Treatment optimisation and pharmacogenetics of systemic and intraperitoneal chemotherapy in colorectal cancer

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TREATMENT OPTIMISATION AND PHARMACOGENETICS OF SYSTEMIC AND INTRAPERITONEAL CHEMOTHERAPY IN COLORECTAL CANCER

EMMA HULSHOF

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CONTENTS

CHAPTER 1

General introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in both males and females in the Netherlands. In 2021, nearly 13,000 patients were newly diagnosed with CRC and the mortality was 4,500 [1]. Approximately 20% of the patients have metastatic disease at the primary diagnosis of CRC [1]. Another approximately 20% develop metastatic disease later on [2]. The most common metastatic sites are the liver, the peritoneum and the lungs. Chances for curation in the metastatic setting of CRC are still low with a 5-years survival of only 12% [1]. Especially in patients with colorectal peritoneal metastasis, survival is found to be worse compared to patients with metastatic disease at other distant sites [3].

In the Netherlands, most patients without distant metastasis undergo surgery (95%). Besides surgery, chemotherapy is one of the CRC treatment modalities, especially in the treatment of advanced and metastatic disease. In total, 61% of the stage III colon cancer patients undergo treatment with adjuvant chemotherapy. In addition, 50% of the patients with distant metastatic disease (stage IV) undergo palliative chemotherapy treatment. [1] However, it is well known that treatment with chemotherapy comes with challenges, such as (severe) adverse events leading to loss of quality of life, treatment discontinuation and sometimes even death. Moreover, chances for curation in the metastatic setting are low. Therefore, there is a large window of opportunity to improve both safety as well as efficacy of chemotherapeutic treatment for the individual patient.

GENETIC BIOMARKERS

A possible approach to improve chemotherapeutic treatment for CRC patients could be the discovery, validation and implementation of new genetic biomarkers. The use of genetic biomarkers allows to identify patients that are at higher risk for severe adverse drug events and to select patients which will benefit the most from chemotherapy. For example, a genotype test that was recommended in 2020 by the European Medicines Agency in order to prevent severe adverse events and even fatal toxicity during fluoropyrimidines treatment was pre-therapeutic genotyping of DPYD in patients treated with fluoropyrimidines [4]. This has led to wider implementation of this upfront genotype test, and has brought us a step closer to personalised medicine and safer dosing of chemotherapy [5].

IMPLEMENTATION OF GENETIC BIOMARKERS

Another genetic biomarker that seems very promising in preventing severe adverse drug events in patients treated with irinotecan is UGT1A1 **28*. Irinotecan is frequently prescribed in patients with metastatic colorectal cancer or pancreatic cancer. Irinotecan is a prodrug that is activated via carboxylesterases in the liver and blood to SN38, which in turn is glucuronidated by UDP-glucuronosyltransferase 1A1 (UGT1A1) in the liver and intestines into SN38-glucuronide (SN38-G). UGT1A1 is the main enzyme responsible for the inactivation of SN38. Genetic variance in the *UGT1A1* gene leads to a decreased activity of the UGT1A1 enzyme [6]. More specific, the *UGT1A1**28 variant leads to a 18–33% reduced expression of *UGT1A1*, which in turn leads to higher SN-38 levels and a hence a higher risk of severe adverse drug events in patient carrying this variant allele [7].

While there is ample evidence in the literature on the association between *UGT1A1*28* and severe toxicity of irinotecan [8, 9] – yet – *UGT1A1* genotyping is not being routinely applied. Therefore, in the first part of this thesis we aim to implement *UGT1A1* genotype-guided dosing of irinotecan in clinical practice.

DISCOVERY AND VALIDATION OF GENETIC BIOMARKERS

Prior to implementation, discovery and validation of new genetic biomarkers is essential. This is especially true for the colorectal peritoneal metastasis population, since survival in patients with colorectal peritoneal metastasis is less favourable compared to patients with other metastatic CRC. The current treatment of colorectal peritoneal metastasis is cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy (CRS + HIPEC), which has already brought a major gain in survival compared to palliative chemotherapy only [10, 11]. HIPEC was added to CRS in order to minimise invisible residual cancer in the peritoneum. However, CRS + HIPEC treatment is not without complications; around 20–40% of the patients experience severe complications and the treatment-related mortality is 3% [12–14]. Moreover, many patients still experience recurrent (peritoneal) disease. A possible solution for this problem would be the use of genetic biomarkers to predict which patients benefit the most from hyperthermic intraperitoneal mitomycin C or oxaliplatin. Therefore, in part II of this thesis we aim to discover genetic biomarkers that are predictive for treatment outcome of colorectal peritoneal metastasis patients treated with CRS + HIPEC.

In short, in this thesis we aim to improve the safety and efficacy of chemotherapeutic drugs in patients with colorectal cancer by individualising drug dosing and choice of drug based on germline genetic biomarkers. The studies presented in this thesis address the following research questions:

Part I: Implementation of *UGT1A1* genotype-guided dosing of irinotecan

- What is the potential value of *UGT1A1* genotype-guided dosing of irinotecan? What is the level of evidence?
- What is the optimal starting dose of irinotecan per *UGT1A1* genotype?
- Is *UGT1A1* genotype-guided dosing of irinotecan less toxic and just as effective as standard dosing of irinotecan in clinical practice?
- Is *UGT1A1* genotype-guided dosing of irinotecan feasible and cost-effective in clinical practice?

Part II: Discovery and validation of genetic biomarkers for hyperthermic intraperitoneal chemotherapy (HIPEC)

- Are genetic biomarkers in the DNA repair pathway associated with treatment outcome of patients treated with CRS + HIPEC with oxaliplatin or mitomycin C?
- Can we identify new genetic biomarkers that are predictive for CRS + HIPEC treatment outcome?
- Are the genetic biomarkers *NQO1*2*, *NQO1*3*, and *POR*28* associated with the efficacy of CRS + HIPEC treatment with mitomycin C?

Part I describes the added value and clinical utility of *UGT1A1* genotype-guided dosing of irinotecan. In **Chapter 2** an overview of the available evidence on *UGT1A1* genotype-guided dosing of irinotecan is provided. **Chapter 3** provides a guideline on *UGT1A1* genotype-guided dosing of irinotecan. With this guideline we aim to aid physicians and pharmacists in the implementation of *UGT1A1* genotype-guided dosing of irinotecan. **Chapter 4** describes a prospective trial on *UGT1A1* genotype-guided dosing of irinotecan in which the safety, feasibility and costs of this strategy is investigated. This pivotal study should provide the evidence whether or not *UGT1A1* genotype-guided dosing of irinotecan should be the new golden standard.

In **Part II** an exploration on genetic biomarkers for the treatment outcome of CRS + HIPEC is described. **Chapter 5** gives an overview of the available literature on the association of genetic biomarkers in the DNA repair pathway and treatment outcome of patients treated with

oxaliplatin or mitomycin C. **Chapter 6** describes a retrospective genome-wide association study on a CRS + HIPEC patient cohort, in order to identify new genetic biomarkers that are associated with treatment outcome. **Chapter 7** describes a retrospective, hypothesis-driven study, in which possible genetic biomarkers for hyperthermic intraperitoneal mitomycin C, based on previous preclinical findings, are clinically validated.

This thesis concludes with a general discussion and future perspectives in **Chapter 8**. Summaries of this thesis in both English and Dutch are presented in **Chapters 9** and **10**.

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PART I

Implementation of *UGT1A1* genotype-guided dosing of irinotecan

CHAPTER 2

Pre-therapeutic *UGT1A1* genotyping to reduce the risk of irinotecan-induced severe toxicity: Ready for prime time

E.C. Hulshof, M.J. Deenen, H.J. Guchelaar, H. Gelderblom

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ABSTRACT

Aim

Pre-therapeutic *UGT1A1* genotyping is not yet routinely performed in most hospitals in patients starting irinotecan chemotherapy. The aim of this position paper was to evaluate the available evidence and to assess the potential value of genotyping of *UGT1A1*28* and *UGT1A1*6* in patients before starting treatment with irinotecan in order to reduce the risk of severe toxicity.

Methods

Literature was selected and assessed based on five pre-specified criteria: 1] level of evidence for associations between *UGT1A1* polymorphisms and irinotecan-induced severe toxicity, 2] clinical validity and utility of pre-therapeutic genotyping of *UGT1A1*, 3] safety and tolerability of irinotecan in carriers of *UGT1A1* polymorphisms, 4] availability of specific dose recommendations for irinotecan in carriers of *UGT1A1* polymorphisms, 5] evidence of cost benefits of pre-therapeutic genotyping of *UGT1A1*.

Results

On all five criteria, study results were favourable for pre-therapeutic genotyping of *UGT1A1*. A high level of evidence (level I) was found for a higher incidence of irinotecan-induced severe toxicity in homozygous carriers of *UGT1A1*28* or *UGT1A1*6*. The clinical validity and utility of this genetic test proved to be acceptable. Dose-finding studies showed a lower maximum tolerated dose in homozygous variant allele carriers, and most of the drug labels and guidelines recommend a dose reduction of 25 to 30% in these patients. Also, pre-therapeutic genotyping of *UGT1A1* is likely to save costs.

Conclusions

Pre-therapeutic genotyping of *UGT1A1* in patients initiating treatment with irinotecan improves patient safety and is likely to be cost-saving, and should therefore become standard of care.

INTRODUCTION

Irinotecan is a commonly applied anti-cancer drug that frequently leads to complications such as severe delayed diarrhoea and neutropenia. Irinotecan is registered for first-line treatment of pancreatic cancer, second-line treatment of colorectal cancer and is also used in other tumour types, such as Ewing sarcoma. Of all treated patients, up to 40% experience CTC grade ≥3 delayed diarrhoea, and up to 50% of the patients experience grade ≥3 neutropenia [1, 2].

Irinotecan is a prodrug that is activated via carboxylesterases in the liver and blood to SN-38, which in turn is glucuronidated in the liver and intestines into SN38-glucuronide (SN38-G) by UDP-glucuronosyltransferase 1A1 (UGT1A1). UGT1A1 is the main enzyme responsible for the inactivation of SN-38 [3].

Several genetic variants within the *UGT1A1* gene are known to be associated with reduced UGT1A1 enzyme activity, and therefore with an increased risk for irinotecan-related severe toxicity [4, 5]. The most well-characterized *UGT1A1* genetic variants are *UGT1A1*28* and *UGT1A1*6*. *UGT1A1*28* is a common tandem repeat polymorphism in the promotor region of the *UGT1A1* gene that leads to reduced enzyme activity, which is also known as Gilbert's syndrome [6, 7]. Homozygous carriers of these variants have a decreased UGT1A1 expression of up to 70% [7]. The polymorphism *UGT1A1*6* is a missense mutation and reduces UGT1A1 enzyme activity to an extent that is comparable to the effect of *UGT1A1*28* [8, 9]. The *UGT1A1*28* polymorphism is highly prevalent in the African, Latino and European population, with a minor allele frequency (MAF) ranging from 32% to 40%, whereas this polymorphism occurs less frequently in the East-Asian population (MAF 12%) and does not occur in the South-Asian population [10]. In contrast, the *UGT1A1*6* polymorphism has the highest MAF in the East-Asian population, i.e. 15%, compared to 0–5% in all other populations [10]. In the Chinese and Japanese population also a combined occurrence of *UGT1A1*6* and *UGT1A1*28* was reported with an incidence ranging from 3–8% [8, 11, 12]. A considerable amount of literature has been published on the association between *UGT1A1* polymorphism and severe toxicity of irinotecan, but so far, *UGT1A1* genotyping is not being routinely applied. Therefore, the aim of this position paper was to evaluate the available evidence and to assess the potential value of pre-therapeutic genotyping of *UGT1A1*28* and *UGT1A1*6* in patients indicated for treatment with irinotecan. The outcomes of this study are relevant for oncologists who prescribe irinotecan in daily practice and for their patients.

METHODS

A literature search was conducted to compile the available evidence on UGT1A1 genotyping in patients treated with irinotecan. We searched PubMed until March 2020 without any limitations on publication year using the following search terms: "irinotecan", "CPT-11", "pharmacogenetics", "cost-effectiveness", "cost-analysis", "UGT1A1", "UGT1A1*6" and "UGT1A1*28". Reference lists in original articles and review articles were manually searched to identify additional potentially relevant publications. In addition, we screened all the available drug labels and guidelines on irinotecan provided on PharmGKB [13].

Publications were included if they reported on at least one of the following subjects: 1] the association between irinotecan-related toxicity and carriership of *UGT1A1*6* or *UGT1A1*28*; 2] *UGT1A1* genotype-guided dose-finding studies for irinotecan; 3] dose recommendations on drug labels or in guidelines for the administration of irinotecan in carriers of *UGT1A1*6* or *UGT1A1*28*; or 4] cost-evaluation of pre-therapeutic *UGT1A1*6* or *UGT1A1*28* genotyping. Publications reporting on liposomal irinotecan were excluded.

To assess the available evidence for pre-therapeutic genotyping of *UGT1A1* in patients treated with irinotecan in a structured and objective manner, data were assessed based on five main criteria, in accordance with standardized guidelines [14–16] on assessing the clinical validity and clinical utility of pharmacogenetic testing.

1. Level of evidence for the association between *UGT1A1* polymorphisms and irinotecan-induced severe toxicity

The following toxicity endpoints were assessed: grade ≥3 neutropenia, grade ≥3 diarrhoea, febrile neutropenia, irinotecan-related hospital admissions, and death. If available, odds ratios or relative risks were reported for each endpoint. The level of evidence for each endpoint was assessed according to the standard operating procedures of the European Society of Medical Oncology [17]. The levels range from V to I, in which level I is the highest level of evidence.

2. Clinical validity and utility of pre-therapeutic genotyping of *UGT1A1*

The clinical validity of pre-therapeutic genotyping of *UGT1A1* describes the accuracy of this genetic test to identify a patient's risk to develop severe toxicity [16]. The clinical validity was assessed by calculating the sensitivity, the specificity and the positive and negative predictive value. In general, a low sensitivity may be expected since other (genetic) factors are also

known to be predictive for irinotecan-induced toxicity and not all toxicity may be attributed to only one single polymorphism.

The clinical utility of pre-therapeutic genotyping of *UGT1A1* describes the ability of genotyping to prevent severe toxicity through differentiation in treatment based on the genotyping results. The clinical utility was assessed by calculating the number needed to treat (NNT; i.e. to apply a dose reduction) and the number needed to genotype (NNG) [14].

Clinical validity and utility parameters were calculated for both *UGT1A1*6* and *UGT1A1*28* for the most important adverse events, that is, grade ≥3 diarrhoea and neutropenia in a recessive genetic model: homozygous versus heterozygous plus wild type.

Since there are no clear cut-off values for deciding whether pre-therapeutic genotyping of *UGT1A1* is clinically valid and utile, values were also compared to the genotype test recently recommended by the European Medicines Agency for the pre-therapeutic genotyping of *DPYD* in patients treated with fluoropyrimidines [18]. A position paper by Lunenburg et al. presented the clinical validity and utility parameters for this genotype test, these parameters were calculated for *DPYD*2A* and *c.2846A>T* for grade ≥3 toxicity [19].

