneration and subsequent endothelial damage. Indeed, levels of endothelial cell-derived microparticles, biomarkers for endothelial injury, were increased in cancer patients during VEGFI therapy.[40] This endothelial damage can predispose to arterial thrombosis by exposing circulating platelets to subendothelial extracellular matrix components, leading to their activation.[67] Additionally, VEGF is an important regulator of vascular homeostasis via its downstream mediators NO and prostacyclin (PGI_2), which are potent vasodilators and inhibitors of platelet aggregation.[39,68] Although an *in vitro* study demonstrated that inhibition of VEGF signaling leads to a reduction of PGI_2 production,[69] this has not yet been verified in clinical studies. In fact, sunitinib treatment led to increased PGI_2 levels in rats.[50] Thus, a reduction in PGI_2 as a cause of a prothrombotic state during VEGFI therapy seems unlikely.

Interestingly, preclinical evidence suggests that plasminogen activator inhibitor-1 (PAI-1), the principal inhibitor of fibrinolysis, plays an important role in VEGFI-induced venous thrombosis formation. In a mouse xenograft model of human lung carcinoma, bevacizumab increased plasma PAI-1 levels and promoted venous thrombus formation, which was largely absent in PAI-$1^{-/-}$ mice or during pharmacological PAI-1 inhibition.[70] Another preclinical study demonstrated that bevacizumab predisposes to thrombosis by formation of immune complexes with VEGF and subsequent stimulation of the platelet FcγRIIa (IgG) receptor, a mechanism similar to heparin-induced thrombocytopenia.[71] Also, impaired venous thrombus resolution has been proposed, which contributes to clinically significant thrombosis, but these prothrombotic mechanisms await clinical verification.[72] In summary, it seems that inhibition of VEGF signaling in patients predominantly affects the arterial vascular beds, rather than the venous system. An explanation for this could be that VEGFI-induced arterial hypertension causes endothelial damage, predisposing to ATE,[66] but more studies are needed to further unravel the mechanisms underlying the prothrombotic properties of this class of anti-neoplastic therapy.

Cardiac toxicity

Cardiac toxicity as a consequence of VEGFIs can range from an asymptomatic small decline in left ventricular ejection fraction (LVEF) and QTc prolongation to severe symptom-

atic heart failure.[73] Although the exact pathophysiology underlying the adverse cardiac sequelae of VEGFIs remain subject of investigation, they seem to reflect both on- and off-target effects of inhibition of VEGF signaling.[73] A previous meta-analysis found comparable incidences of overall cardiac toxicity between direct VEGFIs and multitargeted TKIs with anti-VEGF activity.[66] Notably, VEGFI-induced elevations of systemic blood pressure are thought to potentiate the cardiotoxic effects by increasing ventricular afterload. In transverse aortic constriction mice, a mouse model for pressure overload, administration of a VEGF decoy receptor promoted left ventricular dilatation and loss of cardiac contractile function.[74] Also, in hypertensive mice, sunitinib led to cardiomyocyte apoptosis accompanied by mitochondrial swelling and degenerative changes.[5] Indeed, hypertension is proposed to be a clinical risk factor for VEGFI-induced cardiotoxicity, which further stresses the importance of adequate blood pressure control throughout treatment.[21,29,57] While the majority of these adverse cardiac effects are either subclinical or mild and resolve upon drug withdrawal,[75] they can occur in a substantial proportion of treated patients. In a single-institution clinical trial, 32% of patients with advanced RCC who received sunitinib developed one or multiple forms of cardiac toxicity. Most of these events were low-grade: 24% of patients had abnormal N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels (defined as >300 pg/ml or ≥100% increase compared to elevated levels at baseline), 27% grade 1 heart failure (defined as asymptomatic with laboratory or cardiac imaging abnormalities) and 10% a grade 2 decrease in LVEF (defined as resting LVEF 40-50%; 10-19% drop from baseline).[76] Yet, another study in 3,784 breast cancer patients demonstrated that bevacizumab was associated with a relative risk of 4.7 for developing clinically significant high-grade (≥3) congestive heart failure, with a total incidence of 1.6%.[77] This cardiotoxic potential was further verified by the current largest meta-analysis including 77 studies, which demonstrated that VEGFIs increased the risk of developing cardiac ischemia (odds ratio 2.8) and cardiac dysfunction (odds ratio 1.4).[66] Of note, patients with recent cardiovascular events were excluded in most oncological trials and routine monitoring of cardiac function was rarely performed. Therefore, the reported incidences of adverse cardiac events are likely to be an underestimation of the true incidences in the "real-world" oncological population. Indeed, in a representative oncological patient population in which cardiac function was assessed during each sunitinib treatment cycle, substantially higher incidences of clinically relevant reductions in LVEF (≥10%) and congestive heart failure were found: respectively 28% and 8%.[5] In addition, various TKIs with anti-VEGF activity can lead to prolongation of the QTc-interval,[78] which is a risk factor for Torsades de Pointes; a dangerous ventricular arrythmia that can lead to syncope and sudden cardiac death.[73] Current guidelines on baseline assessment of cardiotoxicity risk, monitoring of cardiotoxicity during VEGFI treatment and thresholds for intervention are predominantly based on expert opinion,

given the absence of prospective studies.[79-83] These recommendations are further discussed in the section on clinical implications and future directions.

Immune Checkpoint Inhibitors (ICIs)

Mechanisms of action and therapeutic indications

The immune system is dedicated to the elimination of pathogens and cells with an unexpected appearance, including tumour cells, and plays an important role in the prevention of cancer. Immune checkpoints are specialized surface proteins that deliver important co-stimulatory or co-inhibitory signals for T cell activation. Under physiological conditions, immune checkpoints are crucial for the maintenance of immune homeostasis and the prevention of autoimmunity. However, tumour cells can hijack this system by expressing ligands for inhibitory immune checkpoints to evade immunosurveillance and to suppress T cell-mediated anti-tumour responses. Immune checkpoint inhibitors (ICIs) are antibodies that block these intrinsic immunosuppressive pathways and can unleash the power of T lymphocytes to destroy tumour cells,[2] which have revolutionised the treatment of various types of cancer. For instance, in case of melanoma, overall survival improved from <10% at 12 months to a median survival of 24 months and a subgroup of >20% that is still alive after 5 years, suggesting cure.[84] In 2018, ICIs have been rewarded the Nobel Prize for Medicine.[85] Currently, ICIs directed at two main immune checkpoint pathways are used in medical oncology: antibodies blocking the inhibitory programmed death-1 (PD-1) receptor or its ligand (PD-L1), and antibodies against the cytotoxic T-lymphocyte antigen-4 (CTLA-4) receptor (**Figure 2**). The main indications for the currently available ICIs are displayed in **Table 2**.

Table 2 Molecular targets and approved indications (as of May 2021) for ICIs

Cellular target	Drug	EMA and FDA-approved indications (*FDA approved indication only)
CTLA-4	Ipilimumab	CRC, HCC*, Melanoma, malignant pleural mesothelioma*, RCC, NSCLC
PD-1	Cemiplimab	Cutaneous SCC
	Pembrolizumab	Cervical cancer*, classical Hodgkin's lymphoma, CRC, gastric or gastro-esophageal junction adenocarcinoma*, HCC*, MCC*, melanoma, NSCLC, PMBCL*, RCC, SCC of the head and neck, UCC
	Nivolumab	CRC*, HCC*, classical Hodgkin's lymphoma, malignant pleural mesothelioma, melanoma, NSCLC, SCC of the head and neck, SCLC*, RCC, UCC
PD-L1	Atezolizumab	HCC, Triple negative breast cancer, NSCLC, UCC
	Avelumab	MCC, RCC, UCC
	Durvalumab	NSCLC, SCLC, UCC*

Abbreviations: CRC, colorectal carcinoma; CTLA-4, cytotoxic T-lymphocyte antigen-4; EMA, European Medicines Agency; FDA, United States Food and Drug Administration; HCC, hepatocellular carcinoma; MCC, Merkel cell carcinoma; NSCLC, non-small cell lung cancer; PD-1, programmed death-1, PD-L1, programmed death ligand-1; PMBCL, primary mediastinal B-cell lymphoma; RCC, renal cell carcinoma; SCC, squamous cell carcinoma; SCLC, small cell lung cancer; UCC, urothelial carcinoma.

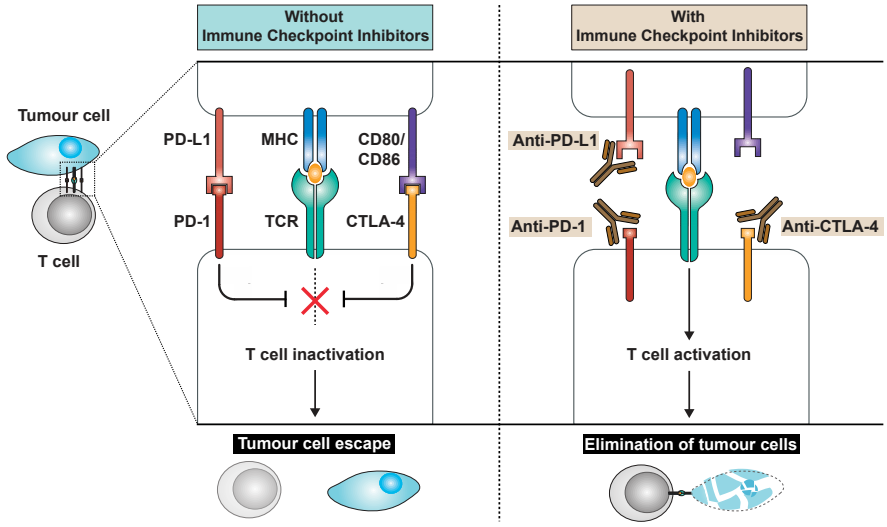

Figure 2. Mechanisms of action of ICIs for the treatment of cancer
Tumour cells are able to evade immunosurveillance by expression of ligands for inhibitory immune checkpoints on the surface of cytotoxic T cells. By blocking these immunosuppressive checkpoints (CTLA-4, PD-1) or the ligand of the latter (PD-L1), immune checkpoint inhibitors (ICIs) lead to T cell activation and subsequent T-cell mediated elimination of tumour cells. Abbreviations: CD80/86, cluster of differentiation 80/86; CTLA-4, cytotoxic T-lymphocyte antigen-4; MHC, major histocompatibility complex; PD-1, programmed death-1; PD-L1, programmed death ligand-1; TCR, T-cell receptor.

Cardiovascular toxicity

By releasing the brakes on the immune system, ICI therapy can lead to a unique spectrum of potentially serious treatment-related toxicity, termed immune-related adverse events (irAEs). These irAEs are thought to arise from multiple mechanisms, particularly aberrant activation of autoreactive T cells that can disrupt immune homeostasis and demonstrate cross-reactivity between tumour neoantigens and normal tissue antigens. ICI-associated irAEs can affect virtually every organ, but the gastrointestinal system, skin, endocrine glands and liver are most often involved.[6] Although less frequent, systemic ICI exposure has also been associated with specific adverse cardiovascular events. Myocarditis and vasculitis are the most common cardiac and vascular toxicities, which will be discussed in the next section. Pericardial disease, Takotsubo cardiomyopathy and cardiac arrythmias have also been reported to be related to ICI therapy in rare cases, however the latter most likely occurs secondary to concurrent irAEs.[3,7,10] Given that ICIs are relatively novel and clinicians may be less familiar with the clinical presentation and diagnosis of irAEs affecting the cardiovascular system, the reported incidence may be an underestimation of the true incidence.[86]

Myocarditis

The exact incidence of myocarditis as a consequence of ICI therapy is unclear. A previous retrospective pharmacovigilance study noted ICI-associated myocarditis in 0.4% of patients,[10] whereas a recent retrospective study found a prevalence of 1.1%.[8] Despite its low occurrence, it is expected that the incidence of ICI-induced myocarditis will increase, given the rapid expansion of indications for ICI therapy and better awareness of this complication among physicians. The clinical course of ICI-associated myocarditis is often fulminant and mortality rates up to 50% have been observed.[10] Mechanisms underlying myocarditis as an irAE remain largely elusive, but previous preclinical and clinical studies have provided some insights. Disruption of the PD-1 receptor in mice led to rapid onset of severe dilated cardiomyopathy and high levels of autoantibodies directed against a cardiac-specific antigen, indicative of autoimmunity.[87] Also, co-administration of ipilimumab and nivolumab in cynomolgus monkeys induced severe myocarditis with infiltration of mononuclear cells in the cardiac tissue, predominantly CD4+ and CD8+ T lymphocytes.[88] In line with this, postmortem histological analyses of cardiac tissue from 2 melanoma patients who developed fatal myocarditis after ipilimumab and nivolumab combination therapy demonstrated substantial myocardial infiltration by T-lymphocytes and macrophages. Interestingly, similar T cell clones were found in tumours, suggesting that myocardial and tumour tissues contain shared antigens or have antigens with high homology.[9] Together, these results indicate that upregulation of PD-1/PD-L1 and CTLA-signaling is a compensatory mechanism to protect cardiac tissue from T-cell induced autoimmunity, which is abrogated during ICI therapy.

The clinical presentation of myocarditis associated with ICI therapy can vary from mild symptoms to life-threatening cardiogenic shock. Patients frequently present with chest pain, dyspnea, fatigue and palpitations. Timely diagnosis is essential given its often fulminant course, but there are no generally accepted diagnostic criteria for ICI-associated myocarditis. Endomyocardial biopsy remains the golden standard, however this is not always feasible and is not routinely performed in clinical practice. Often, myocarditis is diagnosed using a combination of imaging techniques, including electrocardiography (ECG), assessment of cardiac biomarkers (troponin, NT-proBNP), echocardiography and cardiovascular magnetic resonance. A detailed approach for the diagnosis of ICI-myocarditis has been proposed previously.[86,89] Importantly, diagnostic testing should not only be performed to confirm the diagnosis of myocarditis, but also to rule out alternative causes of the clinical manifestations, including cardiac ischemia and complications related to the underlying malignancy.

In a retrospective multi-center case-control study, patients who developed myocarditis were more likely to have received ICI combination therapy, particularly anti-CTLA-4 and anti-PD-1. This suggests that ICI combination therapy is a risk factor for ICI-associated myocarditis, but this has not been prospectively verified.[8] In this same study, every pa-

tient who developed myocarditis had a normal LVEF at baseline, which remained within normal limits in 51% of cases. Contrary, ECG abnormalities and elevated levels of troponin occurred in 89% and 95% of patients diagnosed with myocarditis, respectively. This suggests that particularly the occurrence of ECG abnormalities and elevated troponin levels could be informative diagnostic markers. Given that ICI-associated myocarditis seems to be an early-onset phenomenon, with reported median times to onset of 30 and 34 days after initiation of therapy, intensive monitoring of cardiac toxicity is especially important during the first weeks of treatment.[8,10] Upon diagnosis of ICI-associated myocarditis (grade ≥ 2), discontinuation of ICI therapy and prompt administration of high-dose methylprednisolone (up to 1000 mg/day) followed by oral prednisone (1-2 mg/kg/day) are the cornerstones of treatment.[3,90] In situations where the clinical response to corticosteroids is insufficient, additional immunomodulatory therapies may be required, such as infliximab, tacrolimus, mycophenolate, anti-thymocyte globulin or intravenous immunoglobulins.[90-92]

Vasculitis

Vasculitis is the main reported vascular complication of ICI-therapy. The exact incidence of ICI-induced vasculitis is currently unknown and information about its clinical course and response to therapy is mainly derived from case reports. One recent systematic review of 20 previously published case reports demonstrated that vasculitis as a consequence of ICI therapy predominantly occurs in medium to large vessels, but also vasculitis of the central and peripheral nervous system have been observed [93]. The reported median time of onset was relatively short (3 months), which was in line with a retrospective pharmacovigilance study that found a median time to onset of 55 days among the 82 included cases of ICI-associated vasculitis [10]. Giant cell arteritis (GCA) seems to occur most frequently, affecting medium- to large-size vessels, particularly the temporal arteries. A serious complication of ICI-induced CGA is permanent visual impairment or complete blindness, which was reported in 5 out of 18 (26%) cases in a retrospective study.[10] Like most irAEs, the occurrence of GCA during ICI therapy is thought to be a direct consequence of immune checkpoints inhibition, leading to hyperactive auto-immune responses. Normally, human arteries demonstrate high expression levels of PD-L1, which contributes to an immune privileged state by inhibition of T cell activation. In GCA-patients, PD-L1 expression in the vascular wall is remarkably reduced. This facilitates the infiltration of CD4+ T cells, which can exert proinflammatory effector functions and lead to vasculitis.[94] Therefore, defective PD-1 signaling could be an important determinant in the pathophysiology of ICI-induced CGA,[94] but additional studies are required to further elucidate the underlying mechanisms.

Acceleration of atherosclerosis

Atherosclerosis is a dominant cause of cardiovascular disease such as myocardial infarction and stroke. Hypertension, smoking, diabetes, obesity and high levels of low-density lipoprotein (LDL) cholesterol are established atherosclerotic risk factors. LDL can accumulate in the intima layer of the vascular wall, which is subsequently oxidized and triggers the expression of specific adhesion molecules on the activated endothelium, including vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). This enables the infiltration of predominantly macrophages and $CD4^+$ and $CD8^+$ T cells into the atherosclerotic lesion, which promotes the progression into vulnerable plaques by exerting pro-inflammatory effects.[95] Indeed, the unfavourable role of inflammation has become increasingly clear in the cardiovascular domain.[96-98] Both *in vitro* mechanistic studies and randomized controlled trials applying anti-inflammatory drugs as colchicine or canakinumab (an anti-interleukin-1β mAb) showed that immunosuppressive therapy can decrease the risk for cardiovascular disease.[96,97,99-101]
Immune checkpoints have been reported to play a critical preventive role in the progression of atherosclerosis by inhibition of T-cell driven inflammation in atherosclerotic plaques.[102] Therefore, ICIs are expected to accelerate atherosclerosis and lead to atherosclerosis-related cardiovascular events. In atherosclerosis-prone $Ldlr^{-/-}$ mice, genetic and pharmacological inhibition of PD-1 led to a marked increase in atherosclerotic lesion development and inflammation.[103] Although clinical studies that investigate the effects of ICIs on atherosclerosis are scarce due to the unanticipated long survival outcomes, a large recent retrospective study confirmed the potential of ICIs to exert proatherogenic effects: in 2842 cancer patients who received ICIs, predominantly anti-PD-1 monotherapy, there was a three-fold higher risk of developing atherosclerotic cardiovascular events than in control patients who had received alternative anti-cancer therapy.[11] Interestingly, patients were 1.8 times more likely to experience a cardiovascular event within 2 years after initiation of ICI therapy, compared to the 2-year period prior to ICI initiation. In a smaller group of melanoma patients, this study demonstrated that ICIs accelerated the progression of aortic atherosclerotic plaques, which could be partly attenuated by concomitant usage of statins or corticosteroids. Although this was a retrospective study in which baseline characteristics were not always comparable between treatment groups, these data indicate that ICIs also have relevant proatherogenic effects in the clinical situation. As atherosclerosis is a chronic process leading to complications in the longer term, larger clinical studies in patients receiving ICIs with longer follow-up periods are required. In this way, the potential preventive effects of statins and/or corticosteroids can additionally be investigated. Given that the indications for ICIs are expanding towards the adjuvant setting to prevent metastatic spread and recurrence of cancer, which is expected to lead to prolonged survival outcomes,

better characterization and prevention of these long-term adverse events will become increasingly relevant.

Combined use of VEGFIs and ICIs

Rationale for combined VEGFI and ICI therapy

Unfortunately, an effective anti-tumour response to ICI therapy is not observed in all patients, and a significant proportion of patients demonstrate secondary treatment resistance. To overcome this lack of responsiveness, novel combinatory approaches of ICIs with alternative anti-neoplastic drugs are currently developed. A promising strategy is the combination of ICIs with VEGFIs: mechanistic and clinical studies suggest that these drugs act synergistically to yield superior anti-tumour responses. Next to its role in angiogenesis, VEGF plays an important role in the establishment of an immunosuppressive tumour microenvironment, which impairs anti-tumour immune response via several mechanisms.[13,26,104,105] Firstly, VEGF exerts inhibitory effects on immunostimulatory and immune effector cells, including suppression of dendritic cell maturation and induction of exhaustion and apoptosis of cytotoxic T cells. Secondly, intra-tumoural VEGF promotes the recruitment and activity of immunosuppressive cells, including regulatory T cells (T_{regs}), myeloid-derived suppressor cells and M2-like tumour-associated macrophages. Thirdly, VEGF can create a selective endothelial barrier for cytotoxic T cells by altering the expression of endothelial adhesion molecules (VCAM-1, ICAM-1) and upregulation of immune checkpoints, while still allowing trafficking of immunosuppressive T_{regs}. This selective barrier to infiltration has been referred to as tumour endothelial cell anergy.[15] Fourthly, excessive VEGF production by tumour cells stimulates the formation of a malformed and malfunctional tumour vasculature, characterized by a leaky nature and loose pericyte coverage. Consequently, these abnormal vessels can restrict the entry of cytotoxic drugs and, in the case of ICI therapy, reduced infiltration by anti-tumour immune cells. Targeting these VEGF-induced immunosuppressive mechanisms by administration of VEGFIs promotes an immunosupportive tumour microenvironment, which can greatly potentiate the efficacy of ICI therapy.[104] In turn, T cells activated by ICIs contribute to an immunosupportive tumour microenvironment by secretion of interferon-gamma (IFNγ), establishing a feed-forward loop by further normalising the tumour vasculature (**Figure 3**).[13]

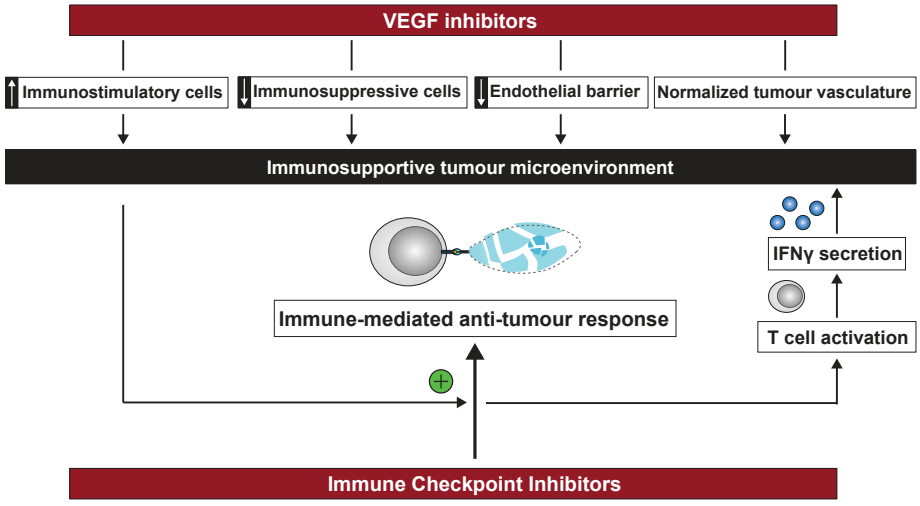

Figure 3 Hypothesised mechanisms leading to synergistic anti-tumour effects of VEGFIs and ICIs
Next to direct anti-tumour effects by targeting angiogenesis, VEGFIs can contribute to an immunosupportive tumour microenvironment (TME) via several mechanisms, including activating effects on immunostimulatory immune cells and inhibitory effects on immunosuppressive immune cells. Also, VEGFIs can decrease selective endothelial barriers for anti-tumour immune cells and normalize the tumour vasculature, allowing their infiltration into tumour tissues. Consequently, this immuunsupportive TME greatly facilitates the anti-tumour effects of T cells, which are activated by ICI therapy. These activated T cells secrete interferon-gamma (IFNγ), which further contributes to the immuunsupportive TME by promotion of vascular normalization, establishing a feed forward loop.[13,104]

The synergistic anti-tumour effects of a combinatory VEGFI/ICI treatment approach have been verified in the clinical situation, displayed by promising results in various tumour types, including RCC and non-small cell lung cancer.[16,106,107] Recently, VEGFI/ICI combination therapy was recommended as first-line therapy for advanced clear cell RCC by the ESMO.[14] Although RCC is currently the only tumour type for which VEGFI/ICI combination therapy has officially been approved, the treatment efficacy of concomitant VEGFI/ICI administration is under investigation in multiple other cancer types, including non-small cell lung cancer, with many studies demonstrating encouraging results.[16,107,108] Therefore, apart from promising combinatory approaches of ICIs with other anti-cancer agents (including conventional chemotherapy, radiotherapy, BRAF/MEK inhibitors and Poly ADP ribose polymerase (PARP) inhibitors) it is to be expected that VEGFI/ICI combination therapy will become an important treatment strategy for various malignancies.[27,109] In the next section, we will speculate on the potential synergistic cardiovascular toxic effects of this combinatory treatment regime and its therapeutic implications.