3. Safety and tolerability of irinotecan in carriers of *UGT1A1* polymorphisms

All available *UGT1A1* genotype-guided dose-finding studies for irinotecan were collected. To compare the outcomes of all the identified studies, relative dose intensities were calculated per study and genotype category and reported in a forest plot. These relative dose intensities were calculated by dividing the recommended dose or maximum tolerable dose reported in each study by the standard conventional dose of irinotecan conform the treatment schedule used in each study, multiplied by 100%.

4. Availability of specific dose recommendations for irinotecan in carriers of *UGT1A1* polymorphisms

Specific dose recommendations per *UGT1A1* genotype category are necessary to provide guidance for oncologists in applying *UGT1A1* genotype-guided dosing. Drug labels and clinical guidelines were screened for the presence of specific dose recommendations per *UGT1A1* genotype category.

5. Evidence of cost benefits of pre-therapeutic genotyping of *UGT1A1*

The implementation of pre-therapeutic *UGT1A1* genotyping will increase treatment costs due to the extra costs for genotyping, but it might also be cost-saving due to the reduction of severe irinotecan-induced toxicity and hospitalisation. All the available cost-analysis publications on pre-therapeutic *UGT1A1* genotyping were assessed.

RESULTS

Based on the selection criteria, a total of 41 publications, 4 drug labels and 3 guidelines were included, specifically resulting in a total of 23, 1, 12, 7 and 5 included publications for criteria 1–5, respectively.

1] Level of evidence for the association between *UGT1A1* polymorphisms and irinotecan-induced severe toxicity

A considerable amount of literature has been published on the increased risk for irinotecan-related toxicity in homozygous *UGT1A1*28* variant allele carriers; this increased risk has been demonstrated in case reports on several [20], sometimes even lethal adverse events [21, 22], in multiple retro- and prospective genetic association studies [23–25] and also in several meta-analyses [26–30]. A similar increased risk for irinotecan-related toxicity in homozygous *UGT1A1*6* variant allele carriers has been reported in several genetic association studies [31–33] and several meta-analyses [34–38].

Carriership of a *UGT1A1* polymorphism was highly associated with grade ≥3 neutropenia and grade ≥3 diarrhoea (level of evidence I). For *UGT1A1*28*, the largest effect size was seen in homozygous carriers compared to heterozygous and wild type patients (recessive model): four [26–29] out of five [26–29, 34] meta-analyses showed a two- to four-fold increased risk of grade ≥3 neutropenia. In all three meta-analyses on *UGT1A1*6*, a similar increased neutropenia risk was observed [34, 35, 37]. For *UGT1A1*28*, a two- to six-fold increased risk of grade ≥3 diarrhoea was observed in four [28–30, 34] out of five [26, 28–30, 34] meta-analyses; in addition, the effect size seemed larger in patients treated at medium or higher doses of irinotecan (>125 mg/m^2). In three meta-analyses reporting on *UGT1A1*6* and severe diarrhoea, homozygotes had a three- to four-fold increased risk compared to wild type patients [36, 38] and a four-fold increased risk compared to heterozygous and wild type patients [34]. A more detailed description of all meta-analyses of studies on the association of *UGT1A1* polymorphisms and grade ≥3 neutropenia and diarrhoea is provided in **Tables 2.1a** and **2.1b**.

Table 2.1a: Association between irinotecan-related severe neutropenia and *UGT1A1* polymorphism

				Association with irinotecan grade ≥ III neutropenia				
Group	n total	Ethnicity	Polymorphism	Comparison	n	Dose (mg/m²)	OR	95% CI
Hoskins et al. 2007 [26]	821	Caucasian	*28	HO vs HE+WT	229	100–125	1.80	0.37–8.84
					410	180	3.22	1.52–6.81
					184	200 – 350	27.8	4.0–195
Hu et al. 2010 [27]	1998	Mainly Caucasian	*28	HO vs HE+WT	1998	80–350	2.20*	1.82–2.66
					300	<150	2.43*	1.34–4.39
					1481	150–250	2.00*	1.62–2.47
					217	≥250	7.22*	3.10–16.78
				HE vs WT	1738	80–350	1.43*	1.16–1.77
					270	<150	2.94*	1.36–6.35
					1288	150–250	1.29*	1.04–1.62
					180	≥250	2.65*	0.70–9.94
Liu et al. 2014 [28]	2015	Caucasian	*28	HO vs HE+WT	2015	80–350	3.44	2.45–4.82
					704	<150	3.63	2.02–6.53
					1311	≥150	3.34	2.21–5.05
				HO vs WT	1095	80–350	4.79	3.28–7.01
					331	<150	6.37	2.69–10.71
					764	≥150	4.64	2.88–7.17
				HE vs WT	1819	80–350	1.90	1.44–2.51
					630	<150	2.01	1.21–3.34
					1189	≥150	1.85	1.32–2.58
Han et al. 2014 [35]	994	Asian	*6	HO vs HE+WT	984	50–350	3.28	1.89–5.69
				HO+HE vs WT	994	50–350	1.54	1.18–2.04
			*6/*28	HO+*6/*28 vs HE+WT	923	50–350	3.28	2.15–4.98

Table 2.1a continues on next page.

Table 2.1a: ***Continued***

				Association with irinotecan grade ≥ III neutropenia				
Group	n total	Ethnicity	Polymorphism	Comparison	n	Dose (mg/m^2)	OR	95% CI
Cheng et al. 2014 [36]	1027	Asian	*6	HO vs WT	576	30–180	4.44	2.42–8.14
					116	<150	9.64	2.05–45.28
					460	≥150	3.95	2.05–7.64
				HE vs WT	933	30–180	1.98	1.45–2.71
					249	<150	4.42	2.27–8.59
					684	≥150	1.55	1.08–2.22
Liu et al. 2017 [29]	6087	Asian and Caucasian	*28	HO vs HE+WT	3668	60–350	4.12	2.36–7.20
				HO+HE vs WT	5232	60–350	2.15	1.71–2.70
				HO vs WT	3575	60–350	5.34	3.05–9.33
				HE vs WT	3948	60–350	1.71	1.41–2.08
Chen et al. 2017 [34]	577	Asian	*6	HO vs HE+WT	277	50–100	4.80	1.62–14.27
				HO+HE vs WT	233	50–100	2.40	1.28–4.49
				HO vs WT	58	50–100	2.16	0.28–16.96
				HE vs WT	182	50–100	2.09	0.66–6.62
			*28	HO vs HE+WT	101	50–100	1.27	0.20–7.94
				HO+HE vs WT	494	50–100	1.47	0.90–2.42
				HO vs WT	45	50–100	1.27	0.20–7.95
				HE vs WT	412	50–100	1.50	0.86–2.62

Table 2.1a: *Continued*

				Association with irinotecan grade ≥ III neutropenia				
Group	n total	Ethnicity	Polymorphism	Comparison	n	Dose (mg/m^2)	OR	95% CI
Zhang et al. 2017 [37]	1140	Asian	*6	HO+HE vs WT	n.r	60–225	2.03	1.54–2.68
					n.r.	<150	2.66	1.10–6.45
					n.r.	≥150	1.97	1.45–2.67
				HO vs WT	n.r.	60–225	2.95	1.83–4.75
					n.r.	<150	3.17	1.11–9.04
					n.r.	≥150	2.89	1.69–4.94
				HE vs WT	n.r.	60–225	1.83	1.36–2.46
					n.r.	<150	2.36	1.28–4.35
					n.r.	≥150	1.65	1.15–2.35
Yang et al. 2018 [38]	6742	Asian	*6	HO vs WT	1466	50–350	3.03	2.05–4.47
				HE vs WT	1928	50–350	1.95	1.34–2.85
		Asian and Caucasian	*28	HO vs WT	2609	50–350	3.50	2.23–5.50
				HE vs WT	3516	50–350	1.91	1.45–2.50

* RR instead of OR. CI = confidence interval, HE = heterozygous carrier, HO = homozygous carrier, n.r. = not reported, OR = odds ratio, RR = relative risk, WT = wild type.

Table 2.1b: Association between irinotecan-related severe diarrhoea and *UGT1A1* polymorphism

				Association with irinotecan grade ≥ III diarrhoea				
Group*	n total	Ethnicity	Polymorphism	Comparison	n	Dose (mg/m^2)	OR	95% CI
Hu et al. 2010 [30]	1065	Asian and Caucasian	*28	HO vs HE+WT	1760	60–350	1.81	1.38–2.39
					355	≤125	1.06	0.57–1.99
					1405	>125	2.06	1.51–2.80
				HE vs WT	1265	60–350	1.73	1.25–2.40
					335	≤125	1.27	0.67–2.42
					930	>125	1.92	1.31–2.82
Liu et al. 2014 [28]	2015	Caucasian	*28	HO vs HE+WT	1980	80–350	1.71	1.18–2.47
					663	<150	1.41	0.82–2.43
					1317	≥150	2.04	1.23–3.38
				HO vs WT	1122	80–350	1.84	1.24–2.72
					348	<150	1.41	0.79–2.51
					774	≥150	2.37	1.39–4.04
				HE vs WT	1794	80–350	1.20	0.93–1.56
					593	<150	1.02	0.70–1.50
					1201	≥150	1.39	0.97–1.98
Cheng et al. 2014 [36]	1027	Asian	*6	HO vs WT	470	30–180	3.51	1.41–7.83
				HE vs WT	719	30–180	1.44	0.84–2.49

Table 2.1b: ***Continued***

				Association with irinotecan grade ≥ III diarrhoea				
Group*	n total	Ethnicity	Polymorphism	Comparison	n	Dose (mg/m^2)	OR	95% CI
Chen et al. 2017 [34]	577	Asian	*6	HO vs HE+WT	307	50–100	6.25	1.51–25.0
				HO+HE vs WT	186	50–100	1.45	0.74–2.84
				HO vs WT	80	50–100	5.93	1.46–24.0
				HE vs WT	182	50–100	1.33	0.60–2.91
			*28	HO vs HE+WT	131	50–100	4.56	1.56–13.18
				HO+HE vs WT	447	50–100	4.90	2.02–11.88
				HO vs WT	104	50–100	17.64	2.58–120.66
				HE vs WT	439	50–100	4.36	1.74–10.91
Yang et al. 2018 [38]	6742	Asian	*6	HO vs WT	651	50–350	4.03	1.98–8.32
				HE vs WT	844	50–350	1.98	1.26–3.11
		Asian and Caucasian	*28	HO vs WT	1817	50–350	1.69	1.20–2.40
				HE vs WT	2521	50–350	1.45	1.07–1.97

* Hoskins et al. did not find an association between irinotecan-related diarrhoea and homozygous carriers of UGT1A1*28, an OR was not reported [26]. CI = confidence interval, HE = heterozygous carrier, HO = homozygous carrier, OR = odds ratio, RR = relative risk, WT = wild type.

Level III and IV evidence was available for the association between *UGT1A1*28* and febrile neutropenia [39–42]. One study reporting on the administration of low doses of irinotecan (50–60 mg/m^2) could not replicate this increased risk [43]. For *UGT1A1*6*, one small study (n=69) reported on an increased risk of febrile neutropenia in heterozygous carriers compared to wild type patients [44].

The carriership of a *UGT1A1*28* allele also increased the risk of hospitalisation due to toxicity (level of evidence III & IV) [39, 41]. No studies on this endpoint have been reported for *UGT1A1*6*. The *UGT1A1*28* variant may also be associated with treatment-related mortality (level of evidence IV); treatment-related fatal neutropenia and bacteraemia occurred in 2 out of 102 (2%) wild type patients compared to 3 out of 26 (11.5%) heterozygous or homozygous *UGT1A1*28* carriers ($p<0.01$) [39]. No studies on *UGT1A1*6* reported on this endpoint.

2] Clinical validity and utility of pre-therapeutic genotyping of *UGT1A1*

The clinical validity and utility parameters were based on event rates reported in the meta-analysis by Yang et al. We selected this meta-analysis because it included Asian as well as Caucasian patients with data on *UGT1A1*6* and *UGT1A1*28*, respectively; besides, it included the highest number of patients and it was the most recent of all the identified meta-analyses [38].

The calculated sensitivity, specificity and positive and negative predictive values for pre-therapeutic *UGT1A1* genotyping are provided in **Table 2.2**. The values proved to be comparable with the values of pre-therapeutic genotyping of *DPYD* in patients treated with fluoropyrimidines [19]. These numbers indicate that pre-therapeutic *UGT1A1* genotyping would not identify all patients that experienced severe diarrhoea or neutropenia, but it would identify almost all the patients that had a good ability to tolerate irinotecan. This test may have false positive results, which may lead to a dose reduction of irinotecan, but this risk is unlikely to be relevant since only the starting dose of irinotecan will be reduced, followed by dose optimisation based on the tolerability of irinotecan in each individual patient. The low number of false negatives is of the highest importance, since the expected severe toxicity of irinotecan in these patients can lead to hospitalisation and delay or even discontinuation of treatment, resulting in a reduced quality of life and treatment failure.

Additionally, the NNT and NNG were calculated. For *UGT1A1*28*, the NNT (i.e. apply a dose reduction) to prevent ≥ grade 3 neutropenia was 9 and to prevent ≥ grade 3 diarrhoea was 14. The NNG to prevent ≥ grade 3 neutropenia and ≥ grade 3 diarrhoea was 79 and 127,

respectively. In view of these results, pre-therapeutic genotyping of *UGT1A1*28* seems even more clinically utile than pre-therapeutic genotyping of *DPYD* in patients treated with fluoropyrimidines, which is mainly due to the higher prevalence of *UGT1A1*28*. For *UGT1A1*6*, the NNT to prevent ≥ grade 3 neutropenia was 8 and the NNT to prevent ≥ grade 3 diarrhoea was 11, while the NNG was 376 and 564, respectively. *UGT1A1*6* seems less clinically utile than pre-therapeutic genotyping of *DPYD* in patients treated with fluoropyrimidines because of the high NNG, which is caused by the low prevalence of this polymorphism. Only 2% of the East-Asian population are homozygous carriers of this polymorphism, and the polymorphism is not present in other populations. See **Table 2.2** for a detailed overview.

Table 2.2: Clinical validity and utility of pre-therapeutic genotyping of *UGT1A1* in patients treated with irinotecan compared to the clinical validity and utility of *DPYD* in patients treated with fluoropyrimidines

	*UGT1A1*6* [38]		*UGT1A1*28* [38]		*DPYD* variants [19]
Parameter	≥ grade 3 neutropenia	≥ grade 3 diarrhoea	≥ grade 3 neutropenia	≥ grade 3 diarrhoea	≥ grade 3 toxicity
Sensitivity	11%	11%	11%	13%	12–15%
Specificity	94%	94%	94%	92%	98%
PPV	33%	20%	30%	22%	20–24%
NPV	80%	89%	82%	85%	96–97%
NNG	376	564	79	127	210–251
NNT	8	11	9	14	5–6

NNG = number needed to genotype, NNT = number needed to treat, NPV = negative predictive value, PPV = positive predictive value.

3] Safety and tolerability of irinotecan in carriers of *UGT1A1* polymorphisms

Several phase 1 *UGT1A1* genotype-guided dose-finding studies have been conducted. In these studies, the maximum tolerable dose (MTD) most often was lower than the standard dose of irinotecan in homozygous carriers of *UGT1A1*6* or *UGT1A1*28* or in compound heterozygous carriers (*UGT1A1*6/*28*) (**Figure 2.1** + **Supplementary Material Table S2.1**). Five [45–49] out of six of these dose-finding studies found a lower MTD than the registered dose of irinotecan and therefore suggest to lower the irinotecan starting dose, with relative dose intensities ranging from 42 to 83% [22, 45–49]. Moreover, the single study that reported a 100% relative dose intensity stated that homozygous carriers may receive irinotecan at a starting dose of 150 mg/m^2, but in subsequent cycles dose reductions or treatment delays were indicated in 12 out of 16 patients (75%) [22].

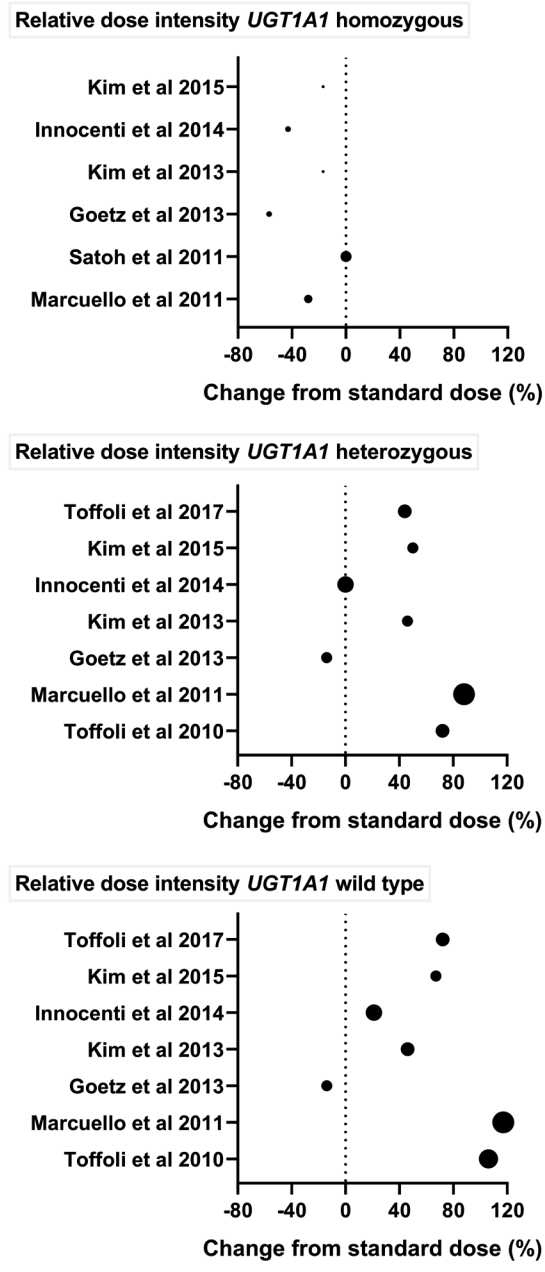

Figure 2.1: Forest-plot of outcomes of dose-finding studies of irinotecan per *UGT1A1* genotype category [22, 45–51].
Each dot represents the outcome of one study, presented as the difference between the maximum tolerable dose (MTD) reported and the standard dose of irinotecan in percentages. The size of each dot indicates the number of patients in each study in comparison to the other studies. Top: homozygous carriers of *UGT1A1*6* or *UGT1A1*28*, middle: heterozygous carriers of *UGT1A1*6* or *UGT1A1*28*, bottom: wild type patients. For the exact numbers see Table S2.1 in the Supplementary material.

In heterozygous carriers of *UGT1A1*6* or *UGT1A1*28* and wild type patients, the MTD was often higher than the standard dose. Five [45, 47, 49–51] out of seven and six [45, 47–51] out of seven dose-finding studies found a higher MTD than the standard dose in heterozygous carriers and wild type patients, with relative dose intensities ranging from 86 to 188% and 86 to 217%, respectively [45–51]. Most of the patients in these dose-finding studies had a relatively low ECOG performance score (ranging from 0 to 1) compared to the real-world population, which might have led to overestimation of the MTD.

Three prospective genotype-guided dosing studies tested the reduced starting dose of irinotecan for homozygous carriers of *UGT1A1*6* or *UGT1A1*28* or *UGT1A1*6/*28* [11, 52, 53] and their findings are in line with the dose-finding studies presented in **Figure 2.1**. Fuji et al. reduced the starting dose of irinotecan from 150 mg/m^2 to 120 mg/m^2 (relative dose intensity 80%) in the homozygous group (n=10), finding no significant differences in adverse events or tumour response compared to the heterozygous carriers and wild type patients (n=43) in this study [11]. Xu et al. conducted a preplanned analysis in the AXEPT trial (XELIRI or FOLFIRI schedule, n=650). Fifty homozygous carriers of *UGT1A1*6* or *UGT1A1*28* or *UGT1A1*6/*28* were enrolled, the starting dose of irinotecan was reduced to 150 mg/m^2 and was well tolerated [53]. Boisdron-Celle et al. conducted a proof of concept trial in which patients intended to be treated with FOLFIRI-cetuximab were stratified by their *UGT1A1*28* genotype and received irinotecan dose intensification provided that treatment was well-tolerated. Eighty-five patients were enrolled, and mean irinotecan doses at 3 months were 247, 210, and 140 mg/m^2 for wild type, heterozygous and homozygous carriers, respectively (relative dose intensities: 137%, 116% and 78%, respectively) [52].

Currently, there is one randomized controlled trial in which 82 wild type patients and heterozygous carriers of *UGT1A1*28* were randomised to receive either high dose-FOLFIRI or standard FOLFIRI [54]. In the high dose-FOLFIRI group, the irinotecan dose was 300 mg/m^2 for wild type patients and 260 mg/m^2 for heterozygous patients. In the control group, the dose was 180 mg/m^2, irrespective of genotype. The authors concluded that *UGT1A1* wild type patients and heterozygous carriers of *UGT1A1*28* may receive higher doses of irinotecan and showed a higher objective response rate compared to those receiving the standard dose (67.5 versus 43.6%; OR=1.73 [95% CI:1.03–2.93, p=0.001]), without a significantly increased risk for severe toxicity (22.5% versus 20.5%).

Table 2.3: Overview of recommendations on drug labels and in guidelines for dosing of irinotecan in homozygous carriers of *UGT1A1* polymorphisms

Organisation	Country/ region	Dose reduction recommended for UGT1A1 polymorphisms?		Dose recommendation	References
Drug labels		*6	*28		
FDA	USA	No	Yes	≥1 dose level reduction	[55]
CBG-MEB	Netherlands	No	No	Not applicable	[60]
PMDA	Japan	Yes	Yes	No specification	[56]
HCSC	Canada	No	Yes	No specification	[57]
Guidelines					
RNPGx-GPCO-unicancer	France	No*	Yes	Dose 180–230 mg/m^2: 25–30% dose reduction Dose ≥240 mg/m^2: contra-indicated	[58]
KNMP-DPWG	Netherlands	Yes	Yes	30% dose reduction	[59]

* The authors mention that this analysis is limited by the fact that other *UGT1A1* deficient variants are relevant in non-Caucasian populations, particularly the *6 and *27 alleles in Asian populations. FDA = Food and Drug Administration, CBG-MEB = Dutch Medicines Evaluation Board, PMDA = Pharmaceuticals and Medical Devices Agency, HCSC = Health Canada/Santé Canada, RNPGx = National Pharmacogenetics Network, GPCO = Group of Clinical Onco-pharmacology, KNMP = Royal Dutch Association for the Advancement of Pharmacy, DPWG = Dutch Pharmacogenetics Working Group.

4] Availability of dose recommendations for irinotecan in carriers of *UGT1A1* polymorphisms

Various dose recommendations for irinotecan in homozygous carriers of *UGT1A1*28* were found on drug labels and in guidelines (**Table 2.3**). Most of the national medicines authorities and guideline working groups recommend to apply a dose reduction of 25 to 30% in homozygous carriers of *UGT1A1*28* [55–59]. Only the Dutch national medicines authority does not recommend dose reduction in homozygous carriers of *UGT1A1*28* treated with conventional irinotecan [60].

For homozygous carriers of *UGT1A1*6*, less information was found on drug labels and in guidelines, which might be due to the fact that this polymorphism only occurs in the Asian population. However, the Japanese drug label states that patients should be selected for treatment based on their stage, general condition and *UGT1A1* genotype, although no specific dose recommendations are provided [56].

Only the French working group mentions dose recommendations for *UGT1A1*28* heterozygous and wild type patients, stating that the administration of an intensified dose of irinotecan (240 mg/m^2) is only possible in wild type patients. In heterozygous patients, dose intensification may be applied in the absence of additional risk factors and under strict medical surveillance [58]. Obviously, this is an off-label dose recommendation.

Moreover, the Clinical Pharmacogenetics Implementation Consortium assigned level A to this gene-drug interaction, indicating that genetic information should be used to change the prescription of this drug [61].

5] Evidence of cost-benefits of pre-therapeutic genotyping of *UGT1A1*

Besides improved patient safety, pre-therapeutic genotyping of *UGT1A1* is also likely to be cost-effective or even cost-saving. To date, four studies [62–65] assessed the cost effectiveness of pre-therapeutic genotyping followed by a 20% to 25% dose reduction of irinotecan in homozygous variant carriers of *UGT1A1*28* in Caucasian populations, or in carriers of both *UGT1A1*6* and *UGT1A1*6*/**28* in a Chinese population, compared to no genotyping. This was assessed with decision-analytic models using clinical and genetic data from literature. All studies concluded that pre-therapeutic genotyping was a cost-saving strategy compared to no genotyping, reporting cost reductions due to pre-therapeutic genotyping ranging from 112 euro up to 596 euro per patient.

Roncato et al. [66] conducted the first retrospective clinical validation study in an Italian hospital setting. They assessed the association between the *UGT1A1*28* genotype and the cost of toxicity management. The mean costs per patient were 812€ for wildtype patients, 1,119€ for heterozygous variant carriers, and 4,886€ for homozygous variant carriers, which illustrates that the costs of irinotecan-related toxicity are significantly higher in patients carrying a homozygous or heterozygous variant of *UGT1A1*28* than in wild type patients. The cost driver was hospitalisation, which accounted for 82% of all toxicity costs. Six out of 22 (27%) homozygous variant carriers were hospitalised for irinotecan-related toxicity, compared to 10 out of 122 (8.2%) heterozygous variant carriers and 6 out of 109 (5.5%) wild type patients.

DISCUSSION

Based on the available literature, we conclude that pre-therapeutic genotyping of *UGT1A1* in patients initiating treatment with irinotecan improves patient safety and is likely to be cost-saving. In this review, the available evidence for pre-therapeutic genotyping of *UGT1A1*6* and *UGT1A1*28* in patients treated with irinotecan was assessed in a structured and objective manner, and data were assessed based on five main criteria.

Level of evidence I exists for the association of *UGT1A1*28* and *UGT1A1*6* and irinotecan-induced severe neutropenia or severe diarrhoea; level III for the association between *UGT1A1*28* and febrile neutropenia, and level III and IV for treatment-related hospitalisation and mortality, respectively. In addition, the clinical validity and utility of pre-therapeutic genotyping of *UGT1A1* proved to be acceptable and comparable with the clinical validity and utility of pre-therapeutic genotyping of *DPYD* in patients treated with fluoropyrimidines. Since this *DPYD* test has recently been recommended by the EMA [18], pre-therapeutic *UGT1A1* genotyping might also be considered clinically valid and utile.

Moreover, the combined conclusion of multiple dose-finding studies indicate that the current standard way of dosing of irinotecan is not safe for homozygous carriers of *UGT1A1*6* or *UGT1A1*28*, whereas wild type patients might even tolerate higher doses of irinotecan. A complementing finding is that the evidence described above has been taken up in various drug labels and guidelines providing specific dose recommendations for irinotecan in homozygous carriers of *UGT1A1*28* or *UGT1A1*6*: most of the national medicines authorities and guideline working groups recommend to apply an initial dose reduction of 25 to 30% in these patients.

Finally, pre-therapeutic genotyping of *UGT1A1* is likely to be cost-saving. Homozygous carriers of *UGT1A1*28* or *UGT1A1*6* were shown to have ~6-fold higher irinotecan-related toxicity costs than wild type patients, mainly due to costs for hospitalisation for toxicity treatment. In comparison, patients carrying a *DPYD* variant seem to have ~4-fold higher toxicity costs than wild type patients [67]. This indicates that the costs of pre-therapeutic genotyping seem to be outweighed by the savings achieved by preventing the costs of toxicity treatment.

A limitation on the available evidence for *UGT1A1* genotype-guided dosing of irinotecan is the absence of a randomized controlled trial on treatment outcome, i.e. overall survival. However, such a trial is hardly feasible and is not likely to be conducted, since at least a roughly estimated 300 homozygous individuals per arm would be needed for sufficient power, requiring a total of at least 6000 patients to be prospectively screened for inclusion. Moreover, with the available evidence favouring pre-therapeutic genotyping, it seems not ethical to randomise patients and patients may not be willing to participate in such a trial. Nonetheless, it is unlikely that genotype-guided dosing for homozygous carriers of *UGT1A1*28* or *UGT1A1*6* will negatively affect overall survival, since the recommended dose reduction leads to equal systemic exposure to SN-38 in these patients as in wild type patients treated with standard-dose therapy [46,68]. Moreover, the addition of other *UGT1A1* variants such as *UGT1A1*93* [4] and variants of other genes encoding for other enzymes such as *UGT1A7* and *UGT1A9* [69] might improve the predictive ability of *UGT1A1* genotype-guided dosing of irinotecan. Of interest, a prospective *UGT1A1*93* genotype-guided dose-finding trial is currently ongoing (https://www.trialregister.nl/ - trial NL6270 (NTR6612)).

Overall, based on this evaluation, all five criteria that were assessed showed that the available evidence is in favour of pre-therapeutic genotyping of *UGT1A1*. We recommend that all patients starting with irinotecan chemotherapy should be genotyped for *UGT1A1*28*; for Asian patients, the *UGT1A1*6* polymorphism should be tested. If a patient is homozygous for *UGT1A1*28* or *UGT1A1*6*, a dose reduction of 25 to 30% should be performed for all dosing regimens of irinotecan. Patients that are compound heterozygous *UGT1A1*6/*28* are considered poor metaboliser. Although less data is available, the available studies and the Japanese drug label suggest to treat these patients conform homozygous carriers of *UGT1A1*6* [11, 22, 35, 56, 65]. Dose-escalation in wild type patients is potentially safe, but there is not enough literature on clinical outcomes, and hence further research is warranted. Due to the presence of a wide interpatient variability in the pharmacokinetic parameters of irinotecan, a step-up based approach based on therapeutic drug monitoring might be of interest [70]. In addition, although turn-around time and costs of *UGT1A1* genotyping may be a challenging issue,

integration of *UGT1A1* genotyping into tumour sequencing programs may potentially enable genome testing without additional genotyping costs [71].

In summary, we conclude that pre-therapeutic genotyping of *UGT1A1* followed by genotype-guided dosing in patients treated with irinotecan is to be favoured over standard treatment and should therefore become standard of care and be implemented in oncology guidelines, such as the NCCN and ESMO guidelines.