Combinatory VEGFI and ICI therapy approach: increased cardiovascular risk?

Currently, the cardiovascular safety profile of combined VEGFI and ICI therapy is unclear, but particular caution is warranted as these agents predispose to cardiovascular toxic-

ity via different mechanisms. First, hypertension seems to predispose to ICI-induced vascular toxicity; in a study including 1,215 patients who received ICI monotherapy or in combination with conventional chemotherapy, hypertensive individuals had a higher chance of developing adverse vascular events (hazard ratio 3.2).[110] Although ICIs do not seem to have direct prohypertensive effects, concomitant administration of VEGFIs causes hypertension in a substantial proportion of patients. For instance, atezolizumab-bevacizumab combination therapy in patients with hepatocellular carcinoma led to any-grade and high-grade (≥3) hypertension in 29.8% and 15.2% of patients, respectively.[111] Moreover, a retrospective clinical trial in lung cancer patients receiving ICIs demonstrated that previous or concomitant VEGFI or TKI therapy was associated with an increased risk for developing major adverse cardiovascular events (hazard ratio: 2.2).[112] Therefore, the prohypertensive effects of VEGFI could synergize with the cardiovascular toxicity of ICIs, although the underlying mechanisms have not been investigated. Most likely, VEGFI-induced hypertension and endothelial damage promote the development of heart failure and atherosclerotic-related cardiovascular events. Also, preclinical models suggest that VEGFIs can promote atherogenesis independent of their prohypertensive effects.[113] This could act in concert with the expected proatherogenic effects of ICI therapy to induce cardiovascular toxicity.[11] **Figure 4** depicts the main cardiovascular toxicity associated with VEGFI and ICI therapy.

As VEGFI/ICI combination therapies are expected to lead to prolonged survival outcomes in many types of cancer, the long-term (atherosclerotic) cardiovascular sequelae

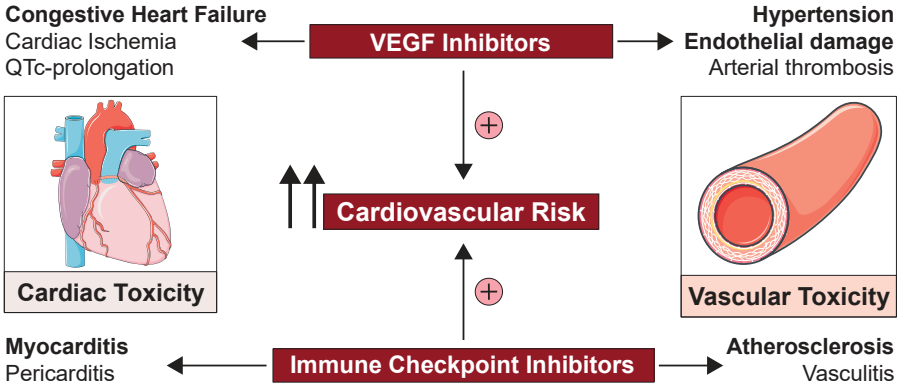

Figure 4 Cardiovascular toxicity associated with VEGFs and ICIs

VEGFIs and ICIs can both lead to specific cardiovascular toxicity, including congestive heart failure, cardiac ischemia, QTc-prolongation, myocarditis, pericarditis, hypertension, acceleration of atherosclerosis, arterial thrombosis and vasculitis. In patients receiving VEGFI/ICI combination therapy, the cardiovascular toxic potentials of each type of treatment are likely to synergize, which greatly enhances cardiovascular risk and predisposes to serious adverse cardiovascular events. For instance, hypertension, which occurs in a substantial proportion of patient receiving VEGFI therapy, seems to be a risk factor for ICI-induced vascular events [110]. Given that significant improvements in survival outcome during VEGFI/ICI combination therapy is expected, long-term adverse cardiovascular sequelae will become increasingly relevant, including atherosclerotic cardiovascular disease.

will become more relevant. Therefore, especially long-term effects of VEGFI/ICI combination regimens need to be observed closely the coming years. Follow-up data from ongoing clinical trials investigating the efficacy of VEGFI/ICI combination therapy will also provide more information about the short- and long-term cardiovascular safety in patients with cancer. Next to VEGFIs, other anti-cancer agents with cardiovascular side effects, such as BRAF/MEK inhibitors or PARP inhibitors, might also gain approval for combinatory administration with ICIs.[109,114,115] Hence, future studies should also focus on the cardiovascular safety of these alternative combination regimens. However, tot review and speculate on the cardiovascular toxicity profiles of all these possible future combinatory approaches with ICIs is beyond the scope of this review.

Clinical implications and future directions

The ESMO, American Society of Clinical Oncology (ASCO) and European Society of Cardiology (ESC) have provided recommendations for the monitoring and treatment cardiovascular toxicity associated with anti-cancer therapies, including VEGFIs and ICIs.[80,83,90] Most of these recommendations are based on expert opinion, due to the absence of prospective clinical evidence. The ESMO, ASCO and ESC guidelines recommend that every patient who will receive anti-cancer therapy with potentially cardiovascular toxic effects should undergo a careful baseline assessment for cardiotoxicity risk stratification.[80,83,90] This baseline assessment should at least include: (1) obtaining a medical history focused on cardiovascular events, previous cancer therapy and cardiovascular risk factors; (2) measurement of blood pressure, blood glucose, and cholesterol levels; (3) measurement of troponin and BNP or NT-proBNP levels; and (4) performing baseline ECG and echocardiography.[80] Also, the type and dosage of anti-cancer therapy should be taken into account.[116,117] This risk stratification aims to select patients at the highest risk to develop cardiovascular toxicity. Usually, this assessment can be done by the oncology care team, but referral to a cardiologist is recommended for patients at the highest risk.[56] The intensity of monitoring for cardiovascular toxicity during VEGFI and ICI treatment will depend on the established cardiovascular risk at baseline.[79,82] It has been proposed that regular measurements of troponin levels at baseline and each ICI treatment cycle might be of additional value.[8] A recent study involving 35 patients with ICI-associated myocarditis demonstrated that 94% had elevated levels of troponin. Nonetheless, elevated troponin levels are not specific for myocarditis, nor for an acute coronary syndrome.[118] Therefore, in most patients, measurement of troponin levels and further diagnostic testing should only be performed in case of symptoms and clinical suspicion of cardiovascular toxicity.[82] This is also in line with recommendations from the ESMO, ASCO and ESC, which suggest performing an ECG and measuring troponin levels at baseline, particularly in patients treated with ICI combination therapy, and to consult a cardiology specialist and perform further diagnostic testing only in the case of symptoms.[82,91]

Routine measurements of troponin and NT-proBNP levels, particularly during the first treatment cycles, should be restricted to the patients at the highest risk.[82,90]

When VEGFIs are to be administered, adequate blood pressure control and screening for hypertensive end-organ damage at baseline is recommended.[30,57] Also, regular monitoring of blood pressure and stringent blood pressure targets for patients receiving VEGFIs have been proposed.[4,29] Therefore, blood pressure values should ideally be <130/80 mmHg, but at least <140/90 mmHg prior to initiation of VEGFI therapy, in line with recommendations from the ESC and the National Cancer Institute's Drug Steering Committee.[30,57] Fortunately, the number of patients who require dose adjustment or treatment discontinuation due to severe VEGFI-induced cardiovascular toxicity is limited. Modest increases in blood pressure during VEGFI can be acceptable, particularly in the metastatic setting. However, substantial rapid increases in blood pressure require prompt intervention as they can lead to acute hypertensive complications and potentiate cardiac toxicity.[4]

The choice of anti-hypertensive therapy is predominantly based on relevant patient comorbidities, expert opinion and clinical experience, given that clinical evidence supporting the use of one anti-hypertensive agent over another is currently lacking.[57,119] Preclinical studies in rats receiving the VEGF-TKI sunitinib or cediranib suggest that calcium channel blockers (CCBs) are more effective than angiotensin-converting enzyme inhibitors (ACEI) to treat VEGFI-induced hypertension,[46] particularly in cases of severe increases in blood pressure (35-50mmHg.[120] Clinically, CCBs are first choice anti-hypertensive treatments for VEGFI-induced hypertension, whereas ACEI or angiotensin receptor blockers (ARB) are first choice in case of concomitant proteinuria, kidney disease and/or left ventricular systolic dysfunction.[57,121] Adequate blood pressure control will become even more important in case of VEGFI/ICI combination therapy, given that hypertension can potentiate atherosclerosis and other ICI-induced adverse vascular events.[110] Notably, non-dihydropyridine CCBs (verapamil, diltiazem) should not be used in combination with most VEGFIs, as they can lead to greatly elevated VEGFI concentrations by inhibition of CYP3A4, the main VEGFI-metabolizing enzyme.

Routine measurement of troponin during VEGFI therapy is currently not recommended. NT-proBNP should be measured every 3 months during treatment, however patients at highest risk for cardiotoxicity should receive measurement at an earlier timepoint (e.g., after 2-4 weeks of treatment initiation).[82] The ESC states that echocardiography should be considered every 4 months during treatment, with an additional early assessment 2-4 weeks after starting treatment in patients with the highest cardiovascular risk.[79] In this way, prompt intervention can be initiated in case cardiac toxicity occurs, either in the form of conventional anti-hypertensive drugs and/or (temporary) withdrawal of VEGFI therapy to prevent permanent myocardial damage.[122,123]

In addition to pharmacological interventions, lifestyle recommendations, including the stimulation of a healthy balanced diet and sufficient physical activity, are indispensable for optimizing cardiovascular health in cancer patients receiving cardiotoxic anti-cancer therapy. This is particularly important for patients who receive anti-cancer therapy in the curative setting or when prolonged survival is expected (e.g., in case of VEGFI/ICI combination therapy). Given the hypothesised roles of VEGFI and ICI therapy in promoting atherosclerosis and the clear association of ICIs with atherosclerosis-related cardiovascular events,[11] we speculate that low thresholds for the initiation of cholesterol-lowering drugs, including statins, are justifiable.

Although most of the therapeutic approaches for the main cardiovascular adverse events associated with VEGFI and ICI therapy have yet to be verified in (prospective) clinical trials, these hypothetical treatment options are summarized in **Table 3**.

Ongoing and future clinical trials should further focus on the long-term cardiovascular toxicity of combined use of VEGFIs and ICIs. In particular, the clinical reevance of their established proatherogenic effects and on additional ways to prevent the development of atherosclerosis should be studied. An interesting candidate for future investigation could be aspirin, which is currently used as secondary prevention for cardiovascular events since its use in primary prevention did not seem to have a positive risk benefit ratio.[124] However, given the previously observed beneficial effects of aspirin on VEGFI-induced hypertension and renal toxicity in a preclinical study,[50] it might also be of use to ameliorate cardiovascular toxicity during VEGFI/ICI combination therapy. In line with preeclampsia, a dose of 160 mg could be beneficial, but both the exact timing and dosing need to be explored. The clinical benefit of aspirin for the prevention of other VEGFI-induced adverse events, including thrombosis, remains unclear due to low reported numbers.[59] Of note, careful monitoring of the occurrence of bleeding events is important when aspirin is added. Thrombocytopenia is a frequently reported side-effect of various VEGFIs and sunitinib and sorafenib can exert inhibitory effects on platelets in a dose-dependent manner.[67] A previous study demonstrated that patients receiving bevacizumab had a slightly elevated risk of bleeding events, compared to the control group (1.9% versus 1.2%).[64] However, these bleeding events were mainly confined to grade 1-2 epistaxis episodes and aspirin usage did not further increase this risk.[125] Interestingly, preclinical observations demonstrate that aspirin can synergize with anti-PD-1 treatment by inhibition of prostaglandin E2 [126] and a recent observational clinical study demonstrated that aspirin treatment was associated with increased objective responses rates in patients treated with PD-1/PD-L1 checkpoint inhibitors.[127] Therefore, future studies should also further investigate if aspirin can further potentiate the anti-tumour efficacy of VEGFI/ICI combination therapies.

Table 3 Putative medical treatment options for the main cardiovascular toxic effects of VEGFIs and ICIs

Drug class	Toxicity	Treatment option(s)	Mechanisms	Remarks
VEGFIs	Hypertension	CCBs	Decrease in systemic vascular resistance by induction vascular smooth muscle cell relaxation	Preclinical [46] and clinical evidence [120] demonstrate therapeutic efficacy Non-dihydropyridine CCBs (verapamil, diltiazem) should be avoided due to cytochrome P450 3A4 inhibition [29,121]
		ACEIs/ARBs	Inhibition of renin-angiotensin-aldosterone signaling	First choice in case of concomitant proteinuria [29,121]
		ET-1 receptor antagonists	Target the upregulation of ET-1 during VEGFI therapy [38,42]	Not currently registered for treatment of systemic hypertension
		Salt restriction	Targets the salt-sensitivity of VEGFI-induced blood pressure rise [47]	Subject of current investigation (Netherlands Trial Register NL7340)
		Aspirin	Targets proposed contributing role of COX signaling to VEGFI-induced hypertension [50]	Preventive effects of COX-1 versus COX-2 inhibition needs further investigation
	Arterial thrombosis	Aspirin	Impaired platelet aggregation	Anti-trombotic effects need to be balanced against possible increased risks of bleeding
	Cardiac toxicity	Anti-hypertensive drugs	Prevention of hypertension as a risk factor for VEGFI-induced cardiac toxicity [21]	The blockers carvedilol and nebivolol could be particularly effective due to an additional increase in NO bioavailability [4]
ICIs	Myocarditis	Corticosteroids	Anti-inflammatory effects	Indicated for ICI-induced myocarditits grade ≥2 [3, 90]
		Immunomodulatory drugs (e.g., infliximab, tacrolimus, mycophenolate, anti-thymocyte globulin, intravenous immunoglobulins)	Anti-inflammatory effects	Add on therapy in situations where the clinical response to corticosteroids is insufficient [90-92]
	Vasculitis	Corticosteroids	Anti-inflammatory effects	-
	Atherosclerosis	Statins	Direct anti-atherogenic effects	-
		Corticosteroids	Reduction in proatherogenic inflammation [11]	Preventive effects largely speculative
		Aspirin	Impaired platelet aggregation and reduction in proatherogenic inflammation	Preventive effects largely speculative
		Anti-hypertensive drugs	Decreased atherosclerotic progression via normalization of blood pressure	-

For most suggested treatment options, clinical trials for this specific indication have not been performed, Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CCB, calcium channel blockers; COX, cyclooxygenase; ET-1, endothelin-1, NO, nitric oxide; VEGF, vascular endothelial growth factor; VEGFI, vascular endothelial growth factor inhibitor.

CONCLUSIONS

The development of novel anti-cancer therapies has substantially improved treatment outcomes for a wide range of malignancies. Although VEGFI and ICI therapy have revolutionized the therapeutic equipment for many types of cancer, both types of agents can lead to adverse effects on the cardiovascular system. VEGFIs most often cause hypertension, arterial thrombosis and congestive heart failure, whereas ICIs are associated with characteristic irAEs that can involve the cardiovascular system, leading to myocarditis, vasculitis, acceleration of atherosclerosis, and other cardiovascular toxicity in rare cases. Therapeutic regimens combining VEGFIs and ICIs have shown promising synergistic anti-tumour responses and are likely to become essential for the treatment of multiple types of cancer. It is to be expected that the superior anti-cancer activity of their combined administration will be mirrored by a substantial increase in adverse cardiovascular events. Future studies should investigate if VEGFI/ICI combination therapy further increases the cardiovascular risk and improved evidence-based clinical guidelines about the screening, monitoring and treatment of adverse cardiovascular effects induced by these agents are highly warranted. A baseline risk stratification for treatment-induced cardiotoxicity and adequate monitoring for the occurrence of cardiovascular events during and after treatment is essential, as early detection facilitates timely intervention. Of note, ICI therapy could also predispose cancer patients to cardiovascular disease in the long term by promotion of inflammation-induced atherosclerotic burden. Given that the indications for ICI therapy are expanding towards the adjuvant setting, prevention of these long-term cardiovascular effects is expected to become an integral part of cardio-oncology care. In this context, statins or aspirin could be interesting candidates for further investigation. Eventually, a multi-disciplinary cardio-oncology approach will optimize cardiovascular health in cancer patients, enabling them to optimally benefit from the unprecedented advances in the field of oncology.

Acknowledgements

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Abbreviations

ACEI, angiotensin-converting enzyme inhibitor; ASCO, American Society of Clinical Oncology; ATE, arterial thromboembolic event; CCB, calcium channel blocker; COX, cyclooxygenase; CTLA-4, cytotoxic T lymphocyte antigen-4; ECG, electrocardiography; ESC, European Society of Cardiology; ESMO, European Society of Medical Oncology; GCA, giant cell arteritis; ICAM-1, intercellular adhesion molecule-1; ICI, immune checkpoint inhibitor; irAE, immune-related ad- verse event; LDL, low-density lipoprotein; LVEF,

left ventricular ejection fraction; mAb, monoclonal antibody; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PAI-1, plasminogen activator inhibitor-1; PARP, poly-ADP ribose polymerase; PD-1, programmed death-1; PD-L1, programmed death ligand-1; PGI_2, prostacyclin; RCC, renal cell carcinoma; ROS, reac- tive oxygen species; TKI, tyrosine kinase inhibitor; T_{reg}, regulatory T cell; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor; VEGFI, VEGF inhibitor; VEGFR, VEGF receptor; VTE, venous thromboembolic event.

REFERENCES

1. Cook KM, Figg WD. Angiogenesis inhibitors: current strategies and future prospects. *CA Cancer J Clin.* 2010;60(4):222-243.
2. Lee L, Gupta M, Sahasranaman S. Immune Checkpoint inhibitors: An introduction to the next-generation cancer immunotherapy. *J Clin Pharmacol.* 2016;56(2):157-169.
3. Ball S, Ghosh RK, Wongsaengsak S, et al. Cardiovascular Toxicities of Immune Checkpoint Inhibitors: JACC Review Topic of the Week. *J Am Coll Cardiol.* 2019;74(13):1714-1727.
4. Touyz RM, Herrmann SMS, Herrmann J. Vascular toxicities with VEGF inhibitor therapies-focus on hypertension and arterial thrombotic events. *J Am Soc Hypertens.* 2018;12(6):409-425.
5. Chu TF, Rupnick MA, Kerkela R, et al. Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. *Lancet (London, England).* 2007;370(9604):2011-2019.
6. Postow MA, Sidlow R, Hellmann MD. Immune-Related Adverse Events Associated with Immune Checkpoint Blockade. *N Engl J Med.* 2018;378(2):158-168.
7. Hu JR, Florido R, Lipson EJ, et al. Cardiovascular toxicities associated with immune checkpoint inhibitors. *Cardiovasc Res.* 2019;115(5):854-868.
8. Mahmood SS, Fradley MG, Cohen JV, et al. Myocarditis in Patients Treated With Immune Checkpoint Inhibitors. *J Am Coll Cardiol.* 2018;71(16):1755-1764.
9. Johnson DB, Balko JM, Compton ML, et al. Fulminant Myocarditis with Combination Immune Checkpoint Blockade. *N Engl J Med.* 2016;375(18):1749-1755.
10. Salem JE, Manouchehri A, Moey M, et al. Cardiovascular toxicities associated with immune checkpoint inhibitors: an observational, retrospective, pharmacovigilance study. *Lancet Oncol.* 2018;19(12):1579-1589.
11. Drobni ZD, Alvi RM, Taron J, et al. Association Between Immune Checkpoint Inhibitors With Cardiovascular Events and Atherosclerotic Plaque. *Circulation.* 2020;142(24):2299-2311.
12. Vaddepally RK, Kharel P, Pandey R, Garje R, Chandra AB. Review of Indications of FDA-Approved Immune Checkpoint Inhibitors per NCCN Guidelines with the Level of Evidence. *Cancers (Basel).* 2020;12(3).
13. Yi M, Jiao D, Qin S, Chu Q, Wu K, Li A. Synergistic effect of immune checkpoint blockade and anti-angiogenesis in cancer treatment. *Mol Cancer.* 2019;18(1):60.
14. Powles T. Recent eUpdate to the ESMO Clinical Practice Guidelines on renal cell carcinoma on cabozantinib and nivolumab for first-line clear cell renal cancer: Renal cell carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2020.
15. Huinen ZR, Huijbers EJM, van Beijnum JR, Nowak-Sliwinska P, Griffioen AW. Anti-angiogenic agents - overcoming tumour endothelial cell anergy and improving immunotherapy outcomes. *Nat Rev Clin Oncol.* 2021.
16. Reck M, Mok TSK, Nishio M, et al. Atezolizumab plus bevacizumab and chemotherapy in non-small-cell lung cancer (IMpower150): key subgroup analyses of patients with EGFR mutations or baseline liver metastases in a randomised, open-label phase 3 trial. *Lancet Respir Med.* 2019;7(5):387-401.
17. Versmissen J, Power JR, Moslehi J. Vascular medicine and cardio-oncology - A new, evolving clinical frontier. *Vasc Med.* 2020;25(3):205-207.
18. Moslehi JJ. Cardiovascular Toxic Effects of Targeted Cancer Therapies. *N Engl J Med.* 2016;375(15):1457-1467.
19. Carmeliet P. VEGF as a key mediator of angiogenesis in cancer. *Oncology.* 2005;69 Suppl 3:4-10.

20. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. *Endocr Rev.* 2004;25(4):581-611.
21. Touyz RM, Herrmann J. Cardiotoxicity with vascular endothelial growth factor inhibitor therapy. *NPJ Precis Oncol.* 2018;2:13.
22. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med.* 1971;285(21):1182-1186.
23. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med.* 2004;350(23):2335-2342.
24. Kim Y, Lin Q, Glazer PM, Yun Z. Hypoxic tumor microenvironment and cancer cell differentiation. *Curr Mol Med.* 2009;9(4):425-434.
25. Jain RK. Antiangiogenesis strategies revisited: from starving tumors to alleviating hypoxia. *Cancer Cell.* 2014;26(5):605-622.
26. Fukumura D, Kloepper J, Amoozgar Z, Duda DG, Jain RK. Enhancing cancer immunotherapy using antiangiogenics: opportunities and challenges. *Nat Rev Clin Oncol.* 2018;15(5):325-340.
27. Neves KB, Montezano AC, Lang NN, Touyz RM. Vascular toxicity associated with anti-angiogenic drugs. *Clin Sci (Lond).* 2020;134(18):2503-2520.
28. Simons M, Gordon E, Claesson-Welsh L. Mechanisms and regulation of endothelial VEGF receptor signalling. *Nature reviews. Molecular cell biology.* 2016;17(10):611-625.
29. van Dorst DCH, Dobbin SJH, Neves KB, et al. Hypertension and Prohypertensive Antineoplastic Therapies in Cancer Patients. *Circ Res.* 2021;128(7):1040-1061.
30. Zamorano JL, Lancellotti P, Rodriguez Muñoz D, et al. 2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines: The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). *Eur Heart J.* 2016;37(36):2768-2801.
31. Li W, Croce K, Steensma DP, McDermott DF, Ben-Yehuda O, Moslehi J. Vascular and Metabolic Implications of Novel Targeted Cancer Therapies: Focus on Kinase Inhibitors. *J Am Coll Cardiol.* 2015;66(10):1160-1178.
32. Maitland ML, Kasza KE, Karrison T, et al. Ambulatory monitoring detects sorafenib-induced blood pressure elevations on the first day of treatment. *Clin Cancer Res.* 2009;15(19):6250-6257.
33. Pandey AK, Singhi EK, Arroyo JP, et al. Mechanisms of VEGF (Vascular Endothelial Growth Factor) Inhibitor-Associated Hypertension and Vascular Disease. *Hypertension.* 2018;71(2):e1-e8.
34. Eechoute K, van der Veldt AA, Oosting S, et al. Polymorphisms in endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF) predict sunitinib-induced hypertension. *Clin Pharmacol Ther.* 2012;92(4):503-510.
35. Tlemsani C, Mir O, Boudou-Rouquette P, et al. Posterior reversible encephalopathy syndrome induced by anti-VEGF agents. *Target Oncol.* 2011;6(4):253-258.
36. Seet RC, Rabinstein AA. Clinical features and outcomes of posterior reversible encephalopathy syndrome following bevacizumab treatment. *Qjm.* 2012;105(1):69-75.
37. Versmissen J, Mirabito Colafella KM, Koolen SLW, Danser AHJ. Vascular Cardio-Oncology: Vascular Endothelial Growth Factor inhibitors and hypertension. *Cardiovasc Res.* 2019;115(5):904-914.
38. Kappers MH, van Esch JH, Sluiter W, Sleijfer S, Danser AH, van den Meiracker AH. Hypertension induced by the tyrosine kinase inhibitor sunitinib is associated with increased circulating endothelin-1 levels. *Hypertension.* 2010;56(4):675-681.
39. Touyz RM, Lang NN, Herrmann J, van den Meiracker AH, Danser AHJ. Recent Advances in Hypertension and Cardiovascular Toxicities With Vascular Endothelial Growth Factor Inhibition. *Hypertension.* 2017;70(2):220-226.

40. Neves KB, Rios FJ, Jones R, Evans TRJ, Montezano AC, Touyz RM. Microparticles from vascular endothelial growth factor pathway inhibitor-treated cancer patients mediate endothelial cell injury. *Cardiovasc Res.* 2019;115(5):978-988.
41. Neves KB, Rios FJ, van der Mey L, et al. VEGFR (Vascular Endothelial Growth Factor Receptor) Inhibition Induces Cardiovascular Damage via Redox-Sensitive Processes. *Hypertension.* 2018;71(4):638-647.
42. Kappers MH, Smedts FM, Horn T, et al. The vascular endothelial growth factor receptor inhibitor sunitinib causes a preeclampsia-like syndrome with activation of the endothelin system. *Hypertension.* 2011;58(2):295-302.
43. Mäki-Petäjä KM, McGeoch A, Yang LL, et al. Mechanisms Underlying Vascular Endothelial Growth Factor Receptor Inhibition-Induced Hypertension: The HYPAZ Trial. *Hypertension.* 2021:Hypertensionaha12016454.
44. Kappers MH, de Beer VJ, Zhou Z, et al. Sunitinib-induced systemic vasoconstriction in swine is endothelin mediated and does not involve nitric oxide or oxidative stress. *Hypertension.* 2012;59(1):151-157.
45. Izzedine H, Massard C, Spano JP, Goldwasser F, Khayat D, Soria JC. VEGF signalling inhibition-induced proteinuria: Mechanisms, significance and management. *Eur J Cancer.* 2010;46(2):439-448.
46. Lankhorst S, Kappers MH, van Esch JH, et al. Treatment of hypertension and renal injury induced by the angiogenesis inhibitor sunitinib: preclinical study. *Hypertension.* 2014;64(6):1282-1289.
47. Lankhorst S, Severs D, Marko L, et al. Salt Sensitivity of Angiogenesis Inhibition-Induced Blood Pressure Rise: Role of Interstitial Sodium Accumulation? *Hypertension.* 2017;69(5):919-926.
48. Levine RJ, Lam C, Qian C, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med.* 2006;355(10):992-1005.
49. Rolnik DL, Wright D, Poon LC, et al. Aspirin versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia. *N Engl J Med.* 2017;377(7):613-622.
50. Mirabito Colafella KM, Neves KB, Montezano AC, et al. Selective ETA versus dual ETA/B receptor blockade for the prevention of sunitinib-induced hypertension and albuminuria in WKY rats. *Cardiovasc Res.* 2019.
51. Rini BI, Cohen DP, Lu DR, et al. Hypertension as a biomarker of efficacy in patients with metastatic renal cell carcinoma treated with sunitinib. *J Natl Cancer Inst.* 2011;103(9):763-773.
52. George S, Reichardt P, Lechner T, Li S, Cohen DP, Demetri GD. Hypertension as a potential biomarker of efficacy in patients with gastrointestinal stromal tumor treated with sunitinib. *Ann Oncol.* 2012;23(12):3180-3187.
53. Hurwitz HI, Douglas PS, Middleton JP, et al. Analysis of early hypertension and clinical outcome with bevacizumab: results from seven phase III studies. *Oncologist.* 2013;18(3):273-280.
54. Duffaud F, Sleijfer S, Litière S, et al. Hypertension (HTN) as a potential biomarker of efficacy in pazopanib-treated patients with advanced non-adipocytic soft tissue sarcoma. A retrospective study based on European Organisation for Research and Treatment of Cancer (EORTC) 62043 and 62072 trials. *Eur J Cancer.* 2015;51(17):2615-2623.
55. Langenberg MH, van Herpen CM, De Bono J, et al. Effective strategies for management of hypertension after vascular endothelial growth factor signaling inhibition therapy: results from a phase II randomized, factorial, double-blind study of Cediranib in patients with advanced solid tumors. *J Clin Oncol.* 2009;27(36):6152-6159.
56. Zamorano JL, Lancellotti P, Rodriguez Munoz D, et al. 2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Prac-

tice Guidelines: The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). *Eur Heart J.* 2016;37(36):2768-2801.

57. Maitland ML, Bakris GL, Black HR, et al. Initial assessment, surveillance, and management of blood pressure in patients receiving vascular endothelial growth factor signaling pathway inhibitors. *J Natl Cancer Inst.* 2010;102(9):596-604.
58. Bick RL. Cancer-associated thrombosis. *N Engl J Med.* 2003;349(2):109-111.
59. Scappaticci FA, Skillings JR, Holden SN, et al. Arterial thromboembolic events in patients with metastatic carcinoma treated with chemotherapy and bevacizumab. *J Natl Cancer Inst.* 2007;99(16):1232-1239.
60. Choueiri TK, Schutz FA, Je Y, Rosenberg JE, Bellmunt J. Risk of arterial thromboembolic events with sunitinib and sorafenib: a systematic review and meta-analysis of clinical trials. *J Clin Oncol.* 2010;28(13):2280-2285.
61. Ranpura V, Hapani S, Chuang J, Wu S. Risk of cardiac ischemia and arterial thromboembolic events with the angiogenesis inhibitor bevacizumab in cancer patients: a meta-analysis of randomized controlled trials. *Acta Oncol.* 2010;49(3):287-297.
62. Nalluri SR, Chu D, Keresztes R, Zhu X, Wu S. Risk of venous thromboembolism with the angiogenesis inhibitor bevacizumab in cancer patients: a meta-analysis. *Jama.* 2008;300(19):2277-2285.
63. Van Cutsem E, Tabernero J, Lakomy R, et al. Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. *J Clin Oncol.* 2012;30(28):3499-3506.
64. Hurwitz HI, Saltz LB, Van Cutsem E, et al. Venous thromboembolic events with chemotherapy plus bevacizumab: a pooled analysis of patients in randomized phase II and III studies. *J Clin Oncol.* 2011;29(13):1757-1764.
65. Totzeck M, Mincu RI, Mrotzek S, Schadendorf D, Rassaf T. Cardiovascular diseases in patients receiving small molecules with anti-vascular endothelial growth factor activity: A meta-analysis of approximately 29,000 cancer patients. *Eur J Prev Cardiol.* 2018;25(5):482-494.
66. Abdel-Qadir H, Ethier JL, Lee DS, Thavendiranathan P, Amir E. Cardiovascular toxicity of angiogenesis inhibitors in treatment of malignancy: A systematic review and meta-analysis. *Cancer Treat Rev.* 2017;53:120-127.
67. Herrmann J. Vascular toxic effects of cancer therapies. *Nat Rev Cardiol.* 2020;17(8):503-522.
68. Tziros C, Freedman JE. The many antithrombotic actions of nitric oxide. *Curr Drug Targets.* 2006;7(10):1243-1251.
69. Neagoe PE, Lemieux C, Sirois MG. Vascular endothelial growth factor (VEGF)-A165-induced prostacyclin synthesis requires the activation of VEGF receptor-1 and -2 heterodimer. *J Biol Chem.* 2005;280(11):9904-9912.
70. Chen N, Ren M, Li R, et al. Bevacizumab promotes venous thromboembolism through the induction of PAI-1 in a mouse xenograft model of human lung carcinoma. *Mol Cancer.* 2015;14:140.
71. Meyer T, Robles-Carrillo L, Robson T, et al. Bevacizumab immune complexes activate platelets and induce thrombosis in FCGR2A transgenic mice. *J Thromb Haemost.* 2009;7(1):171-181.
72. Evans CE, Grover SP, Humphries J, et al. Antiangiogenic therapy inhibits venous thrombus resolution. *Arterioscler Thromb Vasc Biol.* 2014;34(3):565-570.
73. Dobbin SJH, Petrie MC, Myles RC, Touyz RM, Lang NN. Cardiotoxic effects of angiogenesis inhibitors. *Clin Sci (Lond).* 2021;135(1):71-100.
74. Izumiya Y, Shiojima I, Sato K, Sawyer DB, Colucci WS, Walsh K. Vascular endothelial growth factor blockade promotes the transition from compensatory cardiac hypertrophy to failure in response to pressure overload. *Hypertension.* 2006;47(5):887-893.

75. Narayan V, Keefe S, Haas N, et al. Prospective Evaluation of Sunitinib-Induced Cardiotoxicity in Patients with Metastatic Renal Cell Carcinoma. *Clin Cancer Res.* 2017;23(14):3601-3609.
76. Hall PS, Harshman LC, Srinivas S, Witteles RM. The frequency and severity of cardiovascular toxicity from targeted therapy in advanced renal cell carcinoma patients. *JACC.Heart failure.* 2013;1(1):72-78.
77. Choueiri TK, Mayer EL, Je Y, et al. Congestive heart failure risk in patients with breast cancer treated with bevacizumab. *J Clin Oncol.* 2011;29(6):632-638.
78. Kloth JS, Pagani A, Verboom MC, et al. Incidence and relevance of QTc-interval prolongation caused by tyrosine kinase inhibitors. *Br J Cancer.* 2015;112(6):1011-1016.
79. Čelutkienė J, Pudil R, López-Fernández T, et al. Role of cardiovascular imaging in cancer patients receiving cardiotoxic therapies: a position statement on behalf of the Heart Failure Association (HFA), the European Association of Cardiovascular Imaging (EACVI) and the Cardio-Oncology Council of the European Society of Cardiology (ESC). *Eur J Heart Fail.* 2020;22(9):1504-1524.
80. Lyon AR, Dent S, Stanway S, et al. Baseline cardiovascular risk assessment in cancer patients scheduled to receive cardiotoxic cancer therapies: a position statement and new risk assessment tools from the Cardio-Oncology Study Group of the Heart Failure Association of the European Society of Cardiology in collaboration with the International Cardio-Oncology Society. *Eur J Heart Fail.* 2020;22(11):1945-1960.
81. Anker MS, Hadzibegovic S, Lena A, et al. Recent advances in cardio-oncology: a report from the 'Heart Failure Association 2019 and World Congress on Acute Heart Failure 2019'. *ESC Heart Fail.* 2019;6(6):1140-1148.
82. Pudil R, Mueller C, Čelutkienė J, et al. Role of serum biomarkers in cancer patients receiving cardiotoxic cancer therapies: a position statement from the Cardio-Oncology Study Group of the Heart Failure Association and the Cardio-Oncology Council of the European Society of Cardiology. *Eur J Heart Fail.* 2020;22(11):1966-1983.
83. Curigliano G, Lenihan D, Fradley M, et al. Management of cardiac disease in cancer patients throughout oncological treatment: ESMO consensus recommendations. *Ann Oncol.* 2020;31(2):171-190.
84. Ugurel S, Rohmel J, Ascierto PA, et al. Survival of patients with advanced metastatic melanoma: the impact of novel therapies-update 2017. *Eur J Cancer.* 2017;83:247-257.
85. Ledford H, Else H, Warren M. Cancer immunologists scoop medicine Nobel prize. *Nature.* 2018;562(7725):20-21.
86. Zhang L, Reynolds KL, Lyon AR, Palaskas N, Neilan TG. The Evolving Immunotherapy Landscape and the Epidemiology, Diagnosis, and Management of Cardiotoxicity. *JACC: CardioOncology.*0(0).
87. Nishimura H, Okazaki T, Tanaka Y, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science (New York, N.Y.).* 2001;291(5502):319-322.
88. Ji C, Roy MD, Golas J, et al. Myocarditis in Cynomolgus Monkeys Following Treatment with Immune Checkpoint Inhibitors. *Clin Cancer Res.* 2019;25(15):4735-4748.
89. Bonaca MP, Olenchock BA, Salem JE, et al. Myocarditis in the Setting of Cancer Therapeutics: Proposed Case Definitions for Emerging Clinical Syndromes in Cardio-Oncology. *Circulation.* 2019;140(2):80-91.
90. Brahmer JR, Lacchetti C, Schneider BJ, et al. Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol.* 2018;36(17):1714-1768.

91. Palaskas N, Lopez-Mattei J, Durand JB, Iliescu C, Deswal A. Immune Checkpoint Inhibitor Myocarditis: Pathophysiological Characteristics, Diagnosis, and Treatment. *J Am Heart Assoc.* 2020;9(2):e013757.
92. Haanen J, Carbonnel F, Robert C, et al. Management of toxicities from immunotherapy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2017;28(suppl_4):iv119-iv142.
93. Daxini A, Cronin K, Sreih AG. Vasculitis associated with immune checkpoint inhibitors-a systematic review. *Clin Rheumatol.* 2018;37(9):2579-2584.
94. Watanabe R, Zhang H, Berry G, Goronzy JJ, Weyand CM. Immune checkpoint dysfunction in large and medium vessel vasculitis. *Am J Physiol Heart Circ Physiol.* 2017;312(5):H1052-h1059.
95. Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. *Nat Med.* 2011;17(11):1410-1422.
96. Leentjens J, Bekkering S, Joosten LAB, Netea MG, Burgner DP, Riksen NP. Trained Innate Immunity as a Novel Mechanism Linking Infection and the Development of Atherosclerosis. *Circ Res.* 2018;122(5):664-669.
97. Gistera A, Hansson GK. The immunology of atherosclerosis. *Nat Rev Nephrol.* 2017;13(6):368-380.
98. Libby P, Ridker PM, Hansson GK, Leducq Transatlantic Network on A. Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol.* 2009;54(23):2129-2138.
99. Ridker PM, Everett BM, Thuren T, et al. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N Engl J Med.* 2017;377(12):1119-1131.
100. Nidorf SM, Eikelboom JW, Budgeon CA, Thompson PL. Low-dose colchicine for secondary prevention of cardiovascular disease. *J Am Coll Cardiol.* 2013;61(4):404-410.
101. Nidorf SM, Fiolet ATL, Mosterd A, et al. Colchicine in Patients with Chronic Coronary Disease. *N Engl J Med.* 2020;383(19):1838-1847.
102. Kusters PJH, Lutgens E, Seijkens TTP. Exploring immune checkpoints as potential therapeutic targets in atherosclerosis. *Cardiovasc Res.* 2018;114(3):368-377.
103. Bu DX, Tarrio M, Maganto-Garcia E, et al. Impairment of the programmed cell death-1 pathway increases atherosclerotic lesion development and inflammation. *Arteriosclerosis, thrombosis, and vascular biology.* 2011;31(5):1100-1107.
104. Khan KA, Kerbel RS. Improving immunotherapy outcomes with anti-angiogenic treatments and vice versa. *Nat Rev Clin Oncol.* 2018;15(5):310-324.
105. Lee WS, Yang H, Chon HJ, Kim C. Combination of anti-angiogenic therapy and immune checkpoint blockade normalizes vascular-immune crosstalk to potentiate cancer immunity. *Exp Mol Med.* 2020;52(9):1475-1485.
106. Motzer RJ, Penkov K, Haanen J, et al. Avelumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. *N Engl J Med.* 2019;380(12):1103-1115.
107. Socinski MA, Jotte RM, Cappuzzo F, et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. *N Engl J Med.* 2018;378(24):2288-2301.
108. Song Y, Fu Y, Xie Q, Zhu B, Wang J, Zhang B. Anti-angiogenic Agents in Combination With Immune Checkpoint Inhibitors: A Promising Strategy for Cancer Treatment. *Front Immunol.* 2020;11:1956.
109. Meric-Bernstam F, Larkin J, Tabernero J, Bonini C. Enhancing anti-tumour efficacy with immunotherapy combinations. *The Lancet.*
110. Bar J, Markel G, Gottfried T, et al. Acute vascular events as a possibly related adverse event of immunotherapy: a single-institute retrospective study. *Eur J Cancer.* 2019;120:122-131.
111. Finn RS, Qin S, Ikeda M, et al. Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. *N Engl J Med.* 2020;382(20):1894-1905.

112. Chitturi KR, Xu J, Araujo-Gutierrez R, et al. Immune Checkpoint Inhibitor-Related Adverse Cardiovascular Events in Patients With Lung Cancer. *JACC: CardioOncology.* 2019;1(2):182-192.
113. Winnik S, Lohmann C, Siciliani G, et al. Systemic VEGF inhibition accelerates experimental atherosclerosis and disrupts endothelial homeostasis--implications for cardiovascular safety. *Int J Cardiol.* 2013;168(3):2453-2461.
114. Gutzmer R, Stroyakovskiy D, Gogas H, et al. Atezolizumab, vemurafenib, and cobimetinib as first-line treatment for unresectable advanced BRAF(V600) mutation-positive melanoma (IMspire150): primary analysis of the randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet (London, England).* 2020;395(10240):1835-1844.
115. Mincu RI, Mahabadi AA, Michel L, et al. Cardiovascular Adverse Events Associated With BRAF and MEK Inhibitors: A Systematic Review and Meta-analysis. *JAMA Netw Open.* 2019;2(8):e198890.
116. Teske AJ, Linschoten M, Kamphuis JAM, et al. Cardio-oncology: an overview on outpatient management and future developments. *Neth Heart J.* 2018;26(11):521-532.
117. Herrmann J, Lerman A, Sandhu NP, Villarraga HR, Mulvagh SL, Kohli M. Evaluation and management of patients with heart disease and cancer: cardio-oncology. *Mayo Clin Proc.* 2014;89(9):1287-1306.
118. Giannitsis E, Katus HA. Cardiac troponin level elevations not related to acute coronary syndromes. *Nat Rev Cardiol.* 2013;10(11):623-634.
119. Small HY, Montezano AC, Rios FJ, Savoia C, Touyz RM. Hypertension due to antiangiogenic cancer therapy with vascular endothelial growth factor inhibitors: understanding and managing a new syndrome. *Can J Cardiol.* 2014;30(5):534-543.
120. Curwen JO, Musgrove HL, Kendrew J, Richmond GH, Ogilvie DJ, Wedge SR. Inhibition of vascular endothelial growth factor-a signaling induces hypertension: examining the effect of cediranib (recentin; AZD2171) treatment on blood pressure in rat and the use of concomitant antihypertensive therapy. *Clin Cancer Res.* 2008;14(10):3124-3131.
121. Cohen JB, Geara AS, Hogan JJ, Townsend RR. Hypertension in Cancer Patients and Survivors: Epidemiology, Diagnosis, and Management. *JACC CardioOncol.* 2019;1(2):238-251.
122. Schmidinger M, Zielinski CC, Vogl UM, et al. Cardiac toxicity of sunitinib and sorafenib in patients with metastatic renal cell carcinoma. *J Clin Oncol.* 2008;26(32):5204-5212.
123. Dobbin SJH, Cameron AC, Petrie MC, Jones RJ, Touyz RM, Lang NN. Toxicity of cancer therapy: what the cardiologist needs to know about angiogenesis inhibitors. *Heart.* 2018;104(24):1995-2002.
124. Zheng SL, Roddick AJ. Association of Aspirin Use for Primary Prevention With Cardiovascular Events and Bleeding Events: A Systematic Review and Meta-analysis. *JAMA.* 2019;321(3):277-287.
125. Hurwitz H, Saini S. Bevacizumab in the treatment of metastatic colorectal cancer: safety profile and management of adverse events. *Semin Oncol.* 2006;33(5 Suppl 10):S26-34.
126. Zelenay S, van der Veen AG, Böttcher JP, et al. Cyclooxygenase-Dependent Tumor Growth through Evasion of Immunity. *Cell.* 2015;162(6):1257-1270.
127. Cortellini A, Tucci M, Adamo V, et al. Integrated analysis of concomitant medications and oncological outcomes from PD-1/PD-L1 checkpoint inhibitors in clinical practice. *J Immunother Cancer.* 2020;8(2).

CHAPTER 12

PREVENTION OF VASCULAR ENDOTHELIAL GROWTH FACTOR INHIBITOR-INDUCED HYPERTENSION BY DIETARY SODIUM RESTRICTION

Submitted

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ABSTRACT

Background

Vascular endothelial growth factor tyrosine kinase inhibitors (VEGFIs) are effective anticancer agents that often induce a rise in blood pressure. Considering that VEGFI-induced hypertension is sodium-sensitive, we assessed the efficacy and tolerability of a dietary sodium restriction (DSR) for the prevention of VEGFI-induced hypertension.

Methods

In this prospective clinical study, cancer patients who developed VEGFI-induced hypertension (defined as day mean >135/85 mmHg or a rise in systolic and/or diastolic blood pressure ≥20 mmHg) were treated with DSR (<4 g or 70 mmol per day). The primary endpoint of this intervention was to assess the difference in daytime mean arterial blood pressure (MAP) increase between a treatment cycle with and a treatment cycle without dietary sodium restriction. Blood pressure was measured via ambulatory blood pressure monitoring. The DSR was started one week prior to the planned second VGEFI treatment cycle for a period of 5-weeks. Additional plasma and urine samples were collected.

Results

The DSR was applied in sixteen patients. During the first VEGFI treatment cycle without DSR, daytime MAP increased by 15 mmHg (from 95 to 110 mmHg). During the subsequent treatment cycle with DSR, daytime MAP increased by 8 mm Hg from 94 to 102 mmHg. Therefore, DSR significantly reduced the increase in MAP by 7 mmHg (95% CI, 1.3 to 12.0; $P = 0.009$).

Conclusion

DSR is an effective intervention to prevent VEGFI-induced blood pressure rise. DSR therefore should be considered in case of VEGFI-induced blood pressure rise in daily oncology practice.

INTRODUCTION

Vascular endothelial growth factor tyrosine kinase inhibitors (VEGFIs) impair the formation of new blood vessels (neo-angiogenesis) required for growth and metastatic spread of malignant tumours. VEGFI such as cabozantinib, lenvatinib, pazopanib, regorafenib, sorafenib or sunitinib are since long part of regular cancer treatment, and have shown to improve clinical outcomes in renal cell carcinoma, hepatocellular carcinoma, gastrointestinal stromal tumour, some neuro-endocrine tumours and thyroid cancer.[1] Given that VEGFIs do not selectively inhibit neo-angiogenesis, but also affect the existing cardiovascular system, cardiovascular side effects such as -predominantly- hypertension are frequently observed. Hypertension is seen in 25-87% of VEGFI-treated patients, and is considered a biomarker of on-target inhibitory effects of VEGFI. Vascular nephropathy, characterized by proteinuria, is another well-known side-effect of VEGFIs.[2-4] The vascular nephropathy of VEGFI remarkably resemble that of preeclampsia, a complication of pregnancy caused by insufficient angiogenesis of the placenta.[5,6] As we previously showed, both syndromes share a similar pathophysiological pathway involving a rise in endothelin-1 (ET-1), a reduction in renin-angiotensin-aldosterone system (RAAS) activity and an imbalance of the cyclo-oxygenase (COX) products prostacyclin I_2 ($PGI_{2)}$) and thromboxane A_2 (TXA_2).[5-9] Hypertension and/or proteinuria often are dose-limiting toxicity of VEGFI, and frequently necessitate either the prescription of antihypertensive drugs or dose reductions, treatment interruption or early termination of VEGFI treatment.[10,11] Novel effective and easy to handle strategies to treat VEGFI-induced hypertension are, even today, still urgently needed. In preclinical studies in rats, VEGFI-induced hypertension has been demonstrated to be salt sensitive; the increase in blood pressure was higher in animals fed with a high salt diet compared to that in animals in response to a normal salt diet.[12,13] Endothelin-1 (ET-1) is believed to be involved in salt-sensitive hypertension.[14,15] Given these observations, dietary sodium restriction (DSR) is considered an easy-to-use intervention in case of VEGFI–induced hypertension. To proof this, we studied the effects of DSR on VEGFI blood pressure (BP) rise in patients treated with either cabozantinib, lenvatinib, pazopanib, regorafenib, sorafenib or sunitinib.[16]

METHODS

We conducted a prospective, single-center, open-label, intervention study at the Erasmus MC Cancer Institute Rotterdam, the Netherlands. The study was approved by the Medical Ethics Committee from the Erasmus University Medical Center (MEC 2018-155) and complies with the Declaration of Helsinki. The study was registered at the Dutch trial registry (NL7340).