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Supplementary Table S2.1: Overview *UGT1A1* genotype-guided dose-finding studies

Group	Ethnicity	Treatment schedule	Polymorphism	Genotype	n	Standard dose	Recommended dose (MTD)	Relative dose intensity (%)
Toffoli et al. 2010 [50]	Caucasian	q2w combined with 5-FU, leucovorin	*28	WT HE	35 24	180 mg/m^2	370 mg/m^2 310 mg/m^2	206 172
Marcuello et al. 2011 [45]	Caucasian	q2w combined with 5-FU, leucovorin	*28	WT HE HO	42 38 14	180 mg/m^2	390 mg/m^2 340 mg/m^2 130 mg/m^2	217 188 72
Satoh et al. 2011 [22]*	Japanese	q2w monotherapy	*6/*28	HO+*6/*28	21	150 mg/m^2	150 mg/m^2	100
Goetz et al. 2013 [46]**	Caucasian	q3w combined with oxaliplatin, capecitabine	*28	WT HE HO	21 18 11	175 mg/m^2	150 mg/m^2 150 mg/m^2 75 mg/m^2	86 86 43
Kim et al. 2013 [47]	Korean	q3w combined with capecitabine	*6/*28	WT HE HO+*6/*28	23 20 7	240mg/m^2	350 mg/m^2 350 mg/m^2 200 mg/m^2	146 146 83
Innocenti et al. 2014 [48]	Mostly Caucasian	q3w monotherapy	*28	WT HE HO	31 28 9	700 mg	850 mg 700 mg 400 mg	121 100 57
Kim et al. 2015 [49]***	Korean	q2w combined with 5-FU, leucovorin	*6/*28	WT HE HO+*6/*28	19 20 4	180 mg/m^2	300 mg/m^2 270 mg/m^2 150 mg/m^2	167 150 83
Toffoli et al. 2017 [51]	Caucasian	q2w combined with 5-FU, leucovorin, bevacizumab	*28	WT HE	25 23	180 mg/m^2	310 mg/m^2 260 mg/m^2	172 144

* Patients homozygous for the *UGT1A1*28* or *UGT1A1*6* allele can receive irinotecan in a starting dose of 150 mg/m^2, but many required dose reductions or delayed treatment in subsequent cycles. ** Oxaliplatin dose was escalated before irinotecan dose escalation, which might have led to lower MTDs of irinotecan. *** No DLTs were observed for the WT and the HO group at the proposed MTDs, but no further dose escalation was performed. This early stop decision was based on safety concerns based on previous studies and due to poor enrolment in the HO group. HE = heterozygous carrier, HO = homozygous carrier, MTD = maximum tolerable dose, WT = wild type.

CHAPTER 3

Dutch Pharmacogenetics Working Group (DPWG) guideline for the gene–drug interaction between *UGT1A1* and irinotecan

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ABSTRACT

The Dutch Pharmacogenetics Working Group (DPWG) aims to facilitate PGx implementation by developing evidence-based pharmacogenetics guidelines to optimize pharmacotherapy. This guideline describes the starting dose optimization of the anti-cancer drug irinotecan to decrease the risk of severe toxicity, such as (febrile) neutropenia or diarrhoea. Uridine diphosphate glucuronosyl transferase 1A1 (UGT1A1 encoded by the *UGT1A1* gene) enzyme deficiency increases risk of irinotecan-induced toxicity. Gene variants leading to UGT1A1 enzyme deficiency (e.g. *UGT1A1*6*, **28* and **37*) can be used to optimize an individual's starting dose thereby preventing carriers from toxicity. Homozygous or compound heterozygous carriers of these allele variants are defined as *UGT1A1* poor metabolisers (PM). DPWG recommends a 70% starting dose in PM patients and no dose reduction in IM patients who start treatment with irinotecan. Based on the DPWG clinical implication score, *UGT1A1* genotyping is considered "essential", indicating that *UGT1A1* testing must be performed prior to initiating irinotecan treatment.

INTRODUCTION

The role of heritable genetic variation on drug response is referred to as pharmacogenetics (PGx). Personalized medicine, also known as precision medicine, can be achieved with the use of PGx information. Knowledge of an individual's genetic composition for drug metabolizing enzymes, drug transporters, receptors or effector proteins may be used to guide pharmacological treatment. To implement the use of PGx in a clinical setting, guidelines informing physicians are essential. In order to accommodate, the Royal Dutch Pharmacists Association (KNMP) has appointed the Dutch Pharmacogenetics Working Group (DPWG) in 2005, a group of 15 professionals consisting of (clinical) pharmacists, physicians, a general practitioner, clinical pharmacologists, clinical chemists and epidemiologists [1]. The role of the DPWG is to develop evidence-based PGx-guided therapeutic recommendations based on systematic literature review and to implement these into computerized systems used nationwide in The Netherlands for medication prescription, dispensing and monitoring. In order to meet the public request for this information also outside the Dutch pharmacist and physician systems, the DPWG guidelines and future updates are published [2–5].

The current guideline presents the gene-drug interaction between *UGT1A1* and the anti-cancer drug irinotecan. The pharmacotherapeutic rationale for use of irinotecan as well as the cost-effectiveness of PGx-guided dosing is outside the scope of this guideline. This manuscript provides information on the development of this guideline and presents an overview of the PGx therapeutic recommendations. Background information of irinotecan and of the *UGT1A1* gene and its genetic variation is provided. This genetic information is followed by the evidence from literature on the gene-drug interaction between *UGT1A1* and irinotecan. Finally, therapeutic recommendations for the clinic and clinical decision support systems are provided. These DPWG PGx-guided recommendations are also compared to other international guidelines. The goal of this DPWG recommendation is to individualize the starting dose of irinotecan thereby decreasing the risk of severe and potentially fatal toxicity.

DRUG: IRINOTECAN

Irinotecan is a commonly applied anticancer drug and is registered for first-line treatment of pancreatic cancer, the second-line treatment of advanced and metastatic colorectal cancer and several other cancer types, including lung cancer and Ewing sarcoma. Of all treated patients, up to 40% experience common Toxicity Criteria grade ≥ III delayed diarrhoea, and up to 50% of the patients experience grade ≥ III neutropenia [6, 7].

Irinotecan is a prodrug that is converted predominantly by carboxylesterases (CES) in the liver and intestines to the active metabolite SN-38, which has 100 to 1,000-fold higher activity compared to irinotecan. Irinotecan is, besides by CES, also metabolised by CYP3A4/5 in the liver to inactive metabolites. SN-38 is predominantly glucoronidated by UGT1A1 and also by UGT1A6, UGT1A7, UGT1A9 and UGT1A10 to the inactive metabolite SN-38-glucuronide. A schematic overview of the metabolism of irinotecan and its active metabolite SN-38 is depicted in **Figure 3.1**.

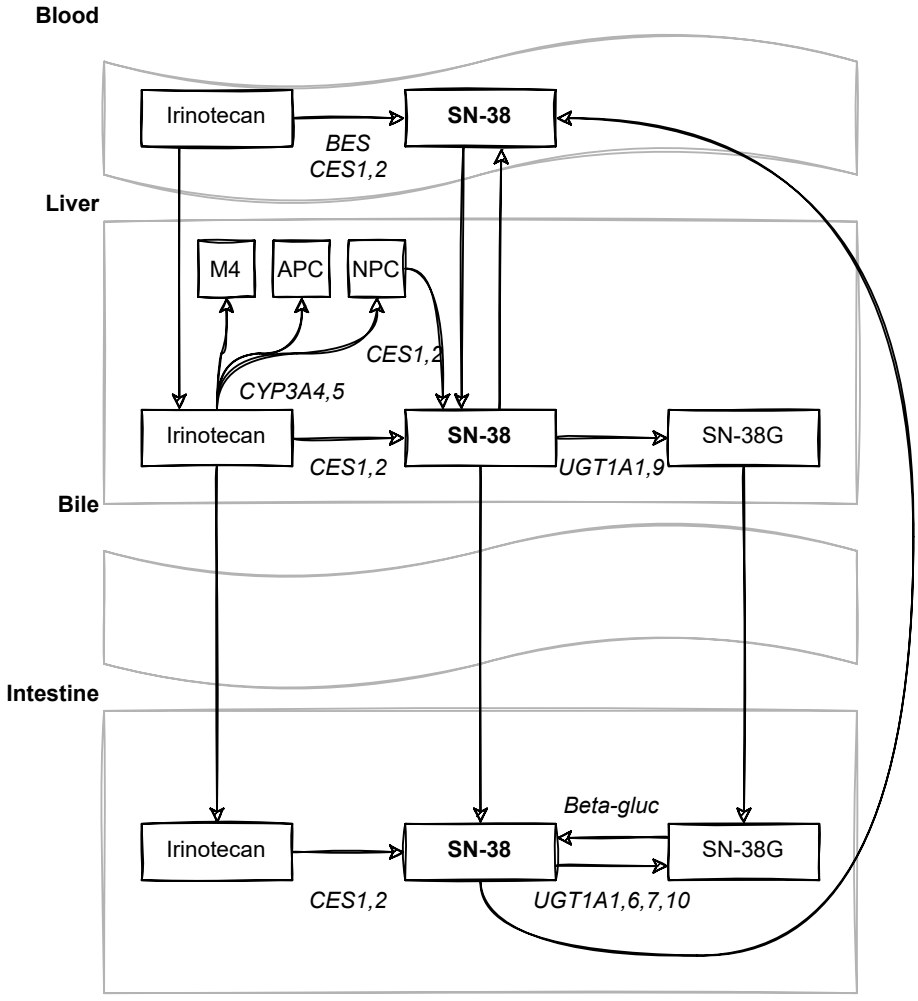

Figure 3.1: Irinotecan metabolism. Irinotecan and its metabolites are presented in rectangles. The active metabolite, SN-38, is presented in bold letters.

Abbreviations: APC = 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin, BES = butyrylcholinesterase, CES = carboxylesterase, CYP = cytochrome P450 enzymes, NPC = 7-ethyl-10-[4-(1-piperidino)-1-amino] carbonyloxycamptothecin, UGT = uridine diphosphate glucuronosyltransferase, SN-38G = SN-38 glucuronide, beta-gluc = beta-glucuronidase.

GENE: URIDINE DIPHOSPHATE GLUCURONOSYL TRANSFERASE 1A1 (*UGT1A1*)

The *UGT1A1* gene coding for the UGT1A1 enzyme is located on chromosome 2 (2q37.1) and consists of 5 exons, of which the first exon at the 5' terminus is unique and exons 2 to 5 are shared with the genes *UGT1A6* and *UGT1A9*.

Many variants exist for *UGT1A1*; more than 100 different alleles have been identified/described in the literature and are often associated with Gilbert syndrome or Crigler-Najjar syndrome. A number of these alleles and their functionality are listed in **Supplementary Table S3.1**. The most studied variation in *UGT1A1* involves a repeat in the promoter region of the *UGT1A1* gene. The number of "TA" tandem repeats in the TATA box of the promoter region varies. The UGT1A1 activity decreases with an increasing number of TA repeats. For example, the *28 variant contains 7 TA repeats instead of 6 TA repeats before the last TA in the TATA box and results in a 67 to 82 percent lower gene expression [8, 9].

The frequency of the various *UGT1A1* alleles shows considerable inter-ethnic variability. The variant allele *28 is abundant in inhabitants in South Asia (41%) and much less frequently in the rest of Asia (10–12%) [10]. The prevalence in Europeans ranges from 22 to 39% [10]. The variant allele *6 is common in several Asian populations and also strongly associated with reduced enzyme activity [11–13]. *UGT1A1*6* has allele frequencies in Japanese, Korean and Chinese populations of 13, 23 and 23%, respectively [14]. An overview of *UGT1A1* allele and genotype frequencies in different populations based on these references and the gnomAD database, is provided in **Supplementary Table S3.2**.

TRANSLATION OF GENOTYPE TO PREDICTED PHENOTYPE

The DPWG has concluded that variants resulting in decreased UGT1A1 metabolic capacity have sufficient evidence to be implemented into clinical care. In the case of the *36 variant, an allele that results in increased UGT1A1 metabolic capacity, there are currently no data to suggest that this results in clinically relevant effects. Therefore, for the time being, this is considered an allele with normal function. Notwithstanding, higher doses of irinotecan could potentially be indicated, but this requires further research. For *UGT1A1*, three different phenotypes are distinguished: normal metaboliser (NM), intermediate metaboliser (IM) and poor metaboliser (PM). The two phenotypes with reduced metabolic capacity (IM and PM) are further subdivided based on whether or not *28 is the only gene variant that results in

decreased metabolic capacity. The genotype-phenotype translation is presented in **Table 3.1**. In addition, an extensive genotype-phenotype translation table that can be used to programme the translation of genotype results into predicted phenotypes in laboratory information systems is provided in **Supplementary Table S3.3**.

Table 3.1: Genotype-phenotype translation

Genotype		Phenotype predicted based on genotype
Description	Examples	
Two alleles with normal (or increased) enzyme activity	*1/*1, *1/*36	NM
*28 and one allele with normal (or increased) enzyme activity	*1/*28, *28/*36	IM (*1/*28)
One allele with decreased enzyme activity other than *28 and one allele with normal (or increased) enzyme activity	*1/*6, *1/*37, *36/*37	IM other
Two *28 alleles	*28/*28	PM (*28/*28)
Two alleles with decreased enzyme activity, of which at least one is not *28	*6/*6, *6/*28, *28/*37	PM other

NM = Normal metaboliser, IM = intermediate metaboliser, PM = poor metaboliser.

GENE-DRUG INTERACTION

Pharmacological mechanism

UGT1A1 is mainly present in the liver and intestines and is the most important enzyme to inactivate irinotecan's active metabolite SN-38. Decreased UGT1A1 activity leads to increased concentrations of SN-38, which in turn could lead to an increased risk of severe toxicities, such as (febrile) neutropenia and diarrhoea [15]. Variations in the *UGT1A1* gene can result in reduced, or even absent enzyme activity. For example, the *UGT1A1*28/*28* genotype leads to an 18–159% increased systemic exposure of SN-38, and SN-38 metabolic clearance decreases by 61% [16–21].

SUPPORTING BODY OF EVIDENCE

A detailed description of the methods used for literature collection, assessment and preparation of the gene-drug monograph has previously been published [1]. In brief, a systematic review of literature was performed, relevant articles were summarized, and therapeutic recom-

mendations were proposed by a scientist of the Royal Dutch Association for the Advancement of Pharmacy (MN). The performed search strategy can be found in **Supplementary Material S3.1** and was conducted until March 19, 2021. The quality of evidence was scored on a 5-point scale ranging from 0 (lowest) to 4 (highest) and the impact of the clinical effect was scored on a 7-point scale ranging from AA# (positive effect) to F (highest negative effect). This clinical impact scale (AA#-F) runs parallel to the Common Terminology Criteria for Adverse Events (CTCAE); where CTCAE grade 5 severity is equal to clinical relevance score F (death) and CTCAE grade 1 severity is equal to clinical relevance score B. The clinical relevance score additionally includes the scores AA#, AA and A, since these do not exist in the CTCAE. These regard AA#: "Positive clinical effect", AA: "No significant clinical and/or kinetic effect", and A: "Significant kinetic effect or not clinically relevant negative effect". The summaries and scores of the articles reviewed to devise this guideline are described in **Supplementary Table S3.4**. The summary and scores of each article were checked by two independent DPWG members. The DPWG made the final decision on the therapeutic recommendations. DPWG guidelines are checked for agreement with current evidence every 5 years in general. An updated version of the guideline will be published if recommendations are altered.