Patients

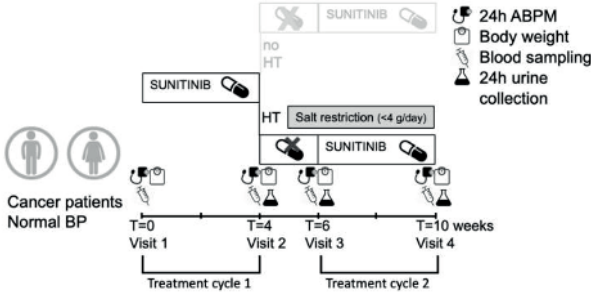
Patients aged ≥ 18 years were eligible if they received on-label treatment cycles of cabozantinib, lenvatinib, pazopanib, sorafenib (continuous dosing), regorafenib (3 weeks on, 1 week off), or sunitinib (continuous dosing or 4 weeks on, 2 weeks off). Patients were included before they started their VEGFI treatment. Exclusion criteria were: use of a diuretic or mineralocorticoid receptor antagonist at baseline and/or weight loss ≥10% in the last six months indicating undernutrition. All patients provided written informed consent prior to study inclusion.

Study design

The primary objective was to investigate if DSR could prevent or diminish the rise in blood pressure as a consequence of VEGFI treatment. Since the blood pressure rise in subsequent treatment cycles is usually of similar magnitude or larger, the rise in the treatment period with the intervention salt restriction was compared with the treatment cycle before the intervention.[6,17] In order to apply the intervention only to patients potentially benefiting most from it, only patients in whom a significant and clinical relevant increase in blood pressure following the first treatment cycle of their assigned VEGFI was observed, were selected. In these patients a 24-hour ambulatory blood pressure monitoring (ABPM) had revealed blood pressure values of ≥ 135/85 mmHg at the end of the first treatment cycle when they started normotensive (day mean <135/85 mmHg), or if they had developed an increase in systolic and/or diastolic blood pressure (SBP, DBP) of at least 20 mmHg during their first treatment cycle. Blood pressure was measured as day mean 24-hour ABPM. The primary outcome of this study was the difference in mean arterial blood pressure (MAP) rise without and with DSR. Secondary outcomes included differences in proteinuria in 24-hour urinary samples and differences in plasma ET-1 levels. The DSR was started one week prior to the planned second treatment cycle to allow for normalization of the blood pressure and to apply DSR during the entire treatment cycle.[17] This meant that for sunitinib the 4 weeks on, 2 weeks off treatment cycle was maintained. For regorafenib the standard rest period of one week was extended by a few days. For continuously applied cabozantinib, lenvatinib, pazopanib, sorafenib or sunitinib, the second treatment cycle was postponed for 1-1.5 weeks (**Figure 1**). Due to the COVID-19 pandemic, from May 2020 onwards home blood pressure measurements were allowed as replacement for 24-hour ABPM according to European Society of Hypertension practice guidelines and recommendations for patients using VEGFIs, as long as all measurements were performed using the same method (i.e., either all 24-hour ABPM or all home blood pressure measurements).[17,18]

Patients were referred to a dietician to be informed about the DSR for 4 (regorafenib) or 5 (all others) weeks according to Dutch guidelines as published previously.[19] In addition to dietary counseling, patients received salt-free bread for the whole intervention period.

A.



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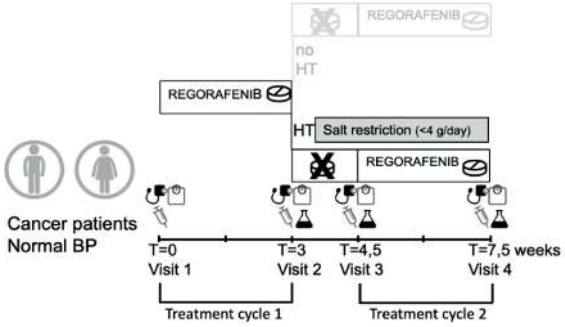

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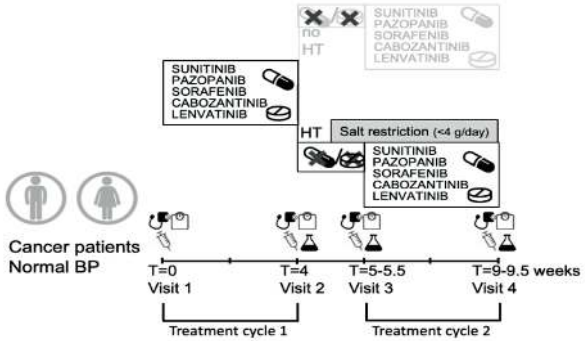

Figure 1. Study design

A. Sunitinib dosing scheme 4 weeks on, 2 weeks off; B. Regorafenib standard dosing scheme 3 weeks on, 1.5 weeks off; C. Continuous dosing of sunitinib, pazopanib, sorafenib, cabozantinib or lenvatinib. Measurements at time points: Visit 1 (baseline): body weight, 24-hour ambulatory blood pressure measurement (ABPM) (or home measurement). Blood: creatinine, sodium, potassium, aldosterone, renin, endothelin (ET-1). Visit 2, Visit 3, Visit 4: body weight, 24-hour ABPM (or home measurement); 24-hour urine: sodium, potassium, protein, creatinine; blood: creatinine, sodium, potassium, renin, aldosterone, ET-1. Visit 2 and Visit 4: trough drug level used VEGFI.

To increase adherence to the diet, patients were contacted by the dietician after one week and halfway through the intervention. In case severe and consistent hypertension occurred despite using DSR (SBP >150 or DBP >95 mmHg three times at home measurement), escape antihypertensive medication was prescribed according to specific study scheme with limited or no direct effect on the renin-angiotensin-aldosterone system (RAAS). The first choice was amlodipine 5 or 10 mg once daily. If a patient was already using a calcium channel blocker, doxazosin 4 or 8 mg once daily could be used.

Measurements

Clinical paramenters (body weight, 24-hour ABPM daytime and overall mean of SBP and DBP based on a non-invasive continuous automatically measurement at home) and blood samples to determine creatinine, sodium, potassium, ET-1, renin, and aldosterone were collected at four time points: visit 1 (baseline, before VEGFI treatment was started), visit 2 (after 4 weeks of treatment and 3 weeks for regorafenib), visit 3 (1-1..5 weeksafter the first VEGFI treatment cycle) and visit 4 (after 4 weeks of treatment and 3 weeks for regorafenib. In addition, 24-hour urine samples (for creatinine, sodium, potassium, protein) were collected at visit 2, visit 3, and visit 4. At visit 1, which coincided with the start of VEGFI treatment when information about the treatment and the current study was provided, asking for 24-hour urine collection was considered too demanding. Drug through levels of the VEGFIs were collected at visit 2 and visit 4. (**Figure 1**).

All study measurements were combined with regular visits and blood sampling for clinical care.

Blood and urine samples were processed by the Department of Clinical Chemistry of the Erasmus MC.

Plasma levels of ET-1 (R&D systems, Minneapolis, USA), PGI2 (6-keto-PGF1α ELISA Kit ADI-900-004, Enzo Life Sciences), TXA2 (TXB2 ELISA Kit ADI-900-002, Enzo Life Sciences) were determined using a chemiluminescent enzyme-linked immunosorbent assay (ELISA). Plasma-renin was measured using a radioimmunometric assay (Cisbio, Saclay, France) and plasma aldosterone was measured by radioimmunoassay (Demeditec, Kiel, Germany), according to the manufacturer's instructions.

Statistical analysis

The blood pressure rise was calculated as the difference in daytime MAP between the end and the start of the VEGFI treatment cycle. Each patient was his/her own control. Aiming for a power of 80% and a one-sided alpha of 5%, 16 patients were required to detect a clinically relevant difference of 10 mmHg (given a standard deviation of 15 mmHg based on previous studies).[6] Since the aim was to show superiority of the dietary sodium restriction a one- sided alpha was chosen to limit the number of patients. Difference in sodium urine and the levels of ET-1 were compared using a paired *t*-test or a Wilcoxon

signed rank test in case of a non-normal distribution. The difference in in urine sodium between visit 2 and visit 4 was analyzed on normally distributed data using the Pearson correlation coefficient. All main endpoints were analyzed according to the intention-to-treat principle. The patient characteristics were described with descriptive statistics. For the biochemical measurements, data were logarithmically transformed before analysis in case of non-normal distribution. Data were analyzed using SPSS Statistics (IBM, version 25.0). $P < 0.05$ was considered statistically significant.

RESULTS

Patients

Patients were recruited between October 2018 and August 2021. Forty-nine patients were screened of which 29 did not meet the inclusion criteria; 15 patients discontinued VEGFI during their first treatment cycle and 14 patients did not develop hypertension as defined in the inclusion criteria. Twenty patients in whom hypertension as defined for inclusion were considered eligible. Four patients were not evaluable for follow up due to various reasons (e.g., non-blood pressure related toxicity, progressive disease) (**Figure 2**).

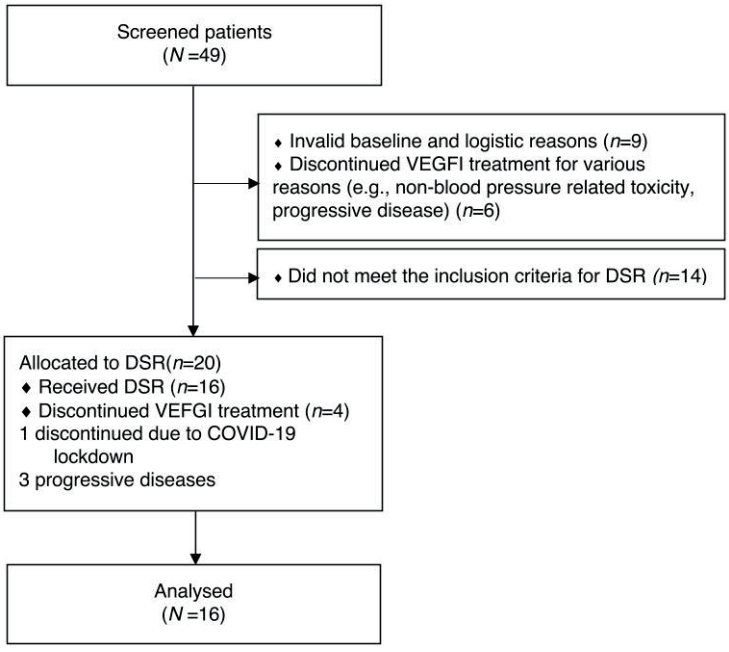

Figure 2. Flow diagram
DSR, dietary sodium restriction; VEGFI, vascular endothelial growth factor inhibitor.

Therefore, ultimately 16 patients were evaluable for the analysis. Patient characteristics are summarized in **Table 1**. The mean age was 65.4 ± 8.8 and 69% were men. Three patients (19 %) had a history of hypertension and were taking antihypertensive drugs prior to treatment cycle one. Before initiation of VEGFI treatment, mean SBP was 129±18 and mean DBP was 78±7 mmHg (24-hour ABPM, n = 12; home measurements, n = 4), respectively.

Table 1. Baseline characteristics of study participants

Characteristics	N=16
Men	11 (69%)
Age, years	65.4 ± 8.8
Hypertension	3 (19%)
Number of antihypertensive medications	0.9 ± 0.4
Angiotensin-converting enzyme inhibitor	1 (6%)
Calcium channel blocker	1 (6%)
ß-blocker	1 (6%)
Body mass index (kg/m^2)	25.5 ± 3.9
eGFR (ml/min, 1.73 m^2)	74.6 ± 18.6
Ambulatory 24-hour daytime BP or home measurements	
Systolic blood pressure (mmHg)	129.3 ± 17.7
Diastolic blood pressure (mmHg)	78.1 ± 7.1
Proteinuria (qualitative measurement)	
Yes	3 (19%)
No	11(68%)
Not available	2 (13%)
Type of treatment and daily dosis	
Cabozantinib	
40 mg	1 (6%)
60 mg	2 (13%)
80-20 mg*	1(6%)
Lenvatinib	
16 mg	1 (6%)
Pazopanib	
800 mg	1 (6%)
Regorafenib	
80 mg	1 (6%)
120 mg	2 (13%)
160 mg	1 (6%)
Sorafenib	
800 mg	1 (6%)

Table 1. Baseline characteristics of study participants (*continued*)

Characteristics	N=16
Sunitinib (4q2)	
50 mg	1 (6%)
37.5 mg	1 (6%)
Sunitinib continuous	
37.5 mg	3 (19%)
Cancer, diagnosis	
GIST	2 (13%)
HCC	4 (25%)
pNET	1 (6%)
RCC	7 (44%)
Thyroid carcinoma	2 (13%)

Data are presented as n (%) and mean ± SD. Abbreviations: 4q2, 4 weeks on 2 weeks off; BP, blood pressure; DBP diastolic blood pressure; eGFR estimated glomerular filtration rate; GIST, gastrointestinal stromal tumour; HCC, hepatocellular carcinoma; pNET, pancreas neuroendocrine tumour; RCC, renal cell carcinoma; SBP, systolic blood pressure. *cometriq

Effects on blood pressure of DSR

At visit 1, the daytime mean arterial pressure (MAP) was 95±10 mmHg, which rose by 15±8 mmHg to 110 mmHg at visit 2 (P = <0.001). At visit 3, the daytime MAP was 94±9 mmHg which rose by 8±4 mmHg to a daytime MAP of 102 mmHg at visit 4. Thus, DSR significantly reduced the VEGFI-induced rise in MAP by 7 mm Hg (95% CI, 1.3 to 12.0; P = 0.009) (**Figure 3**).

In a subgroup analysis of 12 patients in whom the DSR was the only intervention to control VEGFI-induced BP rise the daytime MAP was 18±6 mmHg at visit 2 versus 8 ±5mmHg at visit 4 indicated that DSR was successfully reduced the VEGFI-induced MAP rise by 10 mmHg. In 7 (44%) out of 16 patients, SBP increased to ≥170 mm Hg during the first VEGFI treatment cycle and escape medication was started. In 3 of these 7 patients the added antihypertensive treatment could be discontinued during the stop week, and DSR was sufficient in limiting the BP rise until the end of the study period. In 1 of these 3 patients a dose reduction of the VEGFI treatment was required because of mucositis. Four patients who were prescribed antihypertensive treatment during the first weeks of VEGFI treatment continued this during DSR. To illustrate the effect of DSR, these patients are described in detail in the **Supplementary Data**.

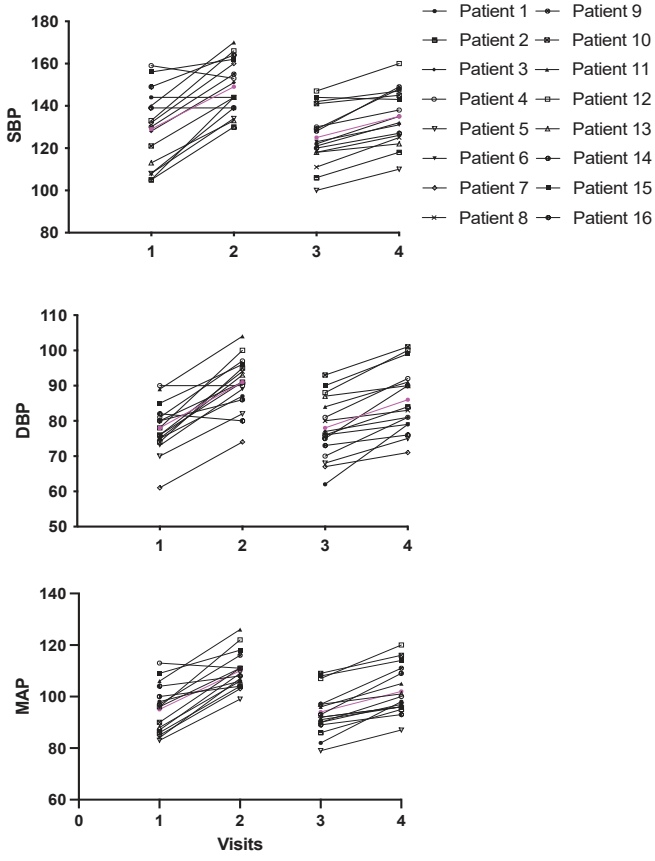

Figure 3. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) before and after treatment with the VEGF inhibitor without and with dietary sodium restriction (DSR).
Time points: 1: baseline; 2: end of treatment cycle 1; 3: baseline treatment cycle with DSR; 4: end of treatment cycle with DSR. In purple mean blood pressure values per visit. Blood pressure values of patients 13, 14, 15 and 16 are home measurements; all others are daytime mean of 24-hour ambulatory blood pressure measurement.

Sodium and protein excretion

Urine sodium excretion decreased from 94 (77-135) (median, IQR)) mmol/L/24-hour at visit 2 to 32 (24-49) mmol/L/24-hour at visit 4 (difference, 62 (53-86) mmol/L; $P < 0.001$). The response in urine sodium confirmed adherence to DSR in all patients, although for some patients it was not visible yet at visit 3, i.e., one week after start of the diet **(Table 2***)*. The difference in urine sodium between visit 2 and visit 4 did not correlate with the difference in rise in MAP between the two treatment cycles (r = - 0.2, P = 0.5). There was no significant effect of DSR on proteinuria, although in 2 patients the proteinuria decreased remarkably during the second treatment cycle (**Supplementary Table 1**).

Table 2. Sodium levels in 24-hour urine collection

	Urine sodium, mmol/L Visit 2	Urine sodium, mmol/L Visit 3	Urine sodium, mmol/L Visit 4
Study patient			
1	76	95	50
2	119	49	81
3	81	48	31
4	209	86	13
5	135	145	66
6	87	12	21
7	133	41	53
8	136	20	32
9	42	31	16
10	114	45	44
11	100	75	30
12	88	42	44
13	87	49	26
14	44	27	23
15	161	38	28
16	39	33	35
Median (IQR)	94 (77-135)	44 (32-69)	32 (24-49)

At visit 2 (before DSR), visit 3 (after start of DSR, before start of VEGFI) and at the end of the DSR (visit 4) per patient. VEGFI; vascular endothelial growth factor inhibitor.
DSR, dietary sodium restriction, VEFGI, vascular endothelial growth factor inhibitor.

Effects on endothelin-1, renin, aldosterone and prostanoids

ET-1 increased on average by 39% in the treatment cycle without DSR which was not seen in the treatment cycle with DSR; the difference between both treatment cycles was not significant. Renin decreased slightly although not significant in the treatment cycle without DSR; at the start of the treatment cycle with DSR, so after start of the DSR, renin levels were as expected higher, and the slight decrease during VEGFI treatment was not seen in the treatment cycle with DSR. Effects on aldosterone followed the same pattern. Plasma 6-keto-$PGF_{1\alpha}$ levels increased almost twofold during VEGFI administration alone ($P = 0.025$), and this rise was not seen during dietary sodium restriction ($P = 0.32$) (**Table 3**). Thus, DSR demonstrated a trend towards reducing the change in plasma 6-keto-$PGF_{1\alpha}$ levels between the start and end of the VEGFI treatment cycle ($P = 0.055$). Plasma levels of TXB_2 showed a non-significant rise during VEGFI treatment, regardless of concomitant dietary sodium restriction.

Table 3. Plasma concentrations of endothelin-1 (ET-1), renine aldosterone, thromboxane B_2, (TXB_2), 6-keto-prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) before and at the end of VEGFI treatment without treatment cycle 1: without dietary sodium restriction, treatment cycle 2: with dietary sodium restriction and with dietary sodium restriction.

Plasma parameter	Treatment cycle 1 (VEGFI)			Treatment cycle 2 (VEGFI + dietary sodium restriction)				
	Start (visit 1)	End (visit 2)	*P*	Start (visit 3)	End (visit 4)	*P*	*P* visit 2 vs. visit 4	*P* Δvisit 2-visit 1 vs. Δvisit 4-visit 3
ET-1, pg/ml	2.1 (1.3-2.9)	2.1 (1.7-3.6)	0.63	1.7 (1.2-3.8)	2.1 (1.4-3.3)	0.30	0.21	0.95
Renin, pg/ml	14.4 (6.8-26.1)	9.9 (5.9-30.7)	0.76	23.3 (11.8-53.3)	21.9 (13.5-51.6)	0.60	0.13	0.98
ET-1/renin ratio	0.29±0.1	0.39±0.1	0.52	0.26±0.1	0.17±0.06	0.47	0.02	0.68
Aldosterone, pg/ml	259 (172-460)	204 (168-420)	0.56	332 (199-420)	332 (226-612)	0.45	0.018	0.45
TXB_2, pg/ml	1351 (834-4062)	1304 (567-2457)	0.94	1740 (1175-2757)	1744 (1082-3788)	0.99	0.23	0.56
6-keto-$PGF_{1\alpha}$, pg/ml	251 (182-414)	585 (274-1098)	0.025	355 (209-399)	316 (252-620)	0.32	0.056	0.055

Data are presented as median (IQR), *P* - value indicates comparison between start and end of the same treatment cycle, *P* Δ indicates comparison of the within-cycle differences between treatment cycle 1 and 2.
VEGFI; vascular endothelial growth factor inhibitor.

VEGFI LEVELS

To ascertain that blood pressure results were not due to lower VEGFI plasma concentrations, VEGFI trough levels were measured without and with DSR at visit 2 and visit 4. No correlation between VEGFI trough levels and blood pressure measurements was found nor VEGFI trough levels and DSR, was found. Due to the heterogeneity in used VEGFIs and treatment schedules, no formal statistical analyses were possible.

TREATMENT SAFETY

During the DSR period 2 patients with antihypertensive treatment during DSR indicated dizziness. After adjusting their antihypertensive treatment, they were symptom-free. There were no other related serious adverse events during the intervention period with DSR. Five patients continued the sodium restriction voluntarily after the end of the study.

DISCUSSION

Hypertension is the most frequently observed side effect of VEGFI. This study demonstrates that the daytime MAP in patients receiving VEGFI treatment is significantly lower by application of DSR. This indicates that DSR is an effective and promising strategy to prevent BP during VEGFI treatment. DSR was applied for a maximum of five weeks to obtain full response on daytime blood pressure. Given the high adherence to the DSR in this study, exemplified by a significant reduction in urinary sodium excretion, dietary sodium restriction appears to be generally well tolerated.

To the best of our knowledge, this is the first prospective study investigating the effect of DSR on the rise in BP during treatment with VEGFIs. The observed effect is in line with the previously demonstrated salt sensitivity of a sunitinib-induced rise in blood pressure in preclinical models.[20]

The pathophysiology of the sodium sensitivity of VEFGI-induced BP rise is incompletely understood. ET-1 is an important factor in VEGFI-induced hypertension [5,6] and is involved in sodium sensitivity: high sodium intake leads to higher ET-1 levels and the vasoconstrictive responses are increased in a high sodium environment.[21,22] However, although a rise of circa 39% in ET-1 levels was observed during VEGFI treatment which was not seen in the treatment cycle with sodium restriction, this difference between both treatment cycles was not significant. This might be explained by the heterogeneity in VEGFI that were used, although the expected effect would be the same. Another explanation may be that ET-1 is released abluminally and plasma levels are not completely representative.[23] Therefore, we cannot exclude that this pathway does play a role in the sodium sensitive hypertension. VEGF appears to have a role in sodium accumulation in the skin by an effect on lymphangiogenesis through the VEGF-3-receptor for VEGF-C[24], which can be abrogated by antibodies against the VEGF3-recepter, also targeted by the studied tyrosine kinase inhibitors.[25] However, an earlier study in rats did not show a difference in lymphangiogenesis during normal diet or high sodium diet during sunitinib treatment.[14] In the current study, we did not measure skin sodium content or lymphangiogenesis. RAAS activation is unlikely to be the initiator of VEGFI-induced BP rise.[26] As earlier, we observed a decrease in renin during VEGFI treatment without dietary sodium restriction, although non-significant.[6] The rise in renin and thus aldosterone in response to sodium restriction is expected; RAAS is activated to promote sodium retention. Interestingly, the non-significant decrease in renin concentration during the treatment cycle without DSR was not observed during the treatment cycle with DSR. Also, the patients not developing hypertension or a clinically relevant blood pressure rise during the first treatment cycle did not have the trend towards lower renin levels at the end of the treatment cycle, suggesting that this decrease in renin is a marker of VEGFI-induced blood pressure rise which is RAAS-independent.

To connect earlier findings showing an effect of acetylsalicylic acid (ASA) on VEGFI-induced blood pressure rise also in line with earlier studies in preeclampsia, we measured prostanoids 6-keto- $PGF_{1\alpha}$ as most stable metabolite of prostacyclin (PGI_2) and thromboxane B_2 (TXB_2). We observed a rise in 6-keto- $PGF_{1\alpha}$ during VEGFI treatment in line with this earlier pre-clinical study.[27] This rise was no longer seen during DSR. In our preclinical study the rise was also blunted by ASA and ET-1 receptor blockers.[27] Although in this study we concluded that the rise in PGI_2 might be compensatory, the absence of rise in treatments attenuating the blood pressure rise more and more suggests that the rise in PGI_2 might be part of the pathophysiology of the blood pressure rise. As we speculated in this earlier study, PGI_2 can elicit vasoconstriction in pathological situations via TXA_2 receptor stimulation, thereby acting as an endothelium derived contracting factor (EDCF).[28,29] The exact contribution of PGI_2 needs further elucidation, for instance by making use of inhibitors that selectively block PGI_2 production (potentially in a renal-specific manner).

The strength of the current study is the prospective design. Although the field of cardio-oncology is expanding rapidly, prospective studies ans intervention studies in particular are scare.

Due to the debilitating effects of COVID-19 on the conduct of our study 24-hour ABPM results of the last four patients undergoing the intervention came from home measurements rather than 24-hour ABPM assessements. These four patients used a validated blood pressure monitor approved by STRIDE (Science and Technology for Regional Innovation and Development in Europe) BP (Blood Pressure) and a standardized form for recording BP, we consider the data just as reliable and reproducible.

Patients with hypertension and on antihypertensive treatment were included in the study. Even though this can be considered a bias to determine the toxicity of VEGFI. However, this represents a real life representation of patients for whom VEGFI treatment are prescribed. A potential complicating factor was the inclusion of patients being exposed to different VEGFI, all of them having a different pharmacodynamics and different on –label treatment schedules. Since BP was equal to or lower than baseline, presence of low concentration of VEGFI do not seem to have a relevant impact on BP. Three patients already had urinary sodium levels of <70 mmol/24-hour at visit 2 of the study, although even in these patients DSR futher lowered sodium excretion. To limit the number of additional measurements at start of the study and therefore no differences between baseline low sodium levels as often seen as a marker of malnutrition,[30] could be observed. However, by excluding patients with significant weight loss prior to start of the treatment the chance of treating patients with severe malnutrition was limited. We did not formally assess quality of life during the DSR period. However, the high adherence to this diet during the study and the treatment and the fact that at least 5 patients wanted to continue DSR after the study period, we assume that the DSR was well tolerated.

In conclusion, results of this study show that DSR is an effective intervention to prevent VEGFI-induced BP rise. DSR therefore should be considered in case of VEGFI-induced BP rise in daily oncology practice.

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REFERENCES

1. Ferrara N, Adamis AP. Ten years of anti-vascular endothelial growth factor therapy. *Nat Rev Drug Discov.* 2016;15(6):385-403.
2. Totzeck M, Mincu RI, Mrotzek S, Schadendorf D, Rassaf T. Cardiovascular diseases in patients receiving small molecules with anti-vascular endothelial growth factor activity: A meta-analysis of approximately 29,000 cancer patients. *Eur J Prev Cardiol.* 2018;25(5):482-494.
3. Versmissen J, Mirabito Colafella KM, Koolen SLW, Danser AHJ. Vascular Cardio-Oncology: Vascular Endothelial Growth Factor inhibitors and hypertension. *Cardiovasc Res.* 2019;115(5):904-914.
4. Zhu X, Stergiopoulos K, Wu S. Risk of hypertension and renal dysfunction with an angiogenesis inhibitor sunitinib: systematic review and meta-analysis. *Acta Oncol.* 2009;48(1):9-17.
5. Kappers MH, Smedts FM, Horn T, et al. The vascular endothelial growth factor receptor inhibitor sunitinib causes a preeclampsia-like syndrome with activation of the endothelin system. *Hypertension.* 2011;58(2):295-302.
6. Kappers MH, van Esch JH, Sluiter W, Sleijfer S, Danser AH, van den Meiracker AH. Hypertension induced by the tyrosine kinase inhibitor sunitinib is associated with increased circulating endothelin-1 levels. *Hypertension.* 2010;56(4):675-681.
7. Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med.* 2004;350(7):672-683.
8. Maynard SE, Min JY, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest.* 2003;111(5):649-658.
9. Mirabito Colafella KM, Neuman RI, Visser W, Danser AHJ, Versmissen J. Aspirin for the prevention and treatment of pre-eclampsia: A matter of COX-1 and/or COX-2 inhibition? *Basic Clin Pharmacol Toxicol.* 2020;127(2):132-141.
10. de Jesus-Gonzalez N, Robinson E, Moslehi J, Humphreys BD. Management of antiangiogenic therapy-induced hypertension. *Hypertension.* 2012;60(3):607-615.
11. Moslehi JJ. Cardiovascular Toxic Effects of Targeted Cancer Therapies. *N Engl J Med.* 2016;375(15):1457-1467.
12. Lankhorst S, Baelde HJ, Clahsen-van Groningen MC, Smedts FM, Danser AH, van den Meiracker AH. Effect of high salt diet on blood pressure and renal damage during vascular endothelial growth factor inhibition with sunitinib. *Nephrol Dial Transplant.* 2016;31(6):914-921.
13. Gu JW, Manning RD, Jr., Young E, Shparago M, Sartin B, Bailey AP. Vascular endothelial growth factor receptor inhibitor enhances dietary salt-induced hypertension in Sprague-Dawley rats. *Am J Physiol Regul Integr Comp Physiol.* 2009;297(1):R142-148.
14. Lankhorst S, Severs D, Marko L, et al. Salt Sensitivity of Angiogenesis Inhibition-Induced Blood Pressure Rise: Role of Interstitial Sodium Accumulation? *Hypertension.* 2017;69(5):919-926.
15. Kohan DE, Rossi NF, Inscho EW, Pollock DM. Regulation of blood pressure and salt homeostasis by endothelin. *Physiol Rev.* 2011;91(1):1-77.
16. Agency. EM. https://www.ema.europa.eu/.
17. Azizi M, Chedid A, Oudard S. Home blood-pressure monitoring in patients receiving sunitinib. *N Engl J Med.* 2008;358(1):95-97.
18. Parati G, Stergiou G, O'Brien E, et al. European Society of Hypertension practice guidelines for ambulatory blood pressure monitoring. *J Hypertens.* 2014;32(7):1359-1366.
19. Bovee DM, Visser WJ, Middel I, et al. A Randomized Trial of Distal Diuretics versus Dietary Sodium Restriction for Hypertension in Chronic Kidney Disease. *J Am Soc Nephrol.* 2020;31(3):650-662.

20. Lankhorst S, Kappers MH, van Esch JH, et al. Treatment of hypertension and renal injury induced by the angiogenesis inhibitor sunitinib: preclinical study. *Hypertension.* 2014;64(6):1282-1289.
21. Khalil RA. Modulators of the vascular endothelin receptor in blood pressure regulation and hypertension. *Curr Mol Pharmacol.* 2011;4(3):176-186.
22. Pollock DM, Pollock JS. Evidence for endothelin involvement in the response to high salt. *Am J Physiol Renal Physiol.* 2001;281(1):F144-150.
23. Wagner OF, Christ G, Wojta J, et al. Polar secretion of endothelin-1 by cultured endothelial cells. *J Biol Chem.* 1992;267(23):16066-16068.
24. Dahlmann A, Linz P, Zucker I, et al. Reduction of Tissue Na(+) Accumulation After Renal Transplantation. *Kidney Int Rep.* 2021;6(9):2338-2347.
25. Wiig H, Schroder A, Neuhofer W, et al. Immune cells control skin lymphatic electrolyte homeostasis and blood pressure. *J Clin Invest.* 2013;123(7):2803-2815.
26. Versmissen J, van Doorn L, Mirabito Colafella KM, Mathijssen RH, Danser AHJ. Sunitinib-induced blood pressure rise does not involve aldosterone: observations in a patient after bilateral adrenalectomy. *J Hypertens.* 2018;36(11):2279-2280.
27. Mirabito Colafella KM, Neves KB, Montezano AC, et al. Selective ETA vs. dual ETA/B receptor blockade for the prevention of sunitinib-induced hypertension and albuminuria in WKY rats. *Cardiovasc Res.* 2020;116(10):1779-1790.
28. Baretella O, Vanhoutte PM. Endothelium-Dependent Contractions: Prostacyclin and Endothelin-1, Partners in Crime? *Adv Pharmacol.* 2016;77:177-208.
29. Vanhoutte PM, Tang EH. Endothelium-dependent contractions: when a good guy turns bad! *J Physiol.* 2008;586(22):5295-5304.
30. Bossi P, Delrio P, Mascheroni A, Zanetti M. The Spectrum of Malnutrition/Cachexia/Sarcopenia in Oncology According to Different Cancer Types and Settings: A Narrative Review. *Nutrients.* 2021;13(6).

SUPPLEMENTARY RESULTS

Supplementary Table 1 Protein levels in 24-hour urine sampling At visit 2 (before dietary sodium restriction (DSR)), visit 3 (after start of DSR, before start of VEGFI) and at the end of the DSR (visit 4) per patient. Visit 2 vs. visit 4. $P = 0.0053$

	Urine protein, g/24h Visit 2	Urine protein, g/24h Visit 3	Urine protein, g/24h Visit 4
Study patient			
1	4.86	0.84	2.15
2	0.12	0.14	0.14
3	0.18	0.11	0.11
4	0.20	0.18	0.14
5	0.26	0.21	0.20
6	0.11	0.11	0.10
7	0.10	0.09	0.08
8	0.10	0.15	0.15
9	0.38	0.17	0.31
10	0.26	0.21	0.26
11	0.17	0.10	0.10
12	0.24	0.23	0.23
13	0.17	0.14	0.14
14	0.34	0.34	0.22
15	1.43	0.7	0.56
16	0.09	0.08	0.08
Median (IQR)	0.19 (0.11-0.32)	0.16 (0.11-0.31)	0.15 (0.10-0.25)

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Supplementary Data detailed case descriptions

Study patient 1

Study patient 1 was a 77-year-old man treated with sunitinib 37.5 mg/day on the 4-weeks on-2 weeks-off schedule for mRCC. At visit 1 (baseline), his 24-hour daytime blood pressure was 144/75 mmHg. After one week of sunitinib treatment, his blood pressure was 172/87 mmHg and he started with amlodipine 5 mg once daily. After 4-weeks of sunitinib treatment, his 24h daytime blood pressure was 144/87 mmHg. Sunitinib treatment was discontinued for 2-weeks according to the regular dosing schedule and dietary salt restriction was initiated. He continued the amlodipine at 5 mg/day. Two weeks later, the 24h daytime blood pressure was 122/62 mmHg. He continued sunitinib treatment at 37.5 mg/day in combination with 5 mg/day amlodipine and the dietary salt restriction. After 4 weeks on this regimen, his daytime blood pressure was 135/79 mmHg.

Study patient 4

Study patient 4 was a 62-year-old man treated with sunitinib 50 mg/day once daily on a 4-weeks on 2-weeks off schedule for mRCC. He started with 5 mg amlodipine once daily immediately after visit 1 measurement of the 24h daytime blood pressure of 159/90 mmHg. After four weeks on sunitinib treatment, his 24h daytime blood pressure was 153/90 mmHg. Sunitinib treatment was discontinued for 2-weeks according to the regular dosing regimen and the dietary salt restriction was initiated. Amlodipine 5 mg/day was continued. Two weeks later, the 24h daytime blood pressure was 130/70 mmHg and he continued the sunitinib treatment at 50 mg/day in combination with 5 mg/day amlodipine and the dietary salt restriction. After 4 weeks of this combination, his daytime blood pressure was 138/81 mm Hg.

Study patient 11

Study patient 11 was a 55-year-old woman treated with sunitinib 37.5 mg once daily continuously for a pancreas neuroendocrine tumor. Her 24h daytime blood pressure at visit 1 was 140/89 mmHg. After a few days, her daytime blood pressure at home rose to 177/104 mmHg and she started 5 mg amlodipine once daily, which was rapidly increased to 10 mg /day. After four weeks of treatment with sunitinib, the daytime 24h blood pressure at visit 2 was 136/95 mm Hg. According to the study protocol, sunitinib was discontinued for one week, amlodipine was reduced to 5 mg/day and the dietary salt restriction was started. At visit 3, the daytime 24h blood pressure was 121/84 mmHg. Two days later, the amlodipine was discontinued because she felt dizzy with a home blood pressure of 115/70 mm Hg. She continued sunitinib treatment with a dose reduction of sunitinib to 25 mg/day due to fatigue. After one week on sunitinib treatment her home blood pressure rose to 150/90mm Hg and amlodipine was resumed at 5 mg/day. At visit 4, the daytime 24h blood pressure was 130/80 mmHg with this combination of treatment.

Study patient 15

Study patient 15 was a 65-year-old man with a history of hypertension treated with 4 mg perindopril once daily. He started with cabozantinib 60 mg/day for mRCC. At visit 1, his daytime blood pressure was 156/85 mmHg. He started on doxazosin 4 mg/day because of previously established hypersensitivity to amlodipine. After one week of treatment, doxazosin was increased to 8 mg/day followed by the addition of lercanidipine 10 mg/day for persistent hypertension. At visit 2, after four weeks of cabozantinib treatment, the daytime blood pressure was 162/96 mmHg and he discontinued cabozantinib treatment according to the study protocol and began the dietary salt restriction. After one week at visit 3, the daytime blood pressure was 144/90 mmHg. He resumed treatment with cabozantinib 60 mg/day. One week after resuming cabozantinib, he reported

dizziness and a home blood pressure of 135/95 mmHg. Lercanidipine was therefore discontinued and doxazocin was reduced to 4 mg and discontinued one week later due to persistent dizziness. After that he reported no more symptoms of dizziness.
At visit 4, 4 weeks of cabozantinib treatment 60 mg/day, perindopril 4 mg/day and the dietary salt restriction, his daytime blood pressure was 143/99 mmHg.

CHAPTER 13

SUMMARY AND GENERAL DISCUSSION

Tolerance to anticancer drugs is determined by drug exposure and drug interactions, but also by side effects and the harm of interventions to prevent or reduce side effects. The studies in this thesis are aimed at understanding and reducing the negative consequences of anticancer treatment. In **Chapter 1,** an introduction is given to side effects and factors that may influence the treatment with the chemotherapeutic agents capecitabine and paclitaxel, and with targeted therapies such as sorafenib. Additionally, an overview is given of the specific objectives of this thesis.

PART I: CHEMOTHERAPY

In Part I (Chapters 2-7), side effects and factors that may influence treatment with the chemotherapeutic drugs capecitabine and paclitaxel were investigated. The research in **Chapter 2** describes a prospective investigation on hand-foot syndrome (HFS) and loss of fingerprints during treatment with capecitabine or tyrosine kinase inhibitors (TKIs) in a cohort of 112 patients. Within 8 weeks of treatment with capecitabine, severe fingerprint loss was noted in 9 patients (14%). Only 4 of these 9 patients had HFS, while HFS was seen in 70% of the complete study population. There was no association between HFS and loss of fingerprints. Grades of HFS during capecitabine treatment were neither associated with the incidence of severe fingerprint loss. Newer drugs such as TKIs are known to develop hand-foot skin reaction (HFSR); a clinically and histopathological variant of HFS.[1] [2] In our study, fingerprint loss was infrequent during TKI treatment (n=1) and did not seem associated with HFSR. As this study had an explorative design, the mechanism of fingerprint loss could not be investigated. To date, there is no study with a plausible explanation for the mechanism underlying loss of fingerprints during capecitabine treatment.[3] Although the loss of fingerprints can be considered a low-risk side effect, and with fingerprints returning within 2 to 4 weeks after cessation of treatment, our findings help to better inform patients about this side effect and to alert them to identification problems in daily life.[4]

Regarding the efficacy of capecitabine treatment, retrospective research pointed out a possible interaction of capecitabine and proton pump inhibitors (PPIs), potentially resulting in a reduced efficacy of capecitabine.[5,6] In **Chapter 3,** we investigated the effects of PPIs on capecitabine absorption in a randomized crossover study in 22 patients. Capecitabine pharmacokinetics were measured in 3 phases: A: with administration of the PPI esomeprazole three hours before capecitabine, B: with capecitabine alone, and C: with cola and esomeprazole administration. When esomeprazole was co-administered with capecitabine, there was an unexpected trend towards a 19% higher mean $AUC_{0\text{-}inf}$ of capecitabine (95%CL, -10% to 57%, P = 0.36) than when capecitabine was

administered alone. As expected, concomitant cola did not reverse the effects observed after esomeprazole. This data illustrates that capecitabine exposure is not negatively influenced by esomeprazole co-treatment and therefore, altered capecitabine pharmacokinetics do not explain the worse clinical outcome of PPI-co-treated cancer patients, which has been observed in previous retrospective cohort analyses. This is in line with other pharmacokinetic interaction studies, in which reduced capecitabine absorption was neither observed after Maalox [7] and rabeprazole [8] administration, nor in patients whom underwent gastrectomy.[9] Hence, no hard conclusions can be drawn on the existence of a true interaction between capecitabine and PPIs. Further prospective research is warranted to validate the presence of a pharmacodynamic drug-drug interaction between capecitabine and PPIs and, if present, to elucidate the mechanisms behind this interaction. Based on our results, we cannot conclude that there is a need to refrain from prescribing PPIs during capecitabine treatment. However, given the overuse of PPIs in cancer patients [10] it is prudent to routinely evaluate whether PPIs are needed during capecitabine treatment and, if possible, consider deprescribing.

Hair loss or chemotherapy-induced alopecia (CIA) is a distressing side effect of anticancer therapy and of paclitaxel in particular.[11] Scalp cooling has a high degree of hair preservation during weekly paclitaxel infusions (59% success rate) more than with anthracycline-based chemotherapy (16% success rate), and is therefore standard of care in the management of hair preservation during paclitaxel treatment.[12] Scalp cooling is currently the only method that has been shown to prevent CIA and is recommended in the 2020 European Society for Medical Oncology (ESMO) guidelines.[13] Despite this guideline, there is no data available regarding the effects of scalp cooling on the exposure of the cytotoxic drugs that are infused. In **Chapter 4** we therefore investigated the influence of scalp cooling on paclitaxel pharmacokinetics in 14 evaluable patients treated with paclitaxel dosed 80-90 mg/m^2, compared with a control group of 24 patients treated with paclitaxel 80-90 mg/m^2 without the use of scalp cooling. With concomitant scalp cooling the exposure of paclitaxel in the systemic circulation was 7% lower (90% CI, -17% to +4%) than without scalp cooling. Therefore, paclitaxel exposure with and without concomitant scalp cooling can be regarded as comparable. In the patients receiving scalp cooling, there were no clear differences in paclitaxel pharmacokinetics between those patients that had CIA (50%) and those that did not. This seems to imply that other factors than pharmacokinetics are more important in explaining CIA, such as individual hair differences (e.g., volume, texture, density or strength).[14 15] Therefore, further research is needed to better advise future patients about the chance of hair preservation with the potential benefit of scalp cooling.[16] In conclusion, our study showed that scalp cooling appears to be a safe intervention for patients treated with a paclitaxel regimen at a dose of 80-90 mg/m^2.

Hypersensitivity reactions (HSRs) are also among the most common side effects during paclitaxel treatment. This is due to Cremophor-EL, the pharmaceutical solvent of paclitaxel.[17] This side effect is usually reduced by premedication consisting of the corticosteroid dexamethasone combined with a histamine 1 (H_1)-receptor antagonist (e.g., clemastine or diphenhydramine) and the histamine 2 (H_2)-receptor antagonist ranitidine.[18,19] In theory, the use of ranitidine is the most controversial of these three drugs as ranitidine may cause HSR by itself in 0.7% of all infusions.[20,21] In contrast to dexamethasone and H_1 receptor antagonists, H_2 receptor antagonists are not recommended in the ESMO guideline for the management of hypersensitivity reactions.[22] It is remarkable that the added value of ranitidine in premedication regimens has never been investigated systematically in a clinical setting. In **Chapter 5**, we therefore describe a pre-post interventional non-inferiority study assessing ranitidine as prophylaxis for paclitaxel infusions. The primary outcome of the study was the incidence of HSR during paclitaxel treatment. Patients receiving their first paclitaxel cycle were included in the study. We studied 183 patients in a pre-intervention group receiving the standard premedication regimen consisting of dexamethasone (10 mg intravenously (IV)), clemastine (2 mg IV) and ranitidine (50 mg IV) and afterwards another 183 patients in the post-interventional group received the experimental premedication regimen without the H_2-antagonist ranitidine. The incidence of HSR ≥ grade 3 was 4.4% (n = 8) in the pre-interventional group compared to 1.6% (n = 3) in the post-interventional group (difference -2.7 % (90% CI, -6.2 to 0.1), and it can be concluded that a premedication regimen without ranitidine was non-inferior to the premedication regimen with ranitidine. Despite the non-randomized study design, our study can be seen as complementary to other large studies done in non-evidence-based intervention strategies to optimize clinical practice,[23] such as omitting calcium/magnesium infusions to protect against oxaliplatin-related neuropathy.[24] Our study also illustrates why the use of premedication regimens during anticancer therapy need to be reconsidered if these are not evidence-based. Our study results contribute directly to reducing the risks for patients and are therefore of daily clinical importance. Within the Erasmus MC Cancer Institute, the premedication regimen without ranitidine was therefore implemented in our institution almost immediately after publication of these results.

For paclitaxel itself, strategies are needed to improve dose individualization, because paclitaxel is characterized by a large inter-individual variability in exposure and a dose-response relationship has been suggested.[25 26] Currently, paclitaxel dose is based on a patient's body surface area (BSA).[27,28] Body size measures can influence the systemic exposure to chemotherapy with the risk of a low exposure and consequently an ineffective treatment or, *vice versa*, a high exposure leading to more side effects. Previous studies demonstrated a wide variety in muscle mass (i.e., skeletal muscle index, SMI) and visceral

adipose tissue (VAT) in patients with identical BSA, which might lead to heterogeneity in chemotherapy-related side effects such as neutropenia.[29-31] In **Chapter 6** we have shown that the dose of paclitaxel cannot be further individualized by correcting for the parameters SMI, VAT and skeletal muscle density (SMD) measured at the third vertebra using a CT scan image slice. These parameters therefore cannot serve as an alternative for BSA-based paclitaxel dosing. It must be taken into account that our study was performed in a homogenous population, predominantly consisting of men treated with a well tolerable paclitaxel dose of 50 mg/m^2 in a curative setting. For drugs with a large interpatient variability in systemic exposure, such as paclitaxel, therapeutic drug monitoring (TDM) has previously been investigated by Joerger et al. in a randomized controlled trial and they found that TDM primarily reduced the incidence of paclitaxel-associated peripheral neuropathy, but did not improve the clinical outcome or the incidence of neutropenia.[32] It should therefore be concluded that for now dosing of paclitaxel based on BSA remains the standard of care.

In contrast to the knowledge of the large inter-individual variability of systemic pharmacokinetics of paclitaxel related to efficacy and side effects, little is known about the correlation of paclitaxel concentrations in tumor tissue and paclitaxel concentrations over time. Particularly in patients with esophageal cancer, intra-tumoral concentrations of paclitaxel may be of interest because a significant proportion of patients do not benefit from paclitaxel treatment or show disease progression shortly after treatment discontinuation.[33,34] Previously, it was shown that systemic paclitaxel exposure and treatment outcome was not correlated in patients with esophageal cancer.[35] We hypothesized that ineffectiveness of treatment could (partly) be explained by low intra-tumoral concentrations of paclitaxel. In addition, ABC efflux transporter expression, known for its role in chemotherapy resistance, and intra-tumoral paclitaxel concentrations overtime, are the first signs of a potential resistance to paclitaxel in esophageal tumors. In **Chapter 7** we describe the results of an exploratory study in 14 esophageal cancer patients with standard of care paclitaxel-based treatment, of whom 78% were treated with neoadjuvant chemo-radiotherapy to identify changes in paclitaxel tissue pharmacokinetics and differences in ABCB1expression. Unfortunately, our primary objective, demonstrating a 25% reduction in intra-tumoral paclitaxel concentrations in the last cycle of weekly paclitaxel in comparison to intra-tumoral esophageal mucosa concentrations of paclitaxel in the first cycle, could not be assessed due to a low amount of tumor cells observed at the last treatment cycle. However, a correlation was seen between intra-tumoral esophageal paclitaxel concentrations and paclitaxel plasma concentrations during the first cycle of paclitaxel treatment. Interestingly, we observed a higher ABCB1 expression in adenocarcinoma than in squamous cell carcinoma, which might lead to a more rapid clearance of paclitaxel in adenocarcinomas and might explain the better treatment ef-

fect of paclitaxel in squamous cell carcinoma.[36,37] Besides the histological differences, both tumor types have a different underlying etiology and generally occur in a different group of patients: risk factors for squamous cell carcinoma are smoking and alcohol abuse, whereas adenocarcinoma is associated with gastroesophageal reflux disease, for example. Despite these major differences, perioperative systemic treatment for both is the same. The differential expression of ABCB1 between these histological subtypes should be used to further individualize systemic treatment for esophageal cancer.