The initial literature search was performed on September 18, 2006, followed by searches on October 27, 2008, March 19, 2014, July 20, 2017 and March 19, 2021. Given the large number of articles, the only articles included after July 2006 were those that included at least 25 subjects with one or more *28 alleles. The only clinical studies included for the period 2008–2017 were meta-analyses, as large individual studies (n>200) were already included in the meta-analyses. From 2008 to 2014 only meta-analyses with mainly White patients were included. Three Asian meta-analyses investigating the effect of *6 and *28 were not included as these are insufficiently relevant to the situation in the Netherlands. For the period after 2014, meta-analyses were included if the effect of *28 was analysed, either alone or in combination with *6. For the period after 2017, clinical studies were only included if they investigated more than 500 patients with the additional requirements of more than 150 cases for case-control studies, and analysis of the effect of *28 in the case of meta-analyses. Pharmacokinetic studies were only included if exposure to or clearance of SN-38 was determined for the *1/*1, *1/*28 and *28/*28 genotypes and if these were the most important genotypes investigated within the population (i.e. studies among Whites) (for the period from 2008 to 2014) or for the *1/*1, *1/*28 and/or *1/*6, and *28/*28 and/or *6/*28 and/or *28/*28 genotypes (for the period from 2014). For the periods from 2008 to 2014 and after 2017, there were no relevant studies investigating the effect of dose adjustments. This means that there were no studies that investigated the effect of approximately 30% lower initial doses for PM compared to the standard dose for NM and IM in this period.

GENERAL CONCLUSION OF EVIDENCE

For *28/*28 and "PM other", there is strong evidence that these genotypes are associated with an increased risk of grade ≥3 toxicity such as neutropenia or diarrhoea. All nine meta-analyses investigating adverse events and 16 of the 23 included studies reported this increased risk. In addition, all seven meta-analyses and three studies investigating the effect of *28/*28 and/or *6/*6 and/or *6/*28 compared with all other genotypes, found that this toxicity risk was also increased for *28/*28 and/or PM patients compared to all other patients. With regard to efficacy, four of the five meta-analyses and eight of the ten studies did not show the *28 and/or *6 variants to be associated with increased effectiveness of treatment. See **Supplementary Table S3.4** and **S3.5** for a detailed description of the literature and the rationale of the therapeutic recommendations. In addition, recently the results of a prospective implementation study of *UGT1A1* genotype-guided dosing of irinotecan in PM patients were published showing that *UGT1A1* genotype-guided dosing of irinotecan in PM patients with applying a 30% dose reduction significantly improved safety while maintaining therapeutic drug exposure [22].

In summary, for *28/*28 and "PM other" there is ample evidence for an increased risk of serious adverse events such as neutropenia or diarrhoea at normal doses (also when compared to all other genotypes/phenotypes), while convincing evidence for an increased efficacy has not been demonstrated. Therefore, the DPWG concludes that a *UGT1A1* gene-drug interaction is present and that it necessitates a dose adjustment of irinotecan. Ongoing debate persists on whether or not there is a clinically relevant higher risk of toxicity in PM patients treated with lower dosages of irinotecan (<150 mg/m^2). However, two meta-analyses [23,24] indicate that the risk of grade 3–4 neutropenia is also elevated at lower doses of irinotecan and therefore the DPWG recommend dose adjustment of irinotecan in all dosing categories.

For *1/*28 and "IM other", a similar amount of evidence is present as for *28/*28 and "PM other". See **Supplementary Table S3.4** and **S3.5** for a detailed description of the literature and the rationale of the therapeutic recommendations. However, *1/*28 is the major group among White populations. The initial standard irinotecan dose derived in earlier phase I studies was therefore mainly driven by the *1/*28 genotype. This is confirmed by Lu et al. 2015 [25], showing that most *1/*28+*1/*1 patients tolerate the standard dose, whereas *28/*28 patients did not. Furthermore, there were negligible dose adjustments calculated for *1/*28 compared to all genotypes (a weighted mean calculated dose adjustment to 95% of the dose for all patients based on 6 studies with a total of 112 patients with the *1/*28

genotype) (**Supplementary Table S3.5**). This means that a priori dose reduction for patients with *1/*28 would lead to subtherapeutic doses for this patient group. Because the kinetic and clinical effects of *28 and *6 are comparable, the same holds true for IM predicted phenotype as a whole. Therefore, the DPWG concludes that a gene-drug interaction is present, but that therapy adjustment is neither required nor advisable in *UGT1A1* IM patients.

Based on the above, the dose for *1/*1 may be increased. As three meta-analyses did not identify a difference in effectiveness of therapy between *1/*28 and *1/*1, an increase for *1/*1 patients has not yet proven to be useful. Therefore, the DPWG decided to refrain from a recommendation for *1/*1.

PHARMACOTHERAPEUTIC RECOMMENDATIONS

The DPWG therapeutic recommendations to optimize the starting dose of irinotecan in patients known to have a variant UGT1A1 predicted phenotype is summarized in **Table 3.2**. In brief, UGT1A1 PM patients, including *28/*28, should receive a 70% starting dose of irinotecan, with the number of 70% primarily based on kinetic data and early dose-finding studies as described below. Further dose titration is possibly guided on neutrophil count and clinical tolerance. For *UGT1A1*1/*28* and *UGT1A1* "IM other" patients no dose reduction is recommended.

Table 3.2: Summary therapeutic recommendations based on *UGT1A1* predicted phenotype for irinotecan

UGT1A1 predicted phenotype	Therapeutic recommendation
PM (*28/*28)	Start with 70% of the normal dose[a]. If the patient tolerates this initial dose, the dose can be increased, guided by the neutrophil count.
PM other	Start with 70% of the normal dose[a]. If the patient tolerates this initial dose, the dose can be increased, guided by the neutrophil count.
IM (*1/*28)	No action required
IM other	No action required

IM = intermediate metaboliser, PM = poor metaboliser.
[a] The normal dose is defined as the dose the patient would receive if he/she would not have a gene variant.

The dose calculation for *28/*28 was based on the SN-38 exposure (area under the curve (AUC)) or clearance in 6 studies with a total of 28 patients with *28/*28. The weighted mean of the calculated dose adjustment is a dose of 58% (range 39–85%, median 53%) of the dose for *1/*1 and a dose of 69% (range 48–92%, median 64%) of the dose for all patients. As the frequency of *1/*1 in Europe is less than 50%, and as caution should be exercised to prevent subtherapeutic doses, the calculated dose compared to all patients was chosen. This is translated into a starting dose of 70% which is more achievable in clinical practice (**Supplementary Table S3.5**). The SN-38 glucuronide/SN-38 area under the curve (AUC) ratios are comparable for *28/*28 and *6/*6, suggestive of a similar effect size on irinotecan metabolism [26]. Therefore, the recommendations for the "PM other" predicted phenotype (for example caused by *6), is the same as the recommendations for the *28/*28 genotype, respectively.

More information on the rationale, kinetic and clinical consequences of these therapeutic recommendations are depicted in **Supplementary Table S3.5**.

Supplementary Table S3.6 provides an overview of suggested pop-up (or look-up) texts for electronic prescribing systems for pharmacists and physicians. These can be used to program alerts into the clinical decision support system (CDSS).

IMPLICATIONS FOR CLINICAL PRACTICE

Ongoing debate persists whether and which single gene-drug pairs should be implemented into routine care. Points of debate include the amount of evidence that is necessary supporting effectiveness of genotyping prior to initiating therapy, cost-effectiveness of PGx testing in the pre-therapeutic setting and its reimbursement [27]. As a consequence, gene–drug pairs which are ready for implementation are hampered in application in clinical practice [28]. In an effort to overcome this inconclusiveness and to direct clinicians on whether or not to order relevant PGx genotyping tests before initiating therapy, the DPWG has developed the Clinical Implication Score. The DPWG Clinical Implication Score for a gene–drug pair can be scored as: essential, beneficial or potentially beneficial. These categories are clarified in **Supplementary Table S3.7**. The development of these categories and the systematic scoring criteria are discussed elsewhere [29]. In brief, the implications for clinical practice are based on four criteria: the clinical effect associated with gene–drug interaction; the level of evidence supporting the associated clinical effect; the number needed to genotype (NNG) in the Dutch population; and the availability of and type of PGx information in the drug label

issued by the Dutch drug agency CBG-MEB. The scores provided for each of these criteria by the DPWG can be found in **Supplementary Table S3.7**. Only gene-drug interactions which are actionable are subject to receiving a Clinical Implication Score.

The Clinical Implication Score of the gene-drug interaction between UGT1A1 and irinotecan is 8 out of the maximum of 10 points. This indicates that genotyping before starting irinotecan is considered "**essential**" for drug safety. Genotyping must be performed before drug therapy has been initiated to guide dose selection. The feasibility and clinical benefit of such an approach has also recently been demonstrated. A recent prospective implementation study on *UGT1A1* genotype-guided dosing of irinotecan in PM patients showed that genotype-guided dosing in PM patients increases safety, provides therapeutic drug exposure, and is cost-effective, and supports the recommendation of a 70% starting dose in UGT1A1 PM patients [22].

DIFFERENCES BETWEEN AVAILABLE PHARMACOGENETIC GUIDELINES

To the best of our knowledge there are two other pharmacogenetic guidelines available on the gene-drug interaction of irinotecan and *UGT1A1*. First, a guideline by the French joint working group comprising the National Pharmacogenetics Network (RNPGx) and the Group of Clinical Onco-pharmacology (GPCO-Unicancer) [30]. Second, an Italian guideline by the Italian association of medical oncologists (AIOM) and the Italian Society of Pharmacology (SIF) [31]. The Clinical Pharmacogenetics Implementation Consortium (CPIC) has no guideline available, but indicates this gene-drug interaction as an actionable PGx [32]. Both guidelines are shortly discussed below.

RNPGx and GPCO-Unicancer

The genotype-phenotype translation in the RNPGx/GPCO guideline is in line with the DPWG guideline; the *36 allele can be interpreted as a *1 allele and the *37 allele as a *28 allele. In addition, in both guidelines pre-treatment *UGT1A1* genotyping is strongly recommended and the advised dose reduction at the first cycle for *28/*28 patients is similar, namely 25–30%.

However, the RNPGx/GPCO guideline does not recommend pre-therapeutic *UGT1A1* genotyping for low irinotecan doses (<180 mg/m^2) because haematological and gastrointestinal toxicities are quite similar regardless of the genotype for low irinotecan doses. In contrast, the DPWG concluded that the risk of grade 3–4 neutropenia is also elevated at lower doses of irinotecan based on two meta-analyses [23, 24] and therefore recommends to genotype all

patients treated with irinotecan. Moreover, in the RNPGx/GPCO guideline it is recommended that *28/*28 patients must not receive high-dose irinotecan (≥240 mg/m^2) because of a much higher risk of haematological toxicity (neutropenia) compared to other genotypes, whereas the DWPG guideline does not advocate a contra-indication for high-dose irinotecan in these patients.

AIOM and SIF

This Italian guideline only provides guidance on the *28 gene variant of *UGT1A1*. They recommend a dose reduction of 30% in *28/*28 patients which is in line with the current DPWG guideline.

CONCLUSION

In conclusion, the DPWG recommends a 70% starting dose in PM patients that start treatment with irinotecan. In IM patients, an a priori dose reduction is not recommended. Based on the DPWG clinical implication score, *UGT1A1* genotyping is considered "essential", therefore directing towards pre-therapeutic *UGT1A1* testing in patients intended for treatment with irinotecan.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY METHODS

Search terms used to perform the literature review of the *UGT1A1* – irinotecan interaction

Search strategy

PubMed was searched for English, Dutch and German articles. For PubMed-searches with the following term: '("humans"[Mesh Terms])' the database was also searched without this term in order to find recent articles.

Complete search strings

Search performed in 2006: irinotecan AND UGT1A1

Search performed in 2008 and 2014: ("irinotecan"[Substance Name] OR irinotecan[Text Word]) AND (UGT* OR "bilirubin uridine-diphosphoglucuronosyl transferase 1A1"[Substance Name] OR "Glucuronosyltransferase"[MeSH] OR "bilirubin"[MeSH Terms] OR bilirubin[Text Word] OR metabolizer OR metaboliser OR polymorph* OR "Polymorphism, Genetic"[MeSH] OR "Pharmacogenetics"[MeSH]) AND (English[lang] OR German[lang] OR Dutch[lang]) AND ("humans"[Mesh Terms])

Search performed in 2017: ("irinotecan" [Supplementary Concept] OR irinotecan) AND ("UGT1A1 enzyme" [Supplementary Concept] OR UGT1A1 OR 1A1) AND (English[lang] OR German[lang] OR Dutch[lang])

Search performed in 2021: ("Irinotecan"[Mesh] OR irinotecan) AND ("UGT1A1 enzyme" [Supplementary Concept] OR UGT1A1 OR 1A1) AND (English[lang] OR German[lang] OR Dutch[lang])

Supplementary Table S3.1: *UGT1A1* alleles and metabolic capacity [1]

Metabolic capacity	*allele	rs-number	HGVS reference sequence		
			NM_000463.3	NP_000454.1	NC_000002.12
Increased functionality	*36	rs3064744	Not applicable	Not applicable	g.233760235TA[6]
Fully functional	*1 (= wild-type)	Not applicable	Not applicable	Not applicable	g.233760235TA[7]
Reduced functionality	*6	rs4148323	c.211G>A	p.Gly71Arg	g.233760498G>A
	*7	rs34993780	c.1456T>G	p.Tyr486Asp	g.233772413T>G
	*8	rs72551343	c.625C>T	p.Arg209Trp	g.233760912C>T
	*9	rs72551348	c.992A>G	p.Gln331Arg	g.233767161A>G
	*12	rs72551341	c.524T>A	p.Leu175Gln	g.233760811T>A
	*27	rs35350960	c.686C>A	p.Pro229Gln	g.233760973C>A
	*28	rs3064744	Not applicable	Not applicable	g.233760235TA[8]
	*29	rs55750087	c.1099C>G	p.Arg367Gly	g.233768234C>G
	*30	rs111033541	c.44T>G	p.Leu15Arg	g.233760331T>G
	*32	rs139607673	c.1006C>T	p.Arg336Trp	g.233767858C>T
	*33	rs72551347	c.881T>C	p.Ile294Thr	g.233767050T>C
	*34	rs1699317728	c.928A>G	p.Met310Val	g.233767097A>G
	*37	rs3064744	Not applicable	Not applicable	g.233760235TA[9]

Supplementary Table S3.1: ***Continued***

Metabolic capacity	*allele	rs-number	HGVS reference sequence		
			NM_000463.3	NP_000454.1	NC_000002.12
Fully dysfunctional (null alleles)	*2	rs587776761	c.877_890delinsA	p.Tyr293fs	g.233767046_233767060delinsA
	*3	rs72551353	c.1124C>T	p.Ser375Phe	g.233768259C>T
	*4	rs72551350	c.1069C>T	p.Gln357Ter	g.233767921C>T
	*5	rs111033539	c.991C>T	p.Gln331Ter	g.233767160C>T
	*10	rs72551349	c.1021C>T	p.Arg341Ter	g.233767873C>T
	*11	rs62625011	c.923G>A	p.Gly308Glu	g.233767092G>A
	*13	rs587776762	c.510CTT[1]	p.Phe171del	g.233760797CTT[1]
	*14	rs72551345	c.826G>C	p.Gly276Arg	g.233761113G>C
	*15	rs7255342	c.529T>C	p.Cys177Arg	g.233760816T>C
	*16	rs72551351	c.1070A>G	p.Gln357Arg	g.233767922A>G
	*17	rs72551354	c.1143C>G	p.Ser381Arg	g.233768278C>G
	*18	rs72551355	c.1201G>C	p.Ala401Pro	g.233768336G>C
	*19	rs1699508733	c.1005G>A	p.Trp335Ter	g.233767857G>A
	*20	rs72551352	c.1102G>A	p.Ala368Thr	g.233768237G>A
	*22	rs758873309	c.875C>T	p.Ala292Val	g.233767044C>T
	*25	rs281865418	c.840C>A	p.Cys280Ter	g.233761127C>A
	*31	rs1559415403	c.1160_1161delinsGT	p.Pro387Arg	g.233768295_233768296delinsGT

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Supplementary Table S3.2: *UGT1A1* allele and genotype frequencies

Population group/region	Prevalence of genotype (%)								Allele frequency (%)		
	*1/*1 TA6/ TA6	*1/*28 TA6/ TA7	*28/*28 TA7/ TA7	*36/*1 TA5/ TA6	*36/*28 TA5/ TA7	*36/*37 TA5/ TA8	*1/*37 TA6/ TA8	*28/*37 TA7/ TA8	*28	*6	*37
Whites	34–38	46–55	11–13	0–2	0	0	0	0–2	33–36		0-2.8
The Netherlands (Whites)	37	54	9						36		
Europe	30–50	40–60	5–15						22–39		
Europe (without Finland)									32	0.2	0.07
Finland									42	4.6	0.05
Africa	20–60	30–50	6–18	4–12	7–8	1–2	3–8	1–14	24–42		1.2–29
African-American	26	33–37	13–19	0–2	5	3	4–15	5–6	36–44		5.7–8.3
African/ African-American									40	0.07	5
Asia	25–75	15–60	2–20	0	0	0	0	0	14–45		0
East Asia									12	15.3	
Japan									10.4	22.2	
South Asia									41	2.0	0.2
South America	55	30	12						27		
Latin-American/ American, mixed ethnicity									31	2.4	0.4
Pacific	75–95	5–20	2						4.5–12		
Ashkenazi Jewish									38	0.5	

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5) Akiyama Y et al. Genetic testing for UGT1A1*28 and *6 in Japanese patients who receive irinotecan chemotherapy. Ann Oncol 2008;19:2089-–90.
6) Genome aggregation database (gnomAD) v2.1.1 (*6) and v3.1.1 (*28 and *37), https://gnomad.broadinstitute.org.

Supplementary Table S3.3: Genotype to predicted phenotype translation to be programmed into laboratory information system

Genotype	rs number variants	Nucleotide at position	Predicted phenotype
UGT1A1: WILDTYPE/WILDTYPE	*UGT1A1*_rs35350960 *UGT1A1*_rs4148323 *UGT1A1*_rs8175347	C:C G:G -:-	*1/*1 (TA6/TA6)
UGT1A1:*28/*37/*28/*37	*UGT1A1*_rs35350960 *UGT1A1*_rs4148323 *UGT1A1*_rs8175347	C:C G:G TA:TA	*28/*28 (TA7/TA7)
UGT1A1:WILDTYPE/*28/*37	*UGT1A1*_rs35350960 *UGT1A1*_rs4148323 *UGT1A1*_rs8175347	C:C G:G -:TA	*1/*28 (TA6/TA7)
UGT1A1:*27/*27	*UGT1A1*_rs35350960 *UGT1A1*_rs4148323 *UGT1A1*_rs8175347	A:A G:G -:-	PM otherwise
UGT1A1:WILDTYPE/*27	*UGT1A1*_rs35350960 *UGT1A1*_rs4148323 *UGT1A1*_rs8175347	C:A G:G -:-	IM otherwise
UGT1A1:WILDTYPE/*27_WILDTYPE/*28/*37	*UGT1A1*_rs35350960 *UGT1A1*_rs4148323 *UGT1A1*_rs8175347	C:A G:G -:TA	PM otherwise
UGT1A1:*6/*6	*UGT1A1*_rs35350960 *UGT1A1*_rs4148323 *UGT1A1*_rs8175347	C:C A:A -:-	PM otherwise
UGT1A1:WILDTYPE/*6	*UGT1A1*_rs35350960 *UGT1A1*_rs4148323 *UGT1A1*_rs8175347	C:C G:A -:-	IM otherwise
UGT1A1:WILDTYPE/*6_WILDTYPE/*28/*37	*UGT1A1*_rs35350960 *UGT1A1*_rs4148323 *UGT1A1*_rs8175347	C:C G:A -:TA	PM otherwise
UGT1A1:WILDTYPE/*27_WILDTYPE/*6	*UGT1A1*_rs35350960 *UGT1A1*_rs4148323 *UGT1A1*_rs8175347	C:A G:A -:-	PM otherwise

This table includes *UGT1A1* alleles with a minor allele frequency ≥1% in either the White, African or Asian population.

According to the allele definition table of PharmGKB, there is no allele including two of the polymorphisms (https://www.pharmgkb.org/haplotype/PA166115865, accessed on 16 December 2022). This suggests that alleles including two or more of these polymorphisms are either very rare or non-existent. For this reason, genotypes with 3 or 4 polymorphisms were not included in the translation table. In addition, in compound heterozygotes, both polymorphisms were considered to be on different alleles.

Supplementary Table S3.4: Literature review of *UGT1A1*-irinotecan interactions supporting the therapeutic guideline to reduce the starting dose in PM patients

The table below follows the KNMP nomenclature for UGT1A1 gene variants. The nomenclature used in the table below may therefore differ from the nomenclature used by the authors in the original publications.

Reference	Code	Effect	Comments
ref. 1 Yang Y et al. UGT1A1*6 and UGT1A1*28 polymorphisms are correlated with irinotecan-induced toxicity: A meta-analysis. Asia Pac J Clin Oncol 2018;14:e479-e489. PMID: 29932297.	Level of evidence score: 3	Meta-analysis of 38 studies with a total of 6742 cancer patients treated with irinotecan, either as combined chemotherapy or as monotherapy. Irinotecan doses in the studies varied from 60 to 375 mg/m^2. 30 studies with a total of 3791 patients (2234x *1/*1, 1182x *1/*28, 275x *28/*28) investigated the effect of *28 on neutropenia. 25 studies with a total of 2780 patients (1568x *1/*1, 963x *1/*28, 249x *28/*28) investigated the effect of *28 on diarrhoea. All 34 studies could be used to investigate the effect of ethnicity, 30 studies to investigate the effect of irinotecan dose and 27 studies to investigate the effect of tumour type. 14 studies with a total of 2072 patients (1322x *1/*1, 606x *1/*6, 144x *6/*6) investigated the effect of *6 on neutropenia. 8 studies with a total of 900 patients (595x *1/*1, 249x *1/*6, 56x *6/*6) investigated the effect of *6 on diarrhoea. All 16 studies could be used to investigate the effect of ethnicity, 14 studies to investigate the effect of irinotecan dose and 11 studies to investigate the effect of tumour type. All studies investigating the effect of *6 were in Asians. Of the 38 studies included in the meta-analysis, 7 were also included separately in this risk analysis (Kweekel 2008, Côté 2007, Massacesi 2006, Toffoli 2006, Innocenti 2004, Rouits 2004, and Font 2003). A later publication of one study was also included in the meta-analysis (McLeod 2006). Of the 38 studies in this meta-analysis, 23 were also included in the meta-analysis of Liu 2017, 12 in the meta-analysis of Liu 2014, 9 in the meta-analysis of Hu 2010 Eur J Cancer, 8 in the meta-analyses of Chen 2014 and Hu 2010 Clin Cancer Res, 7 in the meta-analysis of Han 2014, 4 in the meta-analysis of Hoskins 2007, and 2 in the meta-analysis of Chen 2017. A random-effects model was used for the meta-analyses in case of significant heterogeneity. Otherwise, a fixed-effects model was used. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data extraction was standardised. The authors indicate that the quality of eligible studies was evaluated by surveying the methodologies and trial design, but do not present quality scores for the included studies. Publication bias was analysed, but only with Egger's test and the authors did not specify for which of the four meta-analyses (two outcomes (neutropenia and diarrhoea) and two comparisons (heterozygotes compared to no variant allele and homo-zygotes compared to no variant allele)) they investigated possible publication bias. No publication bias analyses were performed for the subgroups.	Authors' conclusion: 'Both UGT1A1*6 and UGT1A1*28 polymorphisms can be considered as predictors of irinotecan-induced toxicity, with effect varying by race, cancer type and irinotecan dose.'

*28/*28: CTC-AE 4
*1/*28: CTC-AE 4

Results:

ORs (95% CI) for *1/*28 and *28/*28 versus *1/*1:

		28/*28	*1/*28	Incidence for *1/*1 (% of patients)
Neutropenia grade III–IV		OR=3.50 (2.23–5.50) (S)	OR=1.91 (1.45–2.50) (S)	16%
Diarrhoea grade III–IV		OR=1.69 (1.20–2.40) (S)	OR=1.45 (1.07–1.97) (S)	12%
Severe toxicity	All ethnicities	OR=2.28 (1.80–2.88) (S)	OR=1.60 (1.30–1.97) (S)	
	Whites	OR=2.43 (1.44–4.08) (S)	OR=1.59 (1.17–2.17) (S)	
	Asians	OR=2.94 (1.86–4.64) (S)	OR=1.67 (1.29–2.17) (S)	
Severe toxicity	All irinotecan doses	OR=3.07 (2.09–4.52) (S)	OR=1.77 (1.44–2.17) (S)	
	>150 mg/m^2	OR=3.48 (2.25–5.39) (S)	OR=1.81 (1.46–2.25) (S)	
	<150 mg/m^2	NS	NS	
Severe toxicity	All tumour types	OR=2.76 (1.86–4.09) (S)	OR=1.68 (1.37–2.06) (S)	
	Digestive system	OR=2.90 (1.95–4.30) (S)	OR=1.73 (1.40–2.15) (S)	
	Respiratory system	NS	NS	

There was no statistically significant heterogeneity between the studies for the comparison of diarrhoea in *28/*28 versus *1/*1.
The heterogeneity between the studies was significant, but low, for the other comparisons.

There was no publication bias according to the Egger's test.

PM: CTC-AE 4
IM: CTC-AE 4

ORs (95% CI) for *1/*6 and *6/*6 versus *1/*1:

		*6/*6	*1/*6	Incidence for *1/*1 (% of patients)
Neutropenia grade III–IV		OR=3.03 (2.05–4.47) (S)	OR=1.95 (1.34–2.85) (S)	17%
Diarrhoea grade III–IV		OR=4.03 (1.98–8.32) (S)	OR=1.98 (1.26–3.11) (S)	8.6%
Severe toxicity	Asians (= the only ethnicity in the studies)	OR=3.16 (2.25–4.44) (S)	OR=1.95 (1.42–2.66) (S)	
Severe toxicity	All irinotecan doses	OR=3.17 (2.24–4.48) (S)	OR=2.08 (1.46–2.97) (S)	

Supplementary Table S3.4 continues on next page.

Supplementary Table S3.4: ***Continued***

			>150 mg/m^2	OR=2.91 (2.02–4.18) (S)	OR=1.82 (1.28–2.57) (S)	
			<150 mg/m^2	OR=9.42 (2.43–36.5) (S)	OR=3.49 (1.28–9.58) (S)	
		Severe toxicity	All tumour types	OR=3.21 (2.20–4.67) (S)	OR=1.75 (1.22–2.52) (S)	
			Digestive system	OR=3.00 (2.04–4.42) (S)	OR=1.66 (1.18–2.35) (S)	
			Respiratory system (only 1 study)	OR=18.2 (1.56–212) (S)	OR=12.0 (1.02–141) (S)	
		There was moderate heterogeneity between the studies for the comparison of neutropenia in *1/*6 versus *1/*1. There was no statistically significant heterogeneity between the studies for the other comparisons.				
		There was no publication bias according to the Egger's test.				
ref. 2 Tejpar S et al. Clinical and pharmacogenetic determinants of 5-fluorouracyl/ leucovorin/irinotecan toxicity: results of the PETACC-3 trial. Eur J Cancer 2018;99:66-77. PMID: 29909091.	Level of evidence score: 4	574 colon cancer patients were treated with irinotecan 180 mg/m^2 every two weeks in combination with 5-fluorouracil and leucovorin for 6 months. Adverse events were assessed according to the National Cancer Institute Common Toxicity Criteria Grading System. Any grade III or IV toxic event resulted, as per protocol, in a 20% dose reduction for subsequent cycles after toxicity resolution or treatment was postponed. Periods with lowered chemotherapy doses were not included in the analysis of adverse events. Dose reduction was used as a global measure of toxicity. 69.6% of patients had stage III colon cancer. 28.2% of patients developed neutropenia grade III–IV, 9.0% neutropenia grade IV, 10.4% diarrhoea grade III–IV, and 21.2% a serious adverse event. A dose reduction was applied in 30.9% of patients. Comedication other than hormone replacement therapy was not mentioned, but a strong effect of comedication on either UGT1A1 or severe adverse events is not expected. In a parallel arm of this randomised clinical trial, 572 patients were treated with 5-fluorouracil and leucovorin for 6 months, allowing comparison of the effect of *28 in patients treated with and without irinotecan. ORs were determined by multivariate regression analyses. Adjustment was for age, sex, body surface area-sex combination, WHO performance status, bilirubin >0.5x the upper limit of normal, and in case of the neutropenia and dose reduction outcomes also for baseline neutrophils. Genotyping (estimated based on the genotypes of the 568 patients included in the Kaplan-Meier curve): - 234x *1/*1 - 258x *1/*28 - 82x *28/*28 Results:				Authors' conclusion: 'We found that a complex of risk factors is involved in the development of toxicity, including UGT1A1. Parameters that are readily available in clinical practice, notably sex, age and performance status, are stronger predictors than the UGT1A1 *28 genotype.'