PART II: TARGETED ORAL ANTICANCER THERAPY

In Part II (Chapters 8-12), side effects and factors that may influence treatment with targeted anticancer therapy with tyrosine kinase inhibitors such as sorafenib were investigated.

The side effect HFSR can have a major impact on a patient's quality of life and may necessitate dose modifications.[38] Previous *in vitro* and *in vivo* research showed that sorafenib can accumulate in human epidermal keratinocytes mediated by the organic anion transporter 6 (OAT6) and that sorafenib-induced HFSR can be prevented by co-treatment with the OAT6 inhibitor probenecid without negatively impacting the anticancer properties of sorafenib.[39] In **Chapter 8,** we studied the influence of probenecid on the exposure of sorafenib and toxicity in patients with sorafenib treatment and found a significant decrease in sorafenib AUC_{0-12h} by 27% (90% CI, −38% to −14%; $P < 0.01$) when probenecid was used concomitantly with sorafenib. In keratocytes, sorafenib concentrations decreased by the same extent, which suggests that these intracellular concentrations depend on exposure of systemic sorafenib exposure. HFSR occurred in 10 of the 16 patients and was not related to probenecid administration. As probenecid did not seem to influence HFSR incidence or severity and as there was a clear effect on systemic drug exposure, probenecid unfortunately cannot be used for management of HFSR. Overall, there are currently no good treatment option for HFSR other than prophylactic use of urea-based ointments that reduce the incidence of HFSR.[2,13,40]

Thyroid dysfunction is another recognized side effect of sorafenib treatment. The incidence of hypothyroidism during sorafenib treatment ranges from 18-50%.[41] In **Chapter 9** we analyzed a cohort of patients treated with sorafenib for hepatocellular carcinoma (HCC) in order to investigate the effect of sorafenib on thyroid metabolism. Thyroid function tests (i.e., TSH and FT4 (free thyroxine) levels) were determined at baseline and six weeks thereafter until the end of treatment. None of the patients in our cohort had pre-existent hypothyroidism or used drugs that interact with thyroid function, such as dexamethasone. We observed several different mechanisms of thyroid dysfunction in

our cohort. Thyroiditis occurred in 7% of cases and in all patients the antibodies against thyroid peroxidase (TPO) or against the TSH receptor (TSHR) were elevated. Of the other patients about 30% had elevations in TSH or FT4 levels above the normal range which could be explained by suggesting a central effect on the hypothalamic-pituitary-thyroid axis or by reduced peripheral distribution and metabolism of thyroid hormone. In general, sorafenib-induced hypothyroidism occurs after a median of 5 months after start of sorafenib, is irreversible, and is usually caused by subacute thyroiditis.[42,43] Thyroid dysfunction can be managed without dose reduction or discontinuation of sorafenib treatment.[44] For clinical practice it is recommend to measure TSH at the start of sorafenib treatment and at the first day of every treatment cycle.[43,45]

The risk of side effects during treatment with sorafenib,[46] is further increased by potential drug interactions if patients require concomitant medications.[47] In patients who can be treated with a liver transplantation for hepatocellular carcinoma disease, relapses occur in 20% of patients after which sorafenib is an approved treatment option.[48,49] Immunosuppressive therapy such as tacrolimus is the backbone to decrease rejection rates and to improve survival in patients who had a liver transplant.[50] Dose reductions and discontinuation of sorafenib due to side effects occur much more frequently after liver transplantation (15-77% of patients) [51] which may potentially be explained by an interaction between sorafenib and these immunosuppressants. In **Chapter 10** four patients are described who were treated with sorafenib in combination with the immunosuppressant tacrolimus for HCC recurrence after a liver transplantation. Patients were longitudinally monitored for both sorafenib and tacrolimus exposure until sorafenib discontinuation. Over time sorafenib exposure decreased during this combination in all four patients. As sorafenib concentrations decreased --even after tacrolimus was discontinued in two cases– we cannot conclude that tacrolimus influenced sorafenib pharmacokinetics in a predictable way. Exposure to tacrolimus varied between patients: two patients had a decrease in tacrolimus exposure, whereas the two others had an increase in tacrolimus exposure. As we did not find a clear interaction between sorafenib and tacrolimus, we cannot rule out that sorafenib non-adherence played a role in the decrease of sorafenib exposure over time. It is known that 50% of patients on long-term oral anticancer therapy tend not to adhere to the prescribed therapy, which can lead to reduced effectiveness of the therapy and thus less exposure to the treatment.[52]
All four patients experienced severe side effects when sorafenib doses were above 400 mg daily, probably due to a pharmacodynamic interaction between tacrolimus and sorafenib treatment. In addition, the immune-compromised status of these patients may be associated with increased side effects during sorafenib treatment. The mechanism is currently unknown. We therefore recommend that the dose of sorafenib in this

group of patients should not exceed 400 mg per day, as the safest option for this group of patients is to keep them on a tolerable treatment.[53,54]

Targeted therapies including sorafenib, that inhibit the vascular endothelial growth factor (VEGF) signaling pathway, may cause cardio-vascular toxicity.[55,56] This can severely hamper treatment with these drugs, either as monotherapy or in combination with immune checkpoint inhibitors. In **Chapter 11** we discussed the cardiovascular toxicity of VEGF inhibitors (VEGFI) and immune checkpoint inhibitors (ICIs) by summarizing their cardiac and vascular effects. A combination treatment with VEGFIs and ICIs is associated with a significant increase in cardiovascular risk, as both types of drugs synergistically cause cardiovascular side effects. For example, hypertension which occurs in a substantial portion of patients receiving VEGFI therapy, seems to be a risk factor for ICI-induced vascular events.[57] Therefore, a comprehensive baseline assessment of cardiotoxicity such as medical history focused on cardiovascular events, measurement of blood pressure, pre-treatment electrocardiogram, and adequate monitoring of the occurrence of cardiovascular events during and after treatment is essential, as an early detection allows for timely intervention. A multidisciplinary cardio-oncology approach will optimize the cardiovascular health of cancer patients, enabling them to fully benefit from anticancer therapy.[58]

The most frequent and best characterized cardiovascular adverse event in VEGF treatment is hypertension, which is dose-dependent and therefore known as an on-target mechanism.[59] Management of hypertension can be done with conventional anti-hypertensive drugs.[60] However, as in a preclinical study it was shown that hypertension due to sunitinib was salt-sensitive, we aimed to directly target this salt sensitivity to treat VEGFI-induced hypertension.[61] In **Chapter 12** we describe a prospective cohort study in 16 patients with VEGFI-induced hypertension whom were treated with a dietary sodium restriction of less than 4 grams per day.[62] The primary end point was the difference blood pressure rise between a treatment cycle with and without dietary sodium restriction. Blood pressure was measured via ambulatory blood pressure monitoring and plasma and urine samples were collected during each study visit. Sixteen patients underwent the dietary sodium restriction. During the first VEGFI treatment cycle without DSR, daytime MAP increased by 15 mmHg (from 95 to 110 mmHg). During the subsequent treatment cycle with DSR, daytime MAP increased by 8 mm Hg from 94 to 102 mmHg. Therefore, DSR significantly reduced the increase in MAP by 7 mmHg (95% CI, 1.3 to 12.0; $P = 0.009$). The dietary sodium restriction was well tolerated and 5 patients had a desire to continue with the dietary salt restriction after they finished the study. We showed that dietary sodium restriction is a highly effective intervention to reduce blood pressure rise during VEGFI treatment. Furthermore, the study increases awareness of

cardio-oncological problems in the treatment with TKIs and has also shown that patients with cancer are motivated for lifestyle interventions. This fact can serve as a prelude to further self-management during the treatment of oncological patients.[63 64]

CONCLUSIONS AND FUTURE PERSPECTIVES

The results from this thesis have expanded our understanding of the exposure to the chemotherapeutic drugs capecitabine and paclitaxel, and of the PK of VEGFIs, including sorafenib. Although not all of these new insights may lead to policy changes in clinical practice, they may provide recommendations for daily practice and may lead to new studies. For instance, the loss of fingerprints in capecitabine is nowadays mentioned as a side effect in the standard information provided to the patient, the H_2 antagonist ranitidine is safely omitted from paclitaxel premedication regimens at the Erasmus MC Cancer Institute, and lubrication with creams as a preventive intervention for HFSR during sorafenib treatment remains standard of care. With our research on the prescribing of PPIs during the treatment with capecitabine, we tried to nuance the worldwide warning about exposure to this combination. The research into dietary sodium restriction as a new intervention in the treatment of hypertension caused by VEGFIs illustrates that lifestyle interventions can simply reduce polypharmacy in patients with cancer.

The ongoing assessment of the safety and tolerability of drugs is crucial to balance their risks and benefits. As said, management of treatment-related side effects deserves attention. But not only the occurrence and mitigation of side effects are important, but insight in the prognostic information of individual factors is equally important to enable successful anticancer treatment. Knowledge of treatment- related side effects and their impact on patients' quality of life influences the physician's choice for the individual patient.[65] In Europe, including the Netherlands, oncological medicines are regulated by the European Medicines Agency (EMA).[66] Subsequently, the NVMO (Nederlandse Vereniging voor Medische Oncologie) Assessment Committee Oncological Sources (Commissie BOM) [67] assesses the clinical value, treatment methods and treatment indications, based on usually randomized phase III studies of the drug in question. They do this on the basis of the PASKWIL (Palliative, Adjuvant, Specific side effects, Quality of life, Impact of the treatment, Level of evidence)-criteria, including the associated toxicity of the treatment.[68] The assessment process, if applicable, is coordinated with the assessment by the National Health Care Institute.[67]
In clinical practice, (new) cancer drugs are generally prescribed to patients regardless of their individual factors. These patients can be in a worse clinical condition than those who have participated in clinical trials. Hence, the benefit-risk ratio may be less favorable.

Moreover, there are indications that side effects are under-reported in clinical studies.[69] This means that when new treatments come to the market, vigilance must be exercised with regard to side effects, but also the long-term consequences of cancer treatments must be taken into account.[70] During standard of care treatment efforts should therefore also be aimed at observing unreported features of anticancer therapy which can only be noticed at the longer term or with advancing insights and to limit the disease burden by adequately treating frequent side effects as described in this thesis.

Numerous important questions regarding side effects remain: What about gender differences and side effects as a dose individualization strategy, knowing that women on capecitabine treatment need more dose reductions of capecitabine than men due to side effects?[71] What can be done to optimize premedication schedules for other chemotherapeutic agents than paclitaxel? For instance, dexamethasone to prevent HSRs, nausea and vomiting[19,22,72] in the premedication regimen with weekly paclitaxel is of interest too, because of its short- and long-term side effects such as insomnia, agitations, abdominal symptoms, weight gain, skin rash and diabetes mellitus.[73] Several studies to optimize the dose and route of dexamethasone administration have been conducted. However most studies are retrospective, lacking power to detect differences or have controversial results.[74,75] This also applies to a meta-analysis of the effects of oral versus intravenous dexamethasone which showed that premedication with oral dexamethasone is superior to the intravenous dexamethasone treatment in the prevention of paclitaxel-related HSR[76] which is in contrast to the study of Rosello et al. that short-term intravenous dexamethasone was associated with fewer side-effects than oral dexamethasone.[22,77] Therefore the optimal dose and route should be re-evaluated in a confirmatory prospective large study for both patient safety and to convince the medical community with scientifically based results.

Regarding the phenomenon HFSR, until an effective treatment for this side effect is found, it remains vital to provide patients with appropriate education and strict monitor for HFSR during sorafenib treatment, as early detection and timely dose modifications have been shown to improve sorafenib treatment duration and overall survival.[78 79] It is important to recognize HFSR in a timely manner and to perform a skin examination at the start of sorafenib treatment with special attention to the palms of the hands and soles of the feet and to monitor them regularly during the first 6 weeks of treatment.[13] This is best done by healthcare professionals experienced with this phenomenon and the other side effects of sorafenib as a whole.[80]

REFERENCES

1. Li J, Gu J. Hand-foot skin reaction with vascular endothelial growth factor receptor tyrosine kinase inhibitors in cancer patients: A systematic review and meta-analysis. *Crit Rev Oncol Hematol.* 2017;119:50-58.
2. Chen J, Wang Z. How to conduct integrated pharmaceutical care for patients with hand-foot syndrome associated with chemotherapeutic agents and targeted drugs. *J Oncol Pharm Pract.* 2021;27(4):919-929.
3. Zhao J, Zhang X, Cui X, Wang D, Zhang B, Ban L. Loss of Fingerprints as a Side Effect of Capecitabine Therapy: Case Report and Literature Review. *Oncol Res.* 2020;28(1):103-106.
4. NEC. Biometric authentication. *The Top 9 Common Uses of Biometrics in Everyday Life* 2020; https://www.nec.co.nz/market-leadership/publications-media/the-top-9-common-uses-of-biometrics-in-everyday-life/.
5. Chu MP, Hecht JR, Slamon D, et al. Association of Proton Pump Inhibitors and Capecitabine Efficacy in Advanced Gastroesophageal Cancer: Secondary Analysis of the TRIO-013/LOGiC Randomized Clinical Trial. *JAMA Oncol.* 2017;3(6):767-773.
6. Sun J, Ilich AI, Kim CA, et al. Concomitant Administration of Proton Pump Inhibitors and Capecitabine is Associated With Increased Recurrence Risk in Early Stage Colorectal Cancer Patients. *Clin Colorectal Cancer.* 2016;15(3):257-263.
7. Reigner B, Clive S, Cassidy J, et al. Influence of the antacid Maalox on the pharmacokinetics of capecitabine in cancer patients. *Cancer Chemother Pharmacol.* 1999;43(4):309-315.
8. Sekido M, Fujita KI, Kubota Y, et al. Rabeprazole intake does not affect systemic exposure to capecitabine and its metabolites, 5'-deoxy-5-fluorocytidine, 5'-deoxy-5-fluorouridine, and 5-fluorouracil. *Cancer Chemother Pharmacol.* 2019;83(6):1127-1135.
9. Jacobs BAW, Deenen MJ, Joerger M, et al. Pharmacokinetics of Capecitabine and Four Metabolites in a Heterogeneous Population of Cancer Patients: A Comprehensive Analysis. *CPT Pharmacometrics Syst Pharmacol.* 2019;8(12):940-950.
10. Smelick GS, Heffron TP, Chu L, et al. Prevalence of acid-reducing agents (ARA) in cancer populations and ARA drug-drug interaction potential for molecular targeted agents in clinical development. *Mol Pharm.* 2013;10(11):4055-4062.
11. Dunnill CJ, Al-Tameemi W, Collett A, Haslam IS, Georgopoulos NT. A Clinical and Biological Guide for Understanding Chemotherapy-Induced Alopecia and Its Prevention. *Oncologist.* 2018;23(1):84-96.
12. Silva GB, Ciccolini K, Donati A, Hurk CVD. Scalp cooling to prevent chemotherapy-induced alopecia. *An Bras Dermatol.* 2020;95(5):631-637.
13. Lacouture ME, Sibaud V, Gerber PA, et al. Prevention and management of dermatological toxicities related to anticancer agents: ESMO Clinical Practice Guidelines(). *Ann Oncol.* 2021;32(2):157-170.
14. Araoye EF, Stearns V, Aguh C. Considerations for the Use of Scalp Cooling Devices in Black Patients. *J Clin Oncol.* 2020;38(30):3575-3576.
15. Dilawari A, Gallagher C, Alintah P, et al. Does Scalp Cooling Have the Same Efficacy in Black Patients Receiving Chemotherapy for Breast Cancer? *Oncologist.* 2021;26(4):292-e548.
16. Marks DH, Okhovat JP, Hagigeorges D, et al. The effect of scalp cooling on CIA-related quality of life in breast cancer patients: a systematic review. *Breast Cancer Res Treat.* 2019;175(2):267-276.
17. Szebeni J, Alving CR, Savay S, et al. Formation of complement-activating particles in aqueous solutions of Taxol: possible role in hypersensitivity reactions. *Int Immunopharmacol.* 2001;1(4):721-735.

18. Joerger M. Prevention and handling of acute allergic and infusion reactions in oncology. *Ann Oncol.* 2012;23 Suppl 10:x313-319.
19. Taxol (paclitaxel) injection label. Accessed data fda 02/11/ 2020.
20. Demirkan K, Bozkurt B, Karakaya G, Kalyoncu AF. Anaphylactic reaction to drugs commonly used for gastrointestinal system diseases: 3 case reports and review of the literature. *J Investig Allergol Clin Immunol.* 2006;16(3):203-209.
21. Han TY, Jang WS, Yu M, Lee HK, Son SJ, Seo SJ. Anaphylactic reaction to ranitidine (Zantac(R)). *Int J Dermatol.* 2011;50(11):1397-1399.
22. Rosello S, Blasco I, Garcia Fabregat L, Cervantes A, Jordan K, Committee EG. Management of infusion reactions to systemic anticancer therapy: ESMO Clinical Practice Guidelines. *Ann Oncol.* 2017;28(suppl_4):iv100-iv118.
23. Gelderblom H, Zwaveling J. No need for H2-antagonists in premedication regimens for paclitaxel infusions: less is more. *Br J Cancer.* 2021;124(10):1613-1614.
24. Loprinzi CL, Qin R, Dakhil SR, et al. Phase III randomized, placebo-controlled, double-blind study of intravenous calcium and magnesium to prevent oxaliplatin-induced sensory neurotoxicity (N08CB/Alliance). *J Clin Oncol.* 2014;32(10):997-1005.
25. Mathijssen RH, Sparreboom A, Verweij J. Determining the optimal dose in the development of anticancer agents. *Nat Rev Clin Oncol.* 2014;11(5):272-281.
26. de Graan AJ, Elens L, Sprowl JA, et al. CYP3A4*22 genotype and systemic exposure affect paclitaxel-induced neurotoxicity. *Clin Cancer Res.* 2013;19(12):3316-3324.
27. Bins S, Ratain MJ, Mathijssen RH. Conventional dosing of anticancer agents: precisely wrong or just inaccurate? *Clin Pharmacol Ther.* 2014;95(4):361-364.
28. Baker SD, Verweij J, Rowinsky EK, et al. Role of body surface area in dosing of investigational anticancer agents in adults, 1991-2001. *J Natl Cancer Inst.* 2002;94(24):1883-1888.
29. Prado CM, Lima IS, Baracos VE, et al. An exploratory study of body composition as a determinant of epirubicin pharmacokinetics and toxicity. *Cancer Chemother Pharmacol.* 2011;67(1):93-101.
30. Cespedes Feliciano EM, Chen WY, Lee V, et al. Body Composition, Adherence to Anthracycline and Taxane-Based Chemotherapy, and Survival After Nonmetastatic Breast Cancer. *JAMA Oncol.* 2019.
31. Hopkins JJ, Sawyer MB. A review of body composition and pharmacokinetics in oncology. *Expert Rev Clin Pharmacol.* 2017;10(9):947-956.
32. Joerger M, Kraff S, Jaehde U, et al. Validation of a Commercial Assay and Decision Support Tool for Routine Paclitaxel Therapeutic Drug Monitoring (TDM). *Ther Drug Monit.* 2017;39(6):617-624.
33. Chirieac LR, Swisher SG, Ajani JA, et al. Posttherapy pathologic stage predicts survival in patients with esophageal carcinoma receiving preoperative chemoradiation. *Cancer.* 2005;103(7):1347-1355.
34. de Man FM, van Eerden RAG, Oomen-de Hoop E, et al. Efficacy and Toxicity of Weekly Carboplatin and Paclitaxel as Induction or Palliative Treatment in Advanced Esophageal Cancer Patients. *Cancers (Basel).* 2019;11(6).
35. Toxopeus EL, Talman S, van der Gaast A, et al. Induction chemotherapy followed by surgery for advanced oesophageal cancer. *Eur J Surg Oncol.* 2015;41(3):323-332.
36. Toxopeus EL, Nieboer D, Shapiro J, et al. Nomogram for predicting pathologically complete response after neoadjuvant chemoradiotherapy for oesophageal cancer. *Radiother Oncol.* 2015;115(3):392-398.
37. Eyck BM, van Lanschot JJB, Hulshof M, et al. Ten-Year Outcome of Neoadjuvant Chemoradiotherapy Plus Surgery for Esophageal Cancer: The Randomized Controlled CROSS Trial. *J Clin Oncol.* 2021;39(18):1995-2004.

38. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med.* 2008;359(4):378-390.
39. Zimmerman EI, Gibson AA, Hu S, et al. Multikinase Inhibitors Induce Cutaneous Toxicity through OAT6-Mediated Uptake and MAP3K7-Driven Cell Death. *Cancer Res.* 2016;76(1):117-126.
40. Lee YS, Jung YK, Kim JH, et al. Effect of urea cream on sorafenib-associated hand-foot skin reaction in patients with hepatocellular carcinoma: A multicenter, randomised, double-blind controlled study. *Eur J Cancer.* 2020;140:19-27.
41. Daimon M, Kato T, Kaino W, et al. Thyroid dysfunction in patients treated with tyrosine kinase inhibitors, sunitinib, sorafenib and axitinib, for metastatic renal cell carcinoma. *Jpn J Clin Oncol.* 2012;42(8):742-747.
42. Abdulrahman RM, Verloop H, Hoftijzer H, et al. Sorafenib-induced hypothyroidism is associated with increased type 3 deiodination. *J Clin Endocrinol Metab.* 2010;95(8):3758-3762.
43. Basolo A, Matrone A, Elisei R, Santini F. Effects of tyrosine kinase inhibitors on thyroid function and thyroid hormone metabolism. *Semin Cancer Biol.* 2021.
44. Bhattacharya S, Goyal A, Kaur P, Singh R, Kalra S. Anticancer Drug-induced Thyroid Dysfunction. *Eur Endocrinol.* 2020;16(1):32-39.
45. Drui D, Illouz F, Do Cao C, Caron P. Expert opinion on thyroid complications of new anti-cancer therapies: Tyrosine kinase inhibitors. *Ann Endocrinol (Paris).* 2018;79(5):569-573.
46. Rimassa L, Danesi R, Pressiani T, Merle P. Management of adverse events associated with tyrosine kinase inhibitors: Improving outcomes for patients with hepatocellular carcinoma. *Cancer Treat Rev.* 2019;77:20-28.
47. Yu H, Steeghs N, Nijenhuis CM, Schellens JHM, Beijnen JH, Huitema ADR. Practical Guidelines for Therapeutic Drug Monitoring of Anticancer Tyrosine Kinase Inhibitors: Focus on the Pharmacokinetic Targets. *Clinical Pharmacokinetics.* 2014;53(4):305-325.
48. Zimmerman MA, Ghobrial RM, Tong MJ, et al. Recurrence of Hepatocellular Carcinoma Following Liver Transplantation: A Review of Preoperative and Postoperative Prognostic Indicators. *Archives of Surgery.* 2008;143(2):182-188.
49. de'Angelis N, Landi F, Nencioni M, et al. Role of Sorafenib in Patients With Recurrent Hepatocellular Carcinoma After Liver Transplantation. *Prog Transplant.* 2016;26(4):348-355.
50. Dhanasekaran R. Management of Immunosuppression in Liver Transplantation. *Clin Liver Dis.* 2017;21(2):337-353.
51. Li Y, Gao ZH, Qu XJ. The adverse effects of sorafenib in patients with advanced cancers. *Basic Clin Pharmacol Toxicol.* 2015;116(3):216-221.
52. Timmers L, Boons CC, Verbrugghe M, van den Bemt BJ, Van Hecke A, Hugtenburg JG. Supporting adherence to oral anticancer agents: clinical practice and clues to improve care provided by physicians, nurse practitioners, nurses and pharmacists. *BMC Cancer.* 2017;17(1):122.
53. Staufer K, Fischer L, Seegers B, Vettorazzi E, Nashan B, Sterneck M. High toxicity of sorafenib inhibitors. *Clin Sci (Lond).* 2021;135(1):71-100.
57. Bar J, Markel G, Gottfried T, et al. Acute vascular events as a possibly related adverse event of immunotherapy: a single-institute retrospective study. *Eur J Cancer.* 2019;120:122-131.
58. Lyon AR, Dent S, Stanway S, et al. Baseline cardiovascular risk assessment in cancer patients scheduled to receive cardiotoxic cancer therapies: a position statement and new risk assessment tools from the Cardio-Oncology Study Group of the Heart Failure Association of the European Society of Cardiology in collaboration with the International Cardio-Oncology Society. *Eur J Heart Fail.* 2020;22(11):1945-1960.

59. Versmissen J, Mirabito Colafella KM, Koolen SLW, Danser AHJ. Vascular Cardio-Oncology: Vascular Endothelial Growth Factor inhibitors and hypertension. *Cardiovasc Res.* 2019;115(5):904-914.
60. Van Wynsberghe M, Flejeo J, Sakhi H, et al. Nephrotoxicity of Anti-Angiogenic Therapies. *Diagnostics (Basel)*. 2021;11(4).
61. Lankhorst S, Severs D, Marko L, et al. Salt Sensitivity of Angiogenesis Inhibition-Induced Blood Pressure Rise: Role of Interstitial Sodium Accumulation? *Hypertension*. 2017;69(5):919-926.
62. Bovee DM, Visser WJ, Middel I, et al. A Randomized Trial of Distal Diuretics versus Dietary Sodium Restriction for Hypertension in Chronic Kidney Disease. *J Am Soc Nephrol.* 2020;31(3):650-662.
63. Pilic L, Pedlar CR, Mavrommatis Y. Salt-sensitive hypertension: mechanisms and effects of dietary and other lifestyle factors. *Nutr Rev.* 2016;74(10):645-658.
64. Thomas R, Davies N. Lifestyle during and after cancer treatment. *Clin Oncol (R Coll Radiol).* 2007;19(8):616-627.
65. Maillet D, Blay JY, You B, Rachdi A, Gan HK, Peron J. The reporting of adverse events in oncology phase III trials: a comparison of the current status versus the expectations of the EORTC members. *Ann Oncol.* 2016;27(1):192-198.
66. European Medicines Agency. https://clinicaldata.ema.europa.eu/web/cdp. 2019; https://clinicaldata.ema.europa.eu/web/cdp. Accessed October 30, 2021.
67. Nederlandse Vereniging Medische Oncologie. https://www.nvmo.org/bom/. 20. Accessed October 31, 2021.
68. Willemse PH RC. Een toetssteen voor besluitvorming. Nieuwe criteria voor registratie oncologische middelen.*Medisch Contact.* 2001; 20(20):794.
69. Seruga B, Templeton AJ, Badillo FE, Ocana A, Amir E, Tannock IF. Under-reporting of harm in clinical trials. *Lancet Oncol.* 2016;17(5):e209-219.
70. Nurgali K, Jagoe RT, Abalo R. Editorial: Adverse Effects of Cancer Chemotherapy: Anything New to Improve Tolerance and Reduce Sequelae? *Front Pharmacol.* 2018;9:245.
71. Knikman JE, Gelderblom H, Beijnen JH, Cats A, Guchelaar HJ, Henricks LM. Individualized Dosing of Fluoropyrimidine-Based Chemotherapy to Prevent Severe Fluoropyrimidine-Related Toxicity: What Are the Options? *Clin Pharmacol Ther.* 2021;109(3):591-604.
72. Hesketh PJ, Kris MG, Basch E, et al. Antiemetics: American Society of Clinical Oncology Clinical Practice Guideline Update. *J Clin Oncol.* 2017;35(28):3240-3261.
73. Kapugi M, Cunningham K. Corticosteroids. *Orthop Nurs.* 2019;38(5):336-339.
74. de Castro Baccarin AL, Irene MN, de Iracema Gomes Cubero D, et al. The feasibility of dexamethasone omission in weekly paclitaxel treatment for breast cancer patients. *Support Care Cancer.* 2019;27(3):927-931.
75. Lansinger OM, Biedermann S, He Z, Colevas AD. Do Steroids Matter? A Retrospective Review of Premedication for Taxane Chemotherapy and Hypersensitivity Reactions. *J Clin Oncol.* 2021:JCO2101200.
76. Chen FC, Wang LH, Zheng XY, Zhang XM, Zhang J, Li LJ. Meta-analysis of the effects of oral and intravenous dexamethasone premedication in the prevention of paclitaxel-induced allergic reactions. *Oncotarget.* 2017;8(12):19236-19243.
77. de Graaf L, Aarts F. Dexamethason. *Nursing (Maarssen).* 2020;26(12):52-55.
78. Ai L, Xu Z, Yang B, He Q, Luo P. Sorafenib-associated hand-foot skin reaction: practical advice on diagnosis, mechanism, prevention, and management. *Expert Rev Clin Pharmacol.* 2019;12(12):1121-1127.
79. Tovoli F, Ielasi L, Casadei-Gardini A, et al. Management of adverse events with tailored sorafenib dosing prolongs survival of hepatocellular carcinoma patients. *J Hepatol.* 2019;71(6):1175-1183.

80. Liukka M, Steven A, Moreno MFV, et al. Action after Adverse Events in Healthcare: An Integrative Literature Review. *Int J Environ Res Public Health.* 2020;17(13).

APPENDICES

APPENDIX 1

NEDERLANDSE SAMENVATTING

Tolerantie van medicijnen tegen kanker wordt bepaald door blootstelling aan deze medicijnen, de wisselwerking met anderen geneesmiddelen, de bijwerkingen en de nadelen van interventies om deze bijwerkingen te voorkomen of te verminderen. De studies in dit proefschrift zijn gericht op het begrijpen en verminderen van de negatieve gevolgen van behandelingen tegen kanker. In **Hoofdstuk 1** wordt een inleiding gegeven op onderzoek naar bijwerkingen en factoren die van invloed kunnen zijn op de behandeling met twee chemotherapeutische medicijnen, en met doelgerichte therapieën. Daarnaast wordt een overzicht gegeven van de specifieke doelstellingen van dit proefschrift.

DEEL I: CHEMOTHERAPIE

In Deel I (Hoofdstukken 2-7) zijn bijwerkingen en factoren onderzocht die de behandeling met de chemotherapeutica capecitabine en paclitaxel kunnen beïnvloeden. De studie in **Hoofdstuk 2** beschrijft een prospectieve studie naar het hand-voetsyndroom (HFS) en het verlies van vingerafdrukken tijdens de behandeling met capecitabine of tyrosinekinaseremmers (TKI's) in een cohort van 112 patiënten. Binnen 8 weken na start van de behandeling met capecitabine werd een ernstig verlies van vingerafdrukken opgemerkt bij 9 patiënten (14%). Slechts 4 van deze 9 (44%) patiënten hadden een HFS, terwijl HFS werd gezien bij 70% van de gehele onderzoekspopulatie. Er bleek geen verband te zijn tussen HFS en vingerafdrukverlies. Tot op heden is er geen onderzoek gepubliceerd met een plausibele verklaring voor het mechanisme dat ten grondslag ligt aan het verlies van vingerafdrukken tijdens behandeling met capecitabine. Hoewel het verlies van vingerafdrukken kan worden beschouwd als een bijwerking met een laag risico, en de vingerafdrukken binnen 2 tot 4 weken na stopzetting van de behandeling weer terugkeren, helpen onze bevindingen om patiënten beter te informeren over deze bijwerking en om hen te wijzen op identificatieproblemen in het dagelijks leven.

Wat betreft de werkzaamheid van capecitabine wees eerder retrospectief onderzoek op een mogelijke wisselwerking (interactie) tussen capecitabine en de zogenaamde protonpompremmers (PPI's, een vorm van of maagzuurremmers), mogelijk resulterend in een verminderde werkzaamheid van capecitabine. In **Hoofdstuk 3** onderzochten we de effecten van PPI's op de opname (absorptie) van capecitabine vanuit de darmen in de bloedbaan. De farmacokinetiek (blootstelling aan het medicijn) van capecitabine werd gemeten in 22 patienten tijdens 3 fasen: A) met toediening van de PPI esomeprazol drie uur vóór capecitabine, B) met alleen capecitabine en C) met toediening van cola en esomeprazol. Wanneer esomeprazol gelijktijdig met capecitabine werd toegediend, was er een onverwachte trend naar een 19% hogere gemiddelde blootstelling aan capecitabine dan wanneer capecitabine alleen werd toegediend. Deze bevinding illustreert

dat de blootstelling aan capecitabine niet negatief wordt beïnvloed door gelijktijdige behandeling met esomeprazol. De farmacokinetiek van capecitabine verklaart dus niet de slechtere overleving van patiënten met kanker die gelijktijdig met PPI werden behandeld, zoals waargenomen in eerdere retrospectieve cohort analyses. Het is hoogst twijfelachtig of er sprake is van een interactie tussen capecitabine en PPI's. Gezien het overmatige gebruik van PPI's bij patiënten met kanker is het daarentegen wel verstandig om routinematig vast te stellen of PPI's daadwerkelijk nodig zijn tijdens de behandeling met capecitabine en, indien mogelijk, het voorschrijven hiervan te heroverwegen.

Haarverlies (oftewel door chemotherapie geïnduceerde alopecia (CIA)) is een verontrustende bijwerking van antikanker therapie en van paclitaxel in het bijzonder. Hoofdhuidkoeling zorgt in een hoge mate voor haarbehoud tijdens wekelijkse paclitaxel infusies (59% slagingspercentage), en is momenteel de enige methode waarvan is aangetoond dat het CIA kan voorkomen. Ondanks deze toepassing zijn er geen gegevens beschikbaar over de effecten van hoofdhuidkoeling op de blootstelling van de toegediende cytotoxische geneesmiddelen. In **Hoofdstuk 4** onderzochten we daarom de invloed van hoofdhuidkoeling op de farmacokinetiek van paclitaxel bij 38 patiënten die werden behandeld met paclitaxel. Daarvan ondergingen er 14 hoofdhuidkoeling en 24 geen hoofdhuidkoeling. Bij gelijktijdige hoofdhuidkoeling was de blootstelling aan paclitaxel in de systemische circulatie (het bloedplasma) 7% lager dan blootstelling zonder hoofdhuidkoeling. Dit verschil is niet klinisch relevant en valt ruim binnen de statistische marges voor gelijkwaardigheid van de groepen. Concluderend toonde ons onderzoek aan dat hoofdhuidkoeling een veilige interventie lijkt te zijn voor patiënten die worden behandeld met paclitaxel in de onderzochte dosering.

Overgevoeligheidsreacties behoren helaas tot de meest voorkomende bijwerkingen tijdens behandeling met paclitaxel. Dit komt door het middel Cremophor-EL, het oplosmiddel van paclitaxel, wat nodig is om het medicijn aan patiënten te kunnen geven. Deze bijwerking wordt meestal voorkomen of verminderd door premedicatie bestaande uit het corticosteroïd dexamethason in combinatie met een zogenaamde histamine 1 (H_1)-receptorantagonist (bijv. clemastine) en de histamine 2 (H_2)-receptorantagonist ranitidine. Het is des te opmerkelijker dat de toegevoegde waarde van ranitidine aan deze premedicatie regimes nooit systematisch in een klinische setting is onderzocht. In **Hoofdstuk 5** beschrijven we daarom een 'pre-post interventie non-inferioriteit' studie waarin ranitidine wordt beoordeeld als profylaxe voorafgaand aan de paclitaxel infusies. De primaire uitkomst van het onderzoek was de incidentie van HSR tijdens behandeling met paclitaxel. Patiënten die de eerste gift paclitaxel kregen, werden in de studie opgenomen. We bestudeerden 183 patiënten in de pre-interventie groep die het standaard pre-medicatie regime kregen bestaande uit dexamethason, clemastine en ranitidine.

En daarna nog eens 183 patiënten in de post-interventie groep; zij kregen het experimentele premedicatieregime zonder de H_2-antagonist ranitidine. De incidentie van HSR graad 3 was 4.4% in de pre-interventie groep en 1.6% in de post-interventie groep. Hieruit bleek dat ranitidine veilg kan worden weggelaten uit de premedicatie schema's. Dit is inmiddels dan ook doorgevoerd in de dagelijkse praktijk van het Erasmus MC Kanker Instituut. De studieresultaten dragen hiermee direct bij aan het verminderen van de risico's voor patiënten en zijn daarom van groot klinisch belang.

Voor paclitaxel zelf zijn strategieën nodig om de individualisering van de dosis te verbeteren. Paclitaxel wordt gekenmerkt door grote verschillen tussen patienten (interindividuele variabiliteit) in blootstelling en er wordt gesuggereerd dat er dosis-respons relatie is. De dosis van paclitaxel wordt van oudsher gebaseerd op het lichaamsoppervlak van een patiënt. Spier en vetmassa van het lichaam kunnen de systemische blootstelling aan chemotherapie echter ook beïnvloeden, met het risico op een lage(re) blootstelling en bijgevolg in theorie een minder effectieve behandeling of, omgekeerd, een hoge blootstelling met potentieel meer bijwerkingen tot gevolg. In **Hoofdstuk 6** hebben we aangetoond dat de dosis paclitaxel niet verder kan worden geïndividualiseerd door te corrigeren voor de parameters spiermassa, vetweefsel en spierdichtheid (zoals gemeten bij de ruggewervel L3 met behulp van een CT-scan). Deze parameters kunnen daarom niet als alternatief dienen voor de op lichaamsoppervlak gebaseerde dosering van paclitaxel.

In tegenstelling tot de systemische farmacokinetiek van paclitaxel is er weinig bekend over de concentraties van paclitaxel in tumorweefsel, laat staan over veranderingen daarvan in de loop van de tijd. Met name bij patiënten met slokdarmkanker kunnen (lage) intra-tumorale concentraties van paclitaxel van belang zijn, omdat een aanzienlijk deel van de patiënten geen baat heeft van de behandeling met paclitaxel (of ziekteprogressie vertoont kort na beëindiging van de behandeling). We veronderstelden voorafgaand aan ons onderzoek dat de ineffectiviteit van de behandeling (deels) zou kunnen worden verklaard door lage intra-tumorale concentraties van paclitaxel. Als verklaring voor deze mogelijke lage intra-tumorale concentraties hebben we de activiteit onderzocht van het eiwit dat (onder andere) paclitaxel uit (tumor) cellen pompt, namelijk ABCB1. In **Hoofdstuk 7** beschrijven we de resultaten van een verkennend onderzoek bij 14 patiënten met slokdarmkanker die worden behandeld met een standaardbehandeling op basis van paclitaxel, om veranderingen in de farmacokinetiek van paclitaxel in weefsel en verschillen in ABCB1-expressie te identificeren. Helaas kon onze primaire doelstelling niet worden aangetoond vanwege een lage hoeveelheid tumorcellen in de biopten.
Er werd echter wel een correlatie gezien tussen intra-tumorale oesofageale paclitaxel concentraties en paclitaxel plasma-concentraties tijdens de eerste cyclus van de pacli-

taxel behandeling. Interessant genoeg zagen we een hogere ABCB1-expressie in het zogenaamde adenocarcinoom dan in plaveiselcelcarcinoom, een verklaring zou kunnen zijn voor een beperkter effect van de standaardbehandeling bij patiënten met een adenocarcinoom, omdat dit zou kunnen leiden tot een snellere klaring van paclitaxel uit adenocarcinomen. De differentiële expressie van ABCB1 tussen deze histologische subtypes kan worden gebruikt om de systemische behandeling van slokdarmkanker verder te individualiseren.

DEEL II: DOELGERICHTE ORALE ANTIKANKER THERAPIE

In Deel II (Hoofdstukken 8-12) werden bijwerkingen en factoren onderzocht die de behandeling met doelgerichte orale antikanker therapie met tyrosinekinaseremmers, zoals sorafenib, kunnen beïnvloeden. De bijwerking 'hand-voet-huid-reactie' (HFSR) kan een grote impact hebben op de kwaliteit van leven van een patiënt en dosisaanpassingen vereisen. Eerder onderzoek toonde aan dat sorafenib zich kan ophopen in epidermale keratinocyten, gemedieerd door het eiwit 'organische anion transporter 6' (OAT6) en dat door sorafenib-geïnduceerde HFSR kan worden voorkomen door gelijktijdige behandeling met de OAT6-remmer probenecid. In **Hoofdstuk 8** hebben we de invloed van probenecid op de blootstelling aan sorafenib en de toxiciteit bestudeerd en daarbij vonden wij een significante afname van de blootstelling aan sorafenib met 27% wanneer probenecid gelijktijdig met sorafenib werd gebruikt. HFSR trad op bij 10 van de 16 patiënten (63%) en was niet gerelateerd aan probenecid inname. Aangezien probenecid de incidentie of ernst van HFSR niet leek te beïnvloeden en er een duidelijk negatief effect was op de systemische blootstelling aan sorafenib, kan probenecid helaas niet worden gebruikt om HFSR te voorkomen. Over het algemeen zijn geen goede behandelopties voor HFSR, anders dan profylactisch gebruik van op ureum gebaseerde crèmes.

Schildklierdisfunctie is een andere gekende bijwerking van de behandeling met sorafenib. De incidentie van hypothyreoïdie (trage werking van de schildklier) tijdens behandeling met sorafenib varieert van 18-50%. In **Hoofdstuk 9** analyseerden we een cohort patiënten behandeld met sorafenib in verband met hepatocellulair carcinoom (HCC, leverkanker) om het effect van deze behandeling op het schildkliermetabolisme te onderzoeken. Thryreoïditis trad op in 7% van de gevallen en bij alle patiënten waren de antistoffen tegen schildklierperoxidase of tegen de TSH-receptor verhoogd. Sorafenib geïnduceerde hypothyreoïdie treedt op zo'n 5 maanden na aanvang van de behandeling, is onomkeerbaar en wordt meestal veroorzaakt door ontsteking van de schildklier (subacute thryreoïditis). Schildklierdisfunctie kan worden behandeld zonder dat er een dosisverlaging of stopzetting van de behandeling met sorafenib hoeft plaats te vinden.

Voor de klinische praktijk wordt aanbevolen om het TSH te meten aan het begin van de behandeling en op de eerste dag van elke behandelcyclus.

Het risico op bijwerkingen tijdens behandeling met sorafenib wordt verder verhoogd door mogelijke geneesmiddelinteracties wanneer patiënten gelijktijdig andere medicijnen nodig hebben. Van de patiënten die zijn behandeld met een levertransplantatie voor leverkanker, heeft 20% een recidief van de ziekte. Op dat moment is sorafenib een reguliere behandel optie. Immunosuppressieve therapie zoals tacrolimus is nodig om afstoting van de lever te voorkomen en daarmee de overleving te verbeteren bij patiënten die een levertransplantatie hebben ondergaan. Dosisverlaging en stopzetting van sorafenib vanwege bijwerkingen komen veel vaker voor na levertransplantatie (15-77% van de patiënten). Dit kan mogelijk worden verklaard door een interactie tussen sorafenib en deze immunosuppressiva. In **Hoofdstuk 10** worden vier patiënten beschreven die werden behandeld met sorafenib in combinatie met het medicijn tacrolimus voor een recidief van de leverkanker na een eerdere levertransplantatie. Na verloop van tijd nam de blootstelling aan sorafenib af tijdens deze combinatie bij alle vier de patiënten. Aangezien de sorafenib concentraties daalden -- zelfs nadat bij twee patiënten de behandeling met tacrolimus was gestaakt -- konden we niet bevestigen dat tacrolimus de farmacokinetiek van sorafenib op een voorspelbare manier beïnvloedde. Aangezien we geen duidelijke interactie tussen sorafenib en tacrolimus hebben gevonden, kunnen we niet uitsluiten dat therapie-ontrouw aan sorafenib mogelijk een rol speelde bij de waargenomen afname van de blootstelling aan sorafenib in de loop van de tijd. Het is uit eerder onderzoek bekend dat 50% van de patiënten die langdurig orale antikankertherapie krijgen, de neiging hebben zich niet aan de voorgeschreven therapie te houden, wat kan leiden tot mindere blootstelling en daarmee verminderde effectiviteit van de therapie. Alle vier de patiënten in deze serie kregen ernstige bijwerkingen wanneer de dosering sorafenib hoger was dan 400 mg per dag; mogelijk als gevolg van een farmacodynamische interactie tussen tacrolimus en behandeling met sorafenib. Het mechanisme daarachter is momenteel niet bekend. We raden daarom aan sorafenib bij deze groep patiënten niet hoger te doseren dan 400 mg per dag. Dit lijkt de veiligste optie om deze groep patiënten op een verdraagbare behandeling te houden.

Gerichte therapieën die de vasculaire endotheliale groeifactor (VEGF)-signaleringsroute remmen, zoals sorafenib, kunnen hart- en vaatziekten veroorzaken. In **Hoofdstuk 11** werd de cardiovasculaire toxiciteit van VEGF-remmers (VEGFI's) en immuun checkpoint remmers (ICI's) beschreven. Een combinatiebehandeling van VEGFI's en ICI's gaat gepaard met een duidelijke verhoging van het risico op hart- en vaatziekten, aangezien beide soorten geneesmiddelen synergetisch cardiovasculaire bijwerkingen veroorzaken. Zo lijkt hoge bloeddruk, die optreedt bij een groot deel van de patiënten die VEGFI-

therapie krijgen, een risicofactor te zijn voor ICI-geïnduceerde vasculaire bijwerkingen. Een uitgebreide beoordeling zoals medische voorgeschiedenis gericht op hart- en vaatziekten, meten van de bloeddruk, een elektrocardiogram voor start van de behandeling en adequate monitoring van het optreden van cardiovasculaire gebeurtenissen tijdens en na de behandeling met deze geneesmiddelen is belangrijk aangezien een vroege ontdekking van cardiotoxiciteit een adequate interventie mogelijk maakt. Een multidisciplinaire cardio-oncologische benadering is hierbij essentieel om de cardiovasculaire gezondheid van deze kankerpatiënten optimaliseren

De meest voorkomende en best gekarakteriseerde cardiovasculaire bijwerking van de behandeling met VEGFI's is een bloeddrukstijging. De behandeling van deze bloeddrukstijging kan worden gedaan met conventionele medicijnen tegen hoge bloeddruk. Echter, omdat in een preklinische studie bij ratten werd aangetoond dat de bloeddrukstijging als gevolg van de VEGFI sunitinib zoutgevoelig was, wilden we onderzoeken of deze zoutgevoeligheid kan worden gebruikt voor de behandeling van patienten met een VEGFI-geïnduceerde hypertensie. In **Hoofdstuk 12** beschrijven we een prospectieve cohortstudie bij 16 patiënten met VEGFI-geïnduceerde hypertensie die werden behandeld met een zoutbeperkt dieet van minder dan 4 gram zout inname per dag. Het primaire eindpunt van de studie was het gemiddelde verschil in bloeddrukstijging tussen een behandelcyclus met en zonder zoutbeperking. Bij aanvang was de gemiddelde bloeddruk overdag 95 mmHg, en steeg deze tot 110 mmHg na ongeveer 4 weken VEGFI-behandeling, wat een verschil van 15 mmHg opleverde. Tijdens het zoutbeperkt dieet was de gemiddelde bloeddruk 94 mmHg, en steeg deze met 8 mmHg tot een gemiddelde bloeddruk van maar 102 mmHg. De gemiddelde bloeddrukstijging als gevolg van het zoutbeperkt dieet was daarmee significant verminderd met 7 mmHg. De zoutbeperking via de voeding (brood) werd goed verdragen en 5 patiënten hadden de wens om door te gaan met het dieet nadat ze het onderzoek hadden beëindigd. We toonden hiermee aan dat zoutbeperking in de voeding een zeer effectieve behandeling is om de bloeddrukstijging tijdens een VEGFI-behandeling te verlagen. Het onderzoek vergroot tevens het bewustzijn van cardio-oncologische problemen bij de behandeling met VEGFI's en laat zien dat patiënten met kanker gemotiveerd zijn voor leefstijlinterventies. Dit gegeven kan dienen als opmaat naar meer zelfmanagement tijdens de behandeling van oncologische patiënten.

CONCLUSIES EN TOEKOMSTPERSPECTIEVEN

De resultaten van dit proefschrift hebben ons begrip vergroot van de blootstelling aan de chemotherapeutische geneesmiddelen capecitabine en paclitaxel, en aan VEGF-remmers, inclusief sorafenib. Hoewel niet al deze nieuwe inzichten hebben geleid tot beleidsveranderingen in de klinische praktijk, kunnen ze wel als aanbevelingen dienen

voor de dagelijkse praktijk en mogelijk leiden tot nieuwe onderzoeken. Zo wordt het verlies van vingerafdrukken bij capecitabine tegenwoordig als bijwerking genoemd in de standaard voorlichting aan de patiënt, wordt de H_2-receptorantagonist ranitidine weggelaten uit de premedicatie schema's van paclitaxel in het Erasmus MC Kankerinstituut en blijft smeren met crèmes als preventieve interventie voor de hand-voet-huid reactie (HFSR) tijdens de behandeling met sorafenib de standaard aanbeveling. Met ons onderzoek naar het voorschrijven van maagzuurremmers tijdens de behandeling met capecitabine hebben we geprobeerd de wereldwijde waarschuwing over blootstelling aan deze combinatie te nuanceren. Het onderzoek naar zoutbeperking in de voeding als nieuwe interventie bij de behandeling van hypertensie veroorzaakt door VEGF-remmers illustreert dat leefstijlinterventies eenvoudigweg polyfarmacie bij patiënten met kanker kunnen verminderen.