	*28/*28: CTC-AE 4	Results for *28/*28 compared to *1/*1+*1/*28 (neutropenia, and total serious adverse effects) or for *28/*28 versus *1/*28 versus *1/*1 (diarrhoea and dose reduction):		
				incidence for *1/*1+ *1/*28
		Neutropenia grade III–IV	OR_{adj}=2.89 (1.65–5.07) (S)	28% of patients
			Kaplan-Meier curve analysis showed *28/*28 to be associated with more frequent and earlier neutropenia grade III–IV (S).	
			In univariate analysis, there was no difference between *1/*1 and *1/*28.	
			The result was NS in the arm without irinotecan, confirming the result in the arm with irinotecan to be caused by the *28-irinotecan interaction. The percentage of patients with neutropenia grade III-IV in the arm without irinotecan, was 23% of that in the arm with irinotecan (6.4% versus 28.2%).	
		Neutropenia grade IV	OR_{adj}=2.33 (1.03–5.24) (S)	
			The result was NS in the arm without irinotecan, confirming the result in the arm with irinotecan to be caused by the *28-irinotecan interaction. The percentage of patients with neutropenia grade IV in the arm without irinotecan, was 28% of that in the arm with irinotecan (2.5% versus 9.0%).	
		Diarrhoea grade III–IV	Trend for a decrease with increasing number of *28-alleles (p=0.068) (NS).	
			A similar trend, albeit with a somewhat higher p-value (p=0.136), was present in the arm without irinotecan, contradicting the result to be caused by the *28-irinotecan interaction. The percentage of patients with diarrhoea grade III-IV in the arm without irinotecan, was 49% of that in the arm with irinotecan (5.1% versus 10.4%).	
		Total serious adverse events	x 1.7 (S)	0.40 per patient
			The result was NS in the arm without irinotecan, confirming the result in the arm with irinotecan to be caused by the *28-	

Supplementary Table S3.4 continues on next page.

Supplementary Table S3.4: ***Continued***

	*1/*28: CTC-AE 4		irinotecan interaction. For *1/*1+*1/*28, the rate of serious adverse events in the arm without irinotecan, was 58% of the rate in the arm with irinotecan.		
		Dose reduction	OR_{adj} per *28-allele=1.35 (1.01–1.79) (S)		
			The result was NS in the arm without irinotecan, confirming the result in the arm with irinotecan to be caused by the *28-irinotecan interaction. The percentage of patients with dose reduction in the arm without irinotecan, was 50% of that in the arm with irinotecan (15.5% versus 30.9%).		
		Relapse-free survival of stage III patients	*28/*28 showed a trend for a better survival in the arm with irinotecan than in the arm without irinotecan (p=0.07) (NS), but *1/*1+*1/*28 did not.		
		Note: The gene variant 3156G>A was also determined. However, there was a strong association between *28 and 3156G>A and in bivariate logistic regression analysis with both gene variants, only *28 remained significant as predictor for bilirubin >0.5x upper limit of normal and as predictor for neutropenia grade III or grade IV. For this reason, no further analyses were performed for 3156G>A.			
ref. 3 Chen X et al. UGT1A1 polymorphisms with irinotecan-induced toxicities and treatment out-come in Asians with lung cancer: a meta-analysis. Cancer Chemother Pharmacol 2017;79:1109-1117. PubMed PMID: 28502040.	Level of evidence score: 3	Meta-analysis of 9 studies with in total 577 Asian lung cancer patients treated with irinotecan, either as combined chemotherapy or as monotherapy. Irinotecan doses in the studies varied from 50 to 100 mg/m2. In addition, the therapy interval is relatively long in lung cancer treatment. Of the 9 studies included in the meta-analysis, 1 was also included separately in this risk analysis (Han 2006). Of the 9 studies in this meta-analysis, 5 were also included in the meta-analysis of Liu 2017, 3 in the meta-analysis of Han 2014, 2 in the meta-analyses of Dias 2012 and Hu 2010 Eur J Cancer, and 1 in the meta-analysis of Chen 2014. None were included in the meta-analyses of Liu 2014 and Liu 2013 (both colorectal cancer and mainly White), Dias 2014, Hu 2010 Clin Cancer Res and Hoskins 2007. Data on *28 were derived from 9 studies including a total of 524 patients. For diarrhoea, the comparison between *1/*28 and *1/*1 was based on 439 patients from 8 studies of which 78 *1/*28. The comparison between *28/*28 and *1/*1 was based on 104 patients from 3 studies of which 8 *28/*28. For neutropenia, the comparison between *1/*28 and *1/*1 was based on 412 patients from 7 studies of which 71 *1/*28. The comparison between *28/*28 and *1/*1 was based on 81 patients from 2 studies of which 5 *28/*28. For tumour response, the comparison between *1/*28+*28/*28 and *1/*1 was based on 316 patients from 7 studies of which 66 *1/*28+*28/*28. Data on *6 were derived from 6 studies including a total of 441 patients. For diarrhoea, the comparison		Authors' conclusion: 'These data suggest that the UGT1A1*28 polymorphism may not be a suitable biomarker to predict irinotecan (IRI)-induced toxicities and chemotherapy tumour response (TR) in Asians, while UGT1A1*6 polymorphism is associated with a higher risk of IRI-induced neutropenia and diarrhoea, but not IRI-based chemotherapy

<table>
<tr>
<td></td>
<td>*28/*28: CTC-AE 4
*1/*28: Clinical Relevance Score AA</td>
<td>between *1/*6 and *1/*1 was based on 182 patients from 4 studies of which 61 *1/*6. The comparison between *6/*6 and *1/*1 was based on 80 patients from 3 studies of which 4 *6/*6. For neutropenia, the comparison between *1/*6 and *1/*1 was based on 153 patients from 3 studies of which 53 *1/*6. The comparison between *6/*6 and *1/*1 was based on 58 patients from 2 studies of which 3 *6/*6. For tumour response, the comparison between *1/*6+*6/*6 and *1/*1 was based on 182 patients from 4 studies of which 63 *1/*6+*6/*6.
Toxicity was defined as grade 3-4 toxicity and tumour response as the response rate.
A random-effects model was used for the meta-analysis in case of significant heterogeneity. Otherwise, a fixed-effects model was used. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data extraction was standardised.
The authors indicate that the quality of the included studies was evaluated based on information collected from the studies including study design, number of patients, population, mutation detection method, race, histology, Hardy-Weinberg equilibrium, chemotherapy regimen, grade criteria for neutropenia and diarrhoea and definitions of treatment outcome measures, but do not present quality scores for the studies.
Publication bias analysis was not performed.

Results:
<table>
<tr><td colspan="4">ORs (95% CI) for *1/*28 and *28/*28 versus *1/*1:</td></tr>
<tr><td></td><td>*28/*28</td><td>*1/*28</td><td>incidence for *1/*1 (% of patients)</td></tr>
<tr><td>Diarrhoea</td><td>OR=5.93 (1.46–24.0) (S)</td><td>NS</td><td>11%</td></tr>
<tr><td></td><td colspan="2">The association was also significant for *28/*28 versus *1/*1+*1/*28 (OR=6.25 (1.51–25.0) (S)) (3 studies with 131 patients of which 8 *28/*28).</td><td></td></tr>
<tr><td>Neutropenia</td><td>NS</td><td>NS</td><td>30%</td></tr>
<tr><td></td><td colspan="2">There was also no significant association for *28/*28 versus *1/*1+*1/*28 (NS) (2 studies with 101 patients of which 5 *28/*28) and for *1/*28+*28/*28 versus *1/*1 (NS) (8 studies with 494 patients of which 95 *1/*28+ *28/*28).</td><td></td></tr>
<tr><td>Tumour response</td><td colspan="2">NS for *1/*28+*28/*28 versus *1/*1</td><td>54%</td></tr>
<tr><td colspan="4">There was no statistically significant heterogeneity between the studies.</td></tr>
</table>
</td>
<td>TR.’</td>
</tr>
</table>

Supplementary Table S3.4 continues on next page.

Supplementary Table S3.4: ***Continued***

<table>
<tr><td></td><td>PM: CTC-AE 4
IM: CTC-AE 4</td><td>ORs (95% CI) for *1/*6 and *6/*6 versus *1/*1:<table>
<tr><td></td><td>*6/*6</td><td>*1/*6</td><td>incidence for *1/*1 (% of patients)</td></tr>
<tr><td>Diarrhoea</td><td>OR=17.6 (2.58–121) (S)</td><td>OR=4.36 (1.74–10.9) (S)</td><td>8%</td></tr>
<tr><td></td><td colspan="2">The association was also significant for *6/*6 versus *1/*1+*1/*6 (OR=5.26 (1.85–14.3) (S)) (5 studies with 307 patients of which 17 *6/*6).</td><td></td></tr>
<tr><td>Neutropenia</td><td>NS</td><td>NS</td><td>26%</td></tr>
<tr><td></td><td colspan="2">The association was significant for *6/*6 versus *1/*1+*1/*6 (OR=5.00 (1.69–14.3) (S)) (4 studies with 277 patients of which 17 *6/*6).
The association was also significant for *1/*6+*6/*6 versus *1/*1 (OR=2.40 (1.28–4.49) (S)) (4 studies with 233 patients of which 75 *1/*6+*6/*6).</td><td></td></tr>
<tr><td>Tumour response</td><td colspan="2">NS for *1/*6+*6/*6 versus *1/*1</td><td>59%</td></tr>
<tr><td colspan="4">There was no statistically significant heterogeneity between the studies.</td></tr>
</table></td><td></td></tr>
<tr><td>ref. 4
Liu XH et al.
Predictive value of UGT1A1*28 polymorphism in irinotecan-based chemotherapy.
J Cancer 2017;8:691-703.
PubMed PMID: 28367249.</td><td>Level of evidence score: 4</td><td colspan="2">Meta-analysis of 57 clinical trials (58 studies) with in total 6087 patients treated with irinotecan, either as combined chemotherapy or as monotherapy. Irinotecan doses in the studies varied from 60 to 375 mg/m^2. Patients were White in 15 studies, Asian in 40 studies and of mixed ethnicities or not reported in 2 studies. Patients had metastatic colorectal cancer in 29 studies, mixed tumours in 6 studies, metastatic non-small cell lung cancer in 5 studies, advanced gastric cancer in 3 studies, small cell lung cancer in 2 studies, advanced oesophageal cancer in 2 studies and another type of cancer in the remaining 11 studies. The quality of the included studies scored 7–9 points on the 9-point Newcastle-Ottawa Scale.
Of the 57 publications included in the meta-analysis, 11 were also included separately in this risk analysis (Kweekel 2008, Liu 2008, Han 2006, de Jong 2006, Massacesi 2006, Toffoli 2006, Innocenti 2004, Marcuello 2004, Rouits 2004, Font 2003 and Iyer 2002). A later publication of one study was also included in the meta-analysis (McLeod 2006).
Of the 57 publications included in the meta-analysis, 17 were also included in the meta-analysis of Hu 2010 Eur J Cancer, 13 in the meta-analysis of Liu 2014, 10 in the meta-analysis of Hu 2010 Clin Cancer Res, 9 in the meta-analysis of Han 2014, 8 in the meta-analyses of Liu 2013 and Dias 2012, 7 in the meta-analyses of Dias 2014 and Hoskins 2007, and 5 in the meta-analysis of Chen 2014.</td><td>Authors’ conclusion: ‘Our data showed that the UGT1A1*28 polymorphism had a significant relation-ship with toxicity and response to irinotecan-based chemotherapy. This polymorphism may be useful as a monitoring index for cancer patients receiving irinotecan-based chemotherapy.’</td></tr>
</table>

Data on diarrhoea were derived from 44 studies including a total of 4868 patients. The comparison between *1/*28 and *1/*1 was based on 3435 patients from 28 studies. The comparison between *28/*28 and *1/*1 was based on 2610 patients from 17 studies of which 151 *28/*28.
Data on neutropenia were derived from 49 studies including a total of 5232 patients. The comparison between *1/*28 and *1/*1 was based on 3948 patients from 32 studies. The comparison between *28/*28 and *1/*1 was based on 3575 patients from 27 studies of which 219 *28/*28.
Data on tumour response were derived from 18 studies including a total of 2024 patients.
Toxicity was defined as severe toxicity and tumour response as partial or complete remission.
A random-effects model was used for the meta-analysis in case of significant heterogeneity ($p<0.1$). Otherwise, a fixed-effects model was used. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data extraction was standardised. Publication bias was determined by Egger's and Begg's tests. In case of publication bias (a significant Egger's test), a trim and fill method was carried out for adjusting. Publication bias analyses were performed for all comparisons, but not for the subgroups.

Results:

ORs (95% CI) versus *1/*1:			
	*28/*28	*1/*28	incidence for *1/*1 (% of patients)
Diarrhoea			
All patients	OR=3.97 (1.88–8.38) (S)	OR=1.56 (1.25–1.96) (S)	9.3%
	The association was also significant for *28/*28 versus *1/*1+ *1/*28 (OR=3.64 (2.01–6.58) (S)) (24 studies with 3175 patients).		
White patients	NS	NS	13%
	The association was significant for *28/*28 versus *1/*1+ *1/*28 (OR=1.62 (1.03–2.53) (S)) (10 studies with 1211 patients). The association did not reach significance for *1/*28+*28/*28 versus *1/*1 (NS) (11 studies with 1214 patients).		
Asian patients	OR=8.98 (5.21–15.5) (S)	OR=1.85 (1.37–2.50) (S)	8.2%
	The association was also significant for *28/*28 versus *1/*1+ *1/*28 (OR=8.64 (4.14–18.0) (S)) (13 studies with 1917 patients).		
Colorectal cancer	OR=3.53 (1.54–8.09) (S)	OR=1.60 (1.11–2.31) (S)	

Supplementary Table S3.4 continues on next page.