De voortdurende beoordeling van de veiligheid en verdraagbaarheid van geneesmiddelen is van cruciaal belang om de risico's en de voordelen van een antikankerbehandeling tegen elkaar af te wegen. Zoals gezegd verdient de aanpak van behandeling gerelateerde bijwerkingen speciale aandacht. Hierbij is niet alleen het optreden en het verminderen van bijwerkingen belangrijk, maar inzicht in prognostische informatie van individuele factoren is even belangrijk om een succesvolle behandeling tegen kanker mogelijk te maken. Kennis van therapie gerelateerde bijwerkingen en de impact hiervan op de kwaliteit van leven van patiënten beïnvloedt immers de keuze van de arts voor de individuele patiënt.
In de Europese Unie worden de oncologische geneesmiddelen gereguleerd en geregistreerd door het Europees Geneesmiddelenbureau (European Medicines Agency, EMA). Voor de Nederlandse praktijk beoordeelt de NVMO (Nederlandse Vereniging voor Medische Oncologie) -commissie ter Beoordeling van Oncologische Middelen (CieBOM) de klinische waarde van nieuw geregistreerde geneesmiddelen, behandelmethoden en behandelindicaties op het gebied van de medische oncologie op basis van veelal gerandomiseerde fase III-onderzoeken om tot landelijke afstemming te komen binnen de begroepsgroep aangaande het toepassen van nieuwe geneesmiddelen. Dit doen zij op basis van de zogeheten PASKWIL- (Palliatief, Adjuvant, Specifieke bijwerkingen, Kwaliteit van leven, Impact van de behandeling, Level of evidence (niveau van bewijskracht)) criteria. Het beoordelingsproces, indien van toepassing, wordt afgestemd met de beoordeling door Zorginstituut Nederland.
In de klinische praktijk worden (nieuwe) antikanker medicijnen over het algemeen voorgeschreven aan groepen patiënten zonder specifieke aandacht voor hun individuele kenmerken. Deze patiënten kunnen in een slechtere klinische toestand verkeren dan degenen die hebben deelgenomen aan klinische onderzoeken tijdens de ontwikkeling van het betreffende medicijn. Daarom kan de baten-risicoverhouding uiteindelijk min-

der gunstig zijn. Bovendien zijn er aanwijzingen dat (met name zeldzame) bijwerkingen in klinische studies onder-gerapporteerd worden. Dit betekent dat bij het op de markt komen van nieuwe behandelingen waakzaamheid is geboden ten aanzien van bijwerkingen, maar ook met de langetermijngevolgen van antikankerbehandelingen rekening moet worden gehouden. Tijdens de standaardbehandeling moeten de inspanningen daarom enerzijds gericht zijn op het signaleren van niet-gemelde kenmerken van antikankertherapie die pas op langere termijn of met voortschrijdend inzicht kunnen worden opgemerkt en anderzijds op het beperken van de ziektelast door frequente bijwerkingen adequaat te behandelen zoals beschreven in dit proefschrift.

Er zijn nog tal van belangrijke vragen en aandachtspunten betreffende bijwerkingen: hoe zit het met sekseverschillen en bijwerkingen als strategie voor het aanpassen van de dosis, wetende dat vrouwen die een behandeling met capecitabine ondergaan meer dosisverlagingen van capecitabine nodig hebben dan mannen vanwege bijwerkingen? Wat kan er worden gedaan om premedicatie schema's te optimaliseren voor andere chemotherapeutische middelen dan paclitaxel? Zo is dexamethason ter voorkoming van overgevoeligheid reacties, misselijkheid en braken, in het premedicatie schema met wekelijkse paclitaxel ook interessant vanwege de bijwerkingen op korte en lange termijn zoals slapeloosheid, stemmingswisselingen, misselijkheid en opgezette buik, gewichtstoename, huiduitslag en diabetes mellitus. Er zijn verschillende onderzoeken uitgevoerd om de dosis en de toedieningsweg van dexamethason te optimaliseren. De meeste onderzoeken zijn echter retrospectief, missen de omvang om verschillen op te sporen of hebben controversiële (interpretaties van) resultaten. Daarom zou de optimale dosis en route opnieuw geëvalueerd moeten worden in een bevestigende prospectieve grote studie voor zowel de patiëntveiligheid als om de medische gemeenschap te overtuigen met wetenschappelijk onderbouwde resultaten (evidence-based medicine).
Wat betreft het fenomeen HFSR, totdat een effectieve behandeling voor deze bijwerking is gevonden, blijft het van cruciaal belang om patiënten goede voorlichting hierover te geven en op HFSR te controleren tijdens de behandeling met sorafenib, aangezien is aangetoond dat vroege detectie en tijdige dosisaanpassing de behandelduur en de algehele overleving kan verbeteren. Het is belangrijk om het HFSR tijdig te herkennen en bij aanvang van de behandeling een onderzoek van de huid uit te voeren met speciale aandacht voor de handpalmen en voetzolen en deze gedurende de eerste 6 weken van de behandeling regelmatig te controleren. Dit kan het beste worden gedaan door zorgverleners die ervaring hebben met deze en andere bijwerkingen als geheel.

APPENDICES

APPENDIX 2

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APPENDICES

APPENDIX 3

CURRICULUM VITAE

Leni van Doorn werd geboren op 2 juli 1963 te Eindhoven. Na de middelbare school te hebben afgerond in Utrecht, werd zij toegelaten tot de inservice-opleiding verpleegkundige-A in het Streekziekenhuis Prinses Beatrix te Gorinchem en deels in het Linge Ziekenhuis te Leerdam. Na vervolgens een jaar te hebben gewerkt in het Sint Elisabeth Ziekenhuis te Leiderdorp begon zij in 1986 aan de Brede Basis Intensive Care opleiding in het Dijkzigt Ziekenhuis te Rotterdam om vervolgens aldaar als intensive care verpleegkundige te gaan werken op de Heelkunde Intensive Care onder supervisie van prof.dr. H.A. Bruining. Zij werd daar in staat gesteld een klinische studie te begeleiden onder supervisie van prof.dr. C.H.J. van Eijck en dr. A. van der Gaast. In de avonduren volgde zij aan de Open Universiteit het academisch kernprogramma Kunst en cultuurwetenschappen. In 2000 maakte zij de overstap naar de afdeling Interne Oncologie om als researchverpleegkundige te participeren in vroeg klinisch onderzoek. In 2006 kreeg zij de mogelijkheid de opleiding te volgen tot Master Advanced Nursing Practice aan de Hogeschool Leiden met als medisch leermeester dr. F.A.L.M. Eskens. Het afstudeeronderzoek was getiteld `Aanpak van bijwerkingen en informatieverstrekking bij behandeling met sunitinib en sorafenib'. In 2014 studeerde zij af aan de Universiteit van Amsterdam binnen de WO-master of Science in Evidence Based Practice in Health Care. Zij startte haar PhD traject onder leiding van prof.dr. A.H.J. Mathijssen en copromotor dr. S. Bins eind 2016 binnen haar huidige functie als verpleegkundig specialist op de afdeling Interne Oncologie van het Erasmus MC Kanker Instituut te Rotterdam. Datzelfde jaar ontving zij de Meyboom Zorgprijs voor het onderzoek naar verlies van vingerafdrukken bij het medicijn capecitabine. Haar aandachtsgebieden zijn behandeling van patiënten met gastro-intestinale tumoren regulier als in studieverband en behandeling van patiënten die met doelgerichte antikankertherapie worden behandeld.

APPENDICES

APPENDIX 4

LIST OF PUBLICATIONS

Publications included in this thesis

van Doorn L, Heersche N, de Man FM, de Bruijn P, Bijl I, Oomen- de Hoop E, Eskens FALM, van der Gaast A, Mathijssen RHJ, Bins S. Effect of the proton pump inhibitor esomeprazole on the systemic exposure of capecitabine: results of a randomized crossover trial. *Clinical Pharmacology & Therapeutics*. 2022;111(2):455-460

van Eerden RAG*, **van Doorn L***, de Man FM, Heersche N, Doukas M, van den Bosch TPP, Oomen-de Hoop E, de Bruijn P, Bins S, Eman Ibrahim E, Nikkessen S, Friberg, Koolen SLW, Spaander MCW, Mathijssen RHJ. Tissue type differences in ABCB1 expression and paclitaxel tissue pharmacokinetics in patients with esophageal cancer. *Front. Pharmacol.* 2021;12:759146. *shared authorship

van Doorn L*, van Rosmalen MM*, van der Deure WM, Oomen- de Hoop E, Porazzo R, Wijngaard SM, Boere IB, Ibrahim E, Peter de Bruijn, Friberg LE, Koolen SLW, Mathijssen RHJ, Jager A. Effect of scalp cooling on the pharmacokinetics of paclitaxel. *Cancers (Basel)*. 2021;13(15):3915. *shared authorship

van Dorst DCH, **van Doorn L**, Mirabito Colafella KM, Olivier C. Manintveld OC, Hassing HC, Danser AHJ, Mathijssen RHJ, Versmissen J. Cardiovascular toxicity of angiogenesis inhibitors and immune checkpoint inhibitors: synergistic anti-tumour effects at the cost of increased cardiovascular risk? *Clinical Science*. 2021;135;1649-1688.

van Doorn L, Crombag M-R BS, Rier HN, van Vugt JLA, van Kesteren C, Bins S, Mathijssen RHJ, Levin M-D, Koolen SLW. The influence of body composition on the systemic exposure of paclitaxel in esophageal cancer patients. *Pharmaceuticals (Basel)*. 2021;14(1):47

Hussaarts KGAM, **van Doorn L**, Bins S, Sprengers D, de Bruijn P, van Leeuwen RWF, Koolen SLW, van Gelder T, Mathijssen RHJ. Combining sorafenib and immunosuppression in post liver transplantation recipients with hepatocellular carcinoma. *Pharmaceuticals (Basel)*. 2021;14(1):46.

Cox JM*, **van Doorn L***, Malmberg R, Oomen – de Hoop E, Bosch TM, van den Bemt PMLA, Boere IA, Jager A, Mathijssen RHJ, van Leeuwen RWF. The added value of H_2-antagonists in premedication regimens during paclitaxel treatment. *Br J Cancer*. 2021;124(10):1647-1652. *shared authorship

Hussaarts KGAM*, **van Doorn L***, Eechoute K, Damman J, Fu Q, van Doorn N, Eisenmann ED, Gibson AA, Oomen-de Hoop E, de Bruijn P, Baker SD, Koolen SLW, van Gelder T, van

Leeuwen RWF, Mathijssen RHJ, Sparreboom A, Bins S. Influence of probenecid on the pharmacokinetics and pharmacodynamics of sorafenib. *Pharmaceutics.* 2020;12(9)788. *shared authorship

Beukhof CM, **van Doorn L**, Visser TJ, Bins S, Visser WE, van Heerebeek R, van Kemenade FJ, de Rijke YB, de Herder WW, Chaker L, Mathijssen RH, Peeters RP. Sorafenib-Induced Changes in Thyroid Hormone Levels in Patients Treated for Hepatocellular Carcinoma. *J Clin Endocrinol Metab*. 2017;102(8):2922-2929.

van Doorn L, Veelenturf S, Binkhorst L, Bins S, Mathijssen RHJ. Capecitabine and the Risk of Fingerprint Loss. *JAMA Oncol. 2017;3(1):122-123*.

Other publications

Puglisi M, Molife LR, de Jonge MJA, Khan KH, **van Doorn L**, Forster MD, Blanco M, Gutierrez M, Franklin C, Busman T, Yang J, Eskens FALM. A Phase I study of the safety, pharmacokinetics and efficacy of navitoclax plus docetaxel in patients with advanced solid tumors: Findings of a phase I study. *Future Oncol.* 2021;17(21):2747-2758.

Buisman FE, Grünhagen DJ, Homs MYV, Grootscholten C, Filipe WF, Kemeny NE, Cercek A, D'Angelica MI, Donswijk ML, **van Doorn L**, Emmering J, Jarnagin WR, Kingham TP, Klompenhouwer EG, Kok NFM, Kuiper MC, Moelker A, Prevoo W, Versleijen MWJ, Verhoef C, Kuhlmann KFD, Groot Koerkamp B. Adjuvant Hepatic Arterial Infusion Pump Chemotherapy After Resection of Colorectal Liver Metastases: Results of a Safety and Feasibility Study in The Netherlands. *Ann Surg Oncol.* 2019;26(13):4599-4607.

de Man FM, van Eerden RAG, Oomen-de Hoop E, Veraart JN, van Doorn, N, **van Doorn L**, van der Gaast A, Mathijssen RHJ. Efficacy and Toxicity of Weekly Carboplatin and Paclitaxel as Induction or Palliative Treatment in Advanced Esophageal Cancer Patients. Cancers. 2019 Jun13;11(6).

Buisman FE, Homs MYV, Grünhagen DJ, Filipe WF, Bennink RJ, Besselink MGH, Borel Rinkes IHM, Bruijnen RCG, Cercek A, D'Angelica MI, van Delden OM, Donswijk ML, **van Doorn L**, Doornebosch PG, Emmering J, Erdmann JI, IJzerman NS, Grootscholten C, Hagendoorn J, Kemeny NE, Kingham TP, Klompenhouwer EG, Kok NFM, Koolen S, Kuhlmann KFD, Kuiper MC, Lam MGE, Mathijssen RHJ, Moelker A, Oomen-de Hoop E, Punt CJA, te Riele, WW, Roodhart, JML, Swijnenburg RJ, Prevoo W, Tanis PJ, Vermaas M, Versleijen MWJ, Veuger FP, Weterman MJ, Verhoef C, Groot Koerkamp B. Adjuvant hepatic arterial infusion pump chemotherapy and resection versus resection alone in patients with low-risk resectable

colorectal liver metastases – the multicenter randomized controlled PUMP trial. *BMC Cancer*. 2019;19(1):327.

Versmissen J, **van Doorn L**, Mirabito Colafella KM, Mathijssen RH, Danser AHJ. Sunitinib-induced blood pressure rise does not involve aldosterone: observations in a patient after bilateral adrenalectomy. *J Hypertens*. 2018;36(11):2279-2280.

Bins S, **van Doorn L**, Phelps MA, Gibson AA, Hu S, Li L, Vasilyeva A, Du G, Hamberg P, Eskens F, de Bruijn P, Sparreboom A, Mathijssen R, Baker SD. Influence of OATP1B1 Function on the Disposition of Sorafenib-β-D-Glucuronide. *Clin Transl Sci*. 2017;10(4):271-279.

Verheijen RB, Bins S, Mathijssen RH, Lolkema M, **van Doorn L**, Schellens JH, Beijnen JH, Langenberg MH, Huitema AD, Steeghs N. Individualized pazopanib dosing: a prospective feasibility study in cancer patients. *Clin Cancer Res*. 2016;22(23)5738-5746.

Bins S, Lenting A, El Bouazzaoui S, **van Doorn L**, Oomen-de Hoop E, Eskens FA, van Schaik RH, Mathijssen RH. Polymorphisms in SLCO1B1 and UGT1A1 are associated with sorafenib-induced toxicity. *Pharmacogenomics.* 2016;17(14):1483-1490.

van der Linden S, **van Doorn L**, van Eck J, Geilvoet W, Mulders G. Formation of a peer review group for advanced practice nurses: Learning from and with colleagues. *Women's HealthCare*. 2015;34-37.

Lolkema MP, Bohets HH, Arkenau HT, Lampo A, Barale E, De Jonge MJA, **van Doorn L**, Hellemans P, deBono JS, Eskens FALM. The c-Met tyrosine kinase inhibitor JNJ-38877605 causes renal toxicity through species- specific insoluble metabolite formation. *Clin Cancer Res*. 2015;15;21(10):2297-2304.

Oldenhuis CN, Loos WJ, Esteves B, **van Doorn L**, Cotreau MM, Strahs AL, den Hollander MW, Gietema JA, de Vries EGE, Eskens FALM. A Phase Ib Study of the VEGF Receptor Tyrosine Kinase Inhibitor Tivozanib and Modified FOLFOX-6 in Patients with Advanced Gastrointestinal Malignancies. *Clinical Colorectal Cancer*. 2015;14(1):18-24.

Eskens FALM, Tresca P, Tosi D, **van Doorn L**, Fontaine H, van der Gaast A, Veyt-Follet C, Oprea C, Hospitel M, Dieras V. A phase I pharmacokinetic study of the vascular disrupting agent ombrabulin (AVE8062) and docetaxel in advanced solid tumours. *Br J Cancer*. 2014;110(9):2170-2177.

Sprowl JA, **van Doorn L**, Hu S, van Gerven L, de Bruijn P, Li L, Gibson AA, Mathijssen RHJ, Sparreboom A. Conjunctive therapy of cisplatin with the OCT2 inhibitor cimetidine: influence on antitumor efficacy and systemic clearance. *Clin Pharmacol Ther*. 2013;94(5):585-592.

de Graan AJ, Loos WJ, Friberg LE, Baker SD, van der Bol JM, **van Doorn L**, Wiemer EA, van der Holt B, Verweij J, Mathijssen RHJ. Influence of smoking on the pharmacokinetics and toxicity profiles of taxane therapy. *Clin Cancer Res.* 2012;18(16):4425-4432.

Banerij U, **van Doorn L**, Papadatos-Pastos D, Kristeleit R, Debnam P, Tall M, Stewart A, Raynaud F, Garrett MD, Toal M, Hooftman L, De Bono JS, Verweij J, Eskens FALM. A Phase I Pharmacokinetic and Pharmacodynamic Study of CHR-3996, an Oral Class I Selective Histone Deacetylase Inhibitor in Refractory Solid Tumors. *Clin Cancer Res*. 2012;18(9):2687-2694.

Eskens FALM, de Jonge MJA, Bhargava P, Isoe T, Cotreau MM, Esteves B, Hayashi K, Burger H, Thomeer M, **van Doorn L**, Verweij J. Biologic and clinical activity of tivozanib (AV-951, KRN-951), a selective inhibitor of VEGF receptor- 1, -2, and -3 tyrosinekinases, in a 4-week-on 2- week-off schedule in patients with advanced solid tumors. Clin Cancer Res. 2011 Nov 15;17(22);7156-7163.

van Doorn L, Eskens FALM, Visser ThJ, van der Lugt A, Mathijssen RHJ, Peeters R. Sorafenib Induced Thyroiditis in Two Patients with Hepatocellular Carcinoma. *Thyroid.* 2011;21 (2) 197-202.

Eskens FALM, Steeghs N, Verweij J, Bloem JL, Christensen O, **van Doorn L**, Ouwerkerk J, de Jonge MJA, Nortier JWR, Kraetzschmar J, Rajagopalan P, Gelderblom H. Phase I Dose Escalation Study of Telatinib, a Tyrosine Kinase Inhibitor of Vascular Endothelial Growth Factor Recepter 2 and 3, Platelet-Derived Growth Factor Receptor β, and c-Kit, in Patients with Advanced or Metastatic Solid Tumors. *J Clin Oncol*. 2009;27(25):4169-4176.

Kitzen JJEM, de Jonge MJA, Lamers, CHJ, Eskens FALM, van der Biessen D, **van Doorn L**, ter Steeg J, Brandely M, Puozzo Ch, Verweij J. Phase I dose-escalation study of F60008, a novel apoptosis inducer, in patients with advanced solid tumours. *Eur J Cancer.* 2009;45(10):1764-1772.

Eskens FA, Mom CH, Planting AST, Gieteman JA, Amelsberg A, Huisman H, **van Doorn L**, Burger H, Stopfer P, Verweij J, de Vries EGE. A phase I dose escalation study of BIBW 2992, an irreversible dual inhibitor of epidermal growth factor receptor 1 (EGFR) and 2

(HER2) tyrosine kinase in a 2-week on, 2-week off schedule in patients with advanced solid tumours. *Br J Cancer*. 2008;98(1):80-85.

van Meerten E, Eskens F, van Gameren EC, **van Doorn L**, van der Gaast. A. First-line treatment with oxaliplatin and capecitabine in patients with advanced or metastatic oesophageal cancer: a phase II study. *Br J Cancer.* 2007;96(9):1348-1352.

de Jonge MJA, van der Gaast A, Planting AST, **van Doorn L**, Lems A, Boot I, Wanders J, Satomi M, Verweij J. Phase I and pharmacokinetic study of the dolastatin 10 analogue TZT-1027, given on days 1 and 8 of a 3-week cycle in patients with advanced solid tumors. *Clin Cancer Res.* 2005;11(10):3806-3813.

de Jonge MJA, Kaye S, Verweij J, Brock C, Reade S, Scurr M, **van Doorn L**, Verheij C, Loos WJ, Brindley C, Mistry P, Cooper M, Judson I. Phase I and pharmacokinetic study of XR11576, an oral topoisomerase I and II inhibitor, administered on days 1-5 of a 3-weekly cycle in patients with advanced solid tumours. *Br J Cancer*. 2004;91(8):1459-1465.

APPENDICES

APPENDIX 5

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	Copromotor: dr. S. Bins

1. PhD training	**Year**	**Workload (ECTS**
General courses		
BROK Course	2018, 2022	3.0
Scientific Integrity Course Erasmus MC	2019	0.3
Specific courses		
Academic writing and speaking English for professionals	2017	4.0
Microsoft Excel Advanced workshop	2017	0.4
OpenClinica database building	2017	0.3
PhD presentation skills	2017	1.0
Leadership programme	2020	2.0
Oral presentations		
Scientific meeting Medical Oncology Erasmus MC	2016	0.5
5th Daniel den Hoed Day	2017	0.5
3de Lustrum EBP UvA	2017	0.5
Therapeutic Drug Monitoring Oncolytics	2017	0.5
Internal Medicine Clinical Demonstration Erasmus MC	2018	0.5
Retraite Translational Pharmacology Group	2017, 2020	1.0
Translational Pharmacology Meeting	2017- 2021	2.0
Poster presentations		
ESMO Annual Meeting	2017	0.3
MOVD/EDH symposium	2019	0.3
ESMO Annual Meeting	2019	0.3
ESMO Annual Meeting (e-Poster)	2020	0.3
ESC Congress (e-Poster)	2021	0.3
(Inter) national conferences		
ASCO Annual Meeting, Chicago, IL, USA	2016	2.0
ESMO Annual Meetings, Barcelona, Madrid, Virtual	2017, 2019, 2020	6.0
Pharmacology & Therapeutics, Rotterdam	2017	1.0
NVKFB Scientific Spring Meeting Utrecht	2017	0.5
Coeur Symposium Erasmus MC	2017	0.5
Scientific meetings Medical Oncology	2017- 2021	2.5
Regional GI Symposium	2017, 2018, 2021	1.5

Research Day ACE | Pharmacology& therapeutics	2019	0.5
Retraite Translational Pharmacology Group	2017, 2020	0.5
Cardio-Oncology symposium, Virtual	2021	0.5
ESMO World Congress on Gastro Intestinal Cancer, Virtual	2021	1.5
De périphérique van ESMO: 'Verborgen parels uit Parijs'	2021	0.5
Cardiotoxiciteit bij kankertherapie	2021	0.5
Personalized Medicine Research Meetings	2017- 2021	5.0
2. Teaching		
Education oncology nurses, Erasmus MC Cancer Institute	2017- 2021	2.5
Minor medical students	2019	0.25
Supervising Master thesis		
Ivo Bijl (medical student)	2018	1.0
Nadia van Doorn (medical student)	2019	1.0
Niels Heersche (medical student)	2020	1.0
Demi Maaskant (medical student)	2021	1.0
3. Other		
Meijboom Zorgprijs 2016		
V&VN travel grant to attend ESMO 2017 (Madrid, Spain)		
V&VN travel grant to attend ESMO 2019 (Barcelona, Spain)		

APPENDICES

APPENDIX 6

DANKWOORD

Dit proefschrift is het resultaat van een fantastische kans om mezelf wetenschappelijk te ontwikkelen. Ik heb ervan genoten dat ik mij door onderzoek heb kunnen verdiepen in specifieke bijwerkingen van antikankerbehandelingen en ook daarmee een brug te slaan tussen het verpleegkundig- en medisch domein waarin ik als verpleegkundig specialist werkzaam ben. Het contact met de vele patiënten wil ik in dit verband zeker ook noemen. Ik hoop dat de resultaten bijdragen aan een verantwoorde zorg voor de patiënten die deze behandelingen ondergaan.

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