Supplementary Table S3.4: ***Continued***

	*28/*28: CTC-AE 4 *1/*28: CTC-AE 4	patients	The association was also significant for *28/*28 versus *1/*1+ *1/*28 (OR=3.16 (1.61–6.19) (S)) (17 studies with 2656 patients).			
		Non-small cell lung cancer patients	-	NS		
			The association was also not significant for *1/*28+*28/*28 versus *1/*1 (NS) (4 studies with 321 patients).			
		Small cell lung cancer patients	-	NS		
			The association was significant for *28/*28 versus *1/*1+*1/*28 (OR=19.90 (2.57–154) (S)) (2 studies with 64 patients) and for *1/*28+*28/*28 versus *1/*1 (OR=3.95 (1.42–11.0) (S)) (3 studies with 131 patients).			
		Neutropenia				
		All patients	OR=5.34 (3.05–9.33) (S)	OR=1.71 (1.41–2.08) (S)	14%	
			The association was also significant for *28/*28 versus *1/*1+ *1/*28 (OR=4.12 (2.36–7.20) (S)) (28 studies with 3668 patients).			
		White patients	OR=5.39 (3.43–8.47) (S)	OR=1.86 (1.34–2.60) (S)	11%	
			The association was also significant for *28/*28 versus *1/*1+ *1/*28 (OR=3.39 (1.92–5.98) (S)) (12 studies with 1455 patients).			
		Asian patients	OR=4.77 (1.71–13.2) (S)	OR=1.56 (1.07–2.27) (S)	16%	
			The association was also significant for *28/*28 versus *1/*1+ *1/*28 (OR=4.16 (1.44–12.0) (S)) (15 studies with 2154 patients).			
		Colorectal cancer patients	OR=5.07 (2.56–10.0) (S)	OR=1.76 (1.40–2.23) (S)		
			The association was also significant for *28/*28 versus *1/*1+ *1/*28 (OR=3.70 (1.88–7.30) (S)) (20 studies with 2894 patients).			
	*1/*28 + *28/*28: Clinical Relevance Score AA[#]	Non-small cell lung cancer patients	-	NS		
			There was a trend for an increased risk for *1/*28+*28/*28 versus *1/*1 (p=0.064, NS) (4 studies with 351 patients).			
		Tumour response				
		All patients	OR=1.20 (1.07–1.34) (S) for *1/*28+*28/*28 versus *1/*1			
		White patients	OR=1.23 (1.06–1.42) (S) for *1/*28+*28/*28 versus *1/*1			
		Asian patients	NS for *1/*28+*28/*28 versus *1/*1			

		Colorectal cancer patients	OR=1.24 (1.05–1.48) (S) for *1/*28+*28/*28 versus *1/*1		
		Non-small cell lung cancer patients	NS for *1/*28+*28/*28 versus *1/*1		
		Small cell lung cancer patients	NS for *1/*28+*28/*28 versus *1/*1		
		Prospective studies (12 studies, 1292 patients)	NS for *1/*28+*28/*28 versus *1/*1		
		Retrospective studies (4 studies, 538 patients)	OR=1.54 (1.06–2.23) (S) for *1/*28+*28/*28 versus *1/*1		
		For diarrhoea, there was a statistically significant heterogeneity between the studies for the following comparisons: - All patients, *28/*28 versus *1/*1 - All patients, *28/*28 versus *1/*1+*1/*28 - White patients, *1/*28+*28/*28 versus *1/*1 - Colorectal cancer patients, *28/*28 versus *1/*1 - Colorectal cancer patients, *1/*28 versus *1/*1 - Colorectal cancer patients, *28/*28 versus *1/*1+ *1/*28 For the comparisons for all patients, ethnicity and year of publication together accounted for over 90% of the heterogeneity.			
		For neutropenia, there was a statistically significant heterogeneity between the studies for the following comparisons: - All patients, *28/*28 versus *1/*1 - All patients, *28/*28 versus *1/*1+*1/*28 - White patients, *28/*28 versus *1/*1+*1/*28 - Asian patients, *1/*28 versus *1/*1 - Asian patients, *28/*28 versus *1/*1 - Asian patients, *28/*28 versus *1/*1+*1/*28 - Colorectal cancer patients, *28/*28 versus *1/*1 - Colorectal cancer patients, *28/*28 versus *1/*1+ *1/*28			

Supplementary Table S3.4 continues on next page.

Supplementary Table S3.4: ***Continued***

		For the comparisons for all patients, *28/*28 versus *1/*1+ *1/*28, the number of patients accounted for 25% of the heterogeneity and no other factors were found. For tumour response, there was a statistically significant heterogeneity between the studies for the following comparisons: - All patients - Asian patients - Colorectal cancer patients - Retrospective studies There was no publication bias for any of the comparisons mentioned above. Results for all patients were not affected by omitting individual studies in the meta-analyses. For the comparison of *1/*28+*28/*28 versus *1/*1 for all patients, the required sample size for diarrhoea, neutropenia and tumour response was respectively 763, 1162 and 1078 patients. The number of patients in these meta-analyses were higher.	
ref. 5 Lu CY et al. Clinical implication of UGT1A1 promoter polymorphism for irinotecan dose escalation in metastatic colorectal cancer patients treated with bevacizumab combined with FOLFIRI in the first-line setting. Transl Oncol 2015;8:474-9. PubMed PMID: 26692528.	Level of evidence score: 3	70 patients with metastatic colorectal cancer and a life expectancy of more than 3 months were treated with bevacizumab plus FOLFIRI (folinic acid, 5-fluorouracil and irinotecan) and followed for a period of 6 to 34 months (median 22 months). The initial irinotecan dose was 180 mg/m^2 every 2 weeks for patients with the *1/*1 or *1/*28 genotype and 120 mg/m^2 every two weeks (67% of the normal dose) for patients with the *28/*28 genotype. The dose of irinotecan was escalated by 20 to 30 mg/m^2 every two cycles until grade 3/4 adverse events occurred or until the maximum dose of 260 mg/m^2 for *1/*1, 240 mg/m^2 for *1/*28 and 210 mg/m^2 for *28/*28 (81% of the maximum dose for *1/*1 and 88% of the maximum dose of *1/*28) was reached. After the first two treatment cycles, haematological and non-haematological adverse events (including neutropenia, diarrhoea, and nausea/vomiting) were assessed. The response to treatment was assessed radiologically, and the best response was recorded. The first response assessment was usually after the fourth or sixth cycle. Complete response was defined as the disappearance of all target lesions. Partial response was defined as at least a 30% decrease in the sum of the longest diameter from baseline. Progressive disease was defined as either at least a 20% increase in the sum of the longest diameter of target lesions, with the smallest sum of the longest diameters recorded before treatment as reference or the identification of one or more new lesions. Stable disease was defined as neither sufficient shrinkage to quality for partial response nor sufficient increase to qualify for progressive disease. The best response was defined as the best result recorded by the investigators because the confirmatory imaging evidence of response obtained after four to six cycles of chemotherapy was not consistently available. The primary end points were response rate and progression-free survival. The secondary endpoints were toxicity and overall survival. For liver/lung metastatic lesions, metastasectomy was performed after a multidisciplinary team meeting	Authors' conclusion: 'For patients with the UGT1A1 *28/*28 genotype, the starting dose of irinotecan should be decreased to diminish the adverse events of irinotecan. … Our study showed that mCRC patients with UGT1A1 *1/*1 and *1/*28 genotypes could receive escalated doses of irinotecan to obtain a more favourable clinical outcome without significant AEs.'

<table>
<tr>
<td></td>
<td>*28/*28: Clinical Relevance Score A</td>
<td>(25.7% of patients). Patients who underwent metastasectomy achieved better overall survival than those who did not. The comparisons between *28/*28 and *1/*1+*1/*28 were not adjusted for metastasectomy.

Genotyping:
- 65x *1/*1+*1/*28
- 5x *28/*28

Results:
<table>
<tr><td colspan="4">Results for *28/*28 on reduced initial dose compared to *1/*1+*1/*28 on normal initial dose:</td></tr>
<tr><td colspan="2"></td><td>*28/*28</td><td>value for *1/*1+ *1/*28 (incidence in % of patients or maximum dose)</td></tr>
<tr><td colspan="2">Response (either complete or partial)</td><td>x 0.26 (S)</td><td>77%</td></tr>
<tr><td colspan="2" rowspan="2">Disease control rate (either response or stable disease)</td><td>x 0.43 (S)</td><td rowspan="2">94%</td></tr>
<tr><td>The majority of *1/*1+*1/*28 patients (74%) had had a partial response, the majority of *28/*28 patients (60%) had progressive disease.</td></tr>
<tr><td colspan="2">Progression-free survival</td><td>S for *28/*28 versus *1/*28 versus *1/*1 (increase with the number of *1-alleles)</td><td></td></tr>
<tr><td colspan="2">Adverse events grade 3/4</td><td>x 9.7 (S)</td><td>6.2%</td></tr>
<tr><td rowspan="2">Maximum irinotecan dose tolerated</td><td>Mean</td><td>x 0.76 (156 mg/kg) (S)</td><td>206 mg/kg</td></tr>
<tr><td>Largest group (40% of patients)</td><td>x 0.67 (120 mg/kg) (S)</td><td>180 mg/kg</td></tr>
</table>
</td>
<td></td>
</tr>
<tr>
<td>ref. 6
Dias MM et al.
The effect of the UGT1A1*28 allele on survival after irinotecan-based chemotherapy: a</td>
<td>Level of evidence score: 4</td>
<td>Meta-analysis of 11 observational cohort studies (from 10 publications) with in total 1823 patients treated with irinotecan, either as combined chemotherapy or as monotherapy. FOLFI-RI (folinic acid, 5-fluorouracil and irinotecan) was the most commonly administered regimen. Irinotecan doses in the studies varied from 60 mg/m^2 weekly to 350 mg/m^2 every 3 weeks. Additional data were provided for 7 publications through correspondence with the primary study investigators.
Of the 10 publications included in the meta-analysis, 3 were also included separately in this risk analysis (Marcuello 2004, Toffoli 2006, Kweekel 2008). A later version of the publication with two cohort studies</td>
<td>Authors' conclusion:
'In conclusion, the study demonstrates that UGT1A1*28 is unlikely to be strongly prognostic of overall survival for individuals</td>
</tr>
</table>

Supplementary Table S3.4 continues on next page.

Supplementary Table S3.4: *Continued*

<table>
<tr>
<td>collaborative meta-analysis. Pharmacogenomics J 2014;14:424-31. PubMed PMID: 24709690.</td>
<td></td>
<td>(McLeod, 2006) was also included in the meta-analysis.
Of the 10 publications in this meta-analysis, 7 were also included in the meta-analyses of Liu 2013 and Dias 2012. The meta-analyses of Liu 2014, Han 2014, Chen 2014, Hu 2010 Clin Cancer Res, Hu 2010 Eur J Cancer and Hoskins 2007 did not investigate clinical efficacy.
Data on overall survival were derived from 10 studies including a total of 1677 patients. The unadjusted comparison between *1/*28 and *1/*1 was based on 1229 patients from 9 studies of which 605 *1/*28. The adjusted comparison was based on 1040 patients from 7 studies of which 528 *1/*28. The unadjusted comparison between *28/*28 and *1/*1 was based on 919 patients from 10 studies of which 158 *28/*28. The adjusted comparison was based on 626 patients from 7 studies of which 98 *28/*28.
Data on progression-free survival were derived from 10 studies including a total of 1494 patients. The unadjusted comparison between *1/*28 and *1/*1 was based on 1360 patients from 10 studies of which 677 *1/*28. The adjusted comparison was based on 1171 patients from 8 studies of which 584 *1/*28. The unadjusted comparison between *28/*28 and *1/*1 was based on 817 patients from 10 studies of which 134 *28/*28. The adjusted comparison was based on 700 patients from 8 studies of which 113 *28/*28.
The primary end point was overall survival, the secondary end point was progression-free survival. Time to progression, the time from initiation of irinotecan until objective tumour progression, with censoring of death not related to cancer, was used if progression-free survival data were not available.
Hazard ratios or adjusted hazard ratios were calculated for overall and progression-free survival and risks differences for cycles with reduced irinotecan dose.
A random-effects model was used for the meta-analyses of genotype and survival outcomes. A fixed-effects model was used for meta-analyses on the effect of subgroups.
The objectives and methods of this collaborative review were prespecified in a study protocol, of which a copy is available on request. The search and selection strategy was transparent and the data extraction was standardised.
The authors reported which of the included studies confirmed to each of 22 quality criteria.
Publication bias was analysed for all comparisons, but only for overall survival and progression-free survival, not for one of more cycles with reduced irinotecan dose. Publication bias was not analysed for the subgroups.

Results:
<table>
<tr><td colspan="3">Risk versus *1/*1:</td></tr>
<tr><td></td><td>*28/*28</td><td>*1/*28</td></tr>
</table>
</td>
<td>treated with irinotecan. This is in contrast to the strong association previously reported between UGT1A1 *28 and irinotecan-related toxicity.’</td>
</tr>
</table>

	*28/*28: Clinical Relevance Score AA *1/*28: Clinical Relevance Score AA	Overall survival	NS	NS	
			Similar results were found for the adjusted HRs (both NS). Similar results were found for all analysed subgroups (colorectal cancer only, high dose (≥250 mg/m^2 every 3 weeks), intermediate dose (150–<250 mg/m^2 every 2 or 3 weeks), low dose (<150 mg/m^2 weekly), treatment with irinotecan and antimetabolites, treatment with irinotecan and platinum compounds, irinotecan monotherapy, 1st line therapy, 2nd & 3rd line therapy) (NS).		
		Progression-free survival	NS	NS	
			Similar results were found for the adjusted HRs (both NS). Similar results were found for all analysed subgroups (colorectal cancer only, high dose (≥250 mg/m^2 every 3 weeks), intermediate dose (150–<250 mg/m^2 every 2 or 3 weeks), low dose (<150 mg/m^2 weekly), treatment with irinotecan and antimetabolites, treatment with irinotecan and platinum compounds, irinotecan monotherapy, 1st line therapy, 2nd & 3rd line therapy) (NS). A better progression-free survival in *1/*28 compared to *1/*1 was found in the subgroup with 1st line therapy after adjusting (HR_{adj}=0.82; 95% CI: 0.69–0.98) (S). However, this was not confirmed by a significant interaction between 1st line and 2nd & 3rd line (NS).		
		One of more cycles with reduced irinotecan dose	NS	Trend for an increased risk (p=0.07) (NS)	
		For overall survival, there was no statistically significant heterogeneity between the studies, but there was a strong trend for statistically significant heterogeneity for the unadjusted comparison between *28/*28 and *1/*1 (p=0.10). In addition, there was significant heterogeneity for the sub-groups low dose and treatment with irinotecan plus platinum compounds for the comparison between *28/*28 and *1/*1. For progression-free survival, there was no statistically significant heterogeneity between the studies for the comparison between *1/*28 and *1/*1, but there was moderate and significant heterogeneity for the comparison between *28/*28 and *1/*1 (p=0.08). For the unadjusted comparison of the latter, moderate heterogeneity was also found for the subgroups therapy with irinotecan and antimetabolites and 1st line therapy, whereas there was a trend (p=0.10) for the subgroup colorectal cancer only. For the adjusted comparison, there was no significant heterogeneity for the total group and the subgroups mentioned above, but there was a strong and significant heterogeneity for 2nd and 3rd line therapy.			
		There were indications for publication bias or small-study effects for the adjusted overall survival comparison of *28/*28 versus *1/*1. This was attributable to the study of Lara 2009, but exclusion of			

Supplementary Table S3.4 continues on next page.

Supplementary Table S3.4: ***Continued***

<table>
<tr><td></td><td></td><td>this study from the meta-analysis did not substantially alter the results.
There were no indications of publication bias or small-study effects for other comparisons.
8 studies were excluded from the meta-analysis, due to insufficient quantitative data, but included in the systematic review. None of these studies reported a difference in overall and progression-free survival between genotypes (NS).</td><td></td></tr>
<tr><td>ref. 7
Han FF et al.
Associations between UGT1A1*6 or UGT1A1*6/*28 polymorphisms and irinotecan-induced neutropenia in Asian cancer patients.
Cancer Chemother Pharmacol 2014;73:779-88.
PubMed PMID: 24519753.</td><td>Level of evidence score: 3

*28/*28 + PM: CTC-AE 4
PM: CTC-AE 4</td><td>Meta-analysis of 19 studies with in total 1671 Asian patients treated with irinotecan, either as combined chemotherapy or as monotherapy. Irinotecan doses in the studies varied from 50 mg/m^2 on day 1, 8 and 15 every 4 weeks to 350 mg/m^2.
Of the 19 studies included in the meta-analysis, 2 were also included separately in this risk analysis (Han 2006 and Minami 2007).
Of the 19 studies in this meta-analysis, 13 were included in the meta-analysis of Chen 2014. The meta-analyses of Liu 2014, Hu 2010 Clin Cancer Res and Hoskins 2007 did not investigate Asian patients. The meta-analyses of Liu 2013, Dias 2012, Hu 2010 Eur J Cancer did not investigate neutropenia risk.
The comparison between *28/*28 + *6/*28 + *6/*6 and *1/*28 + *1/*6 + *1/*1 was based on 923 patients from 11 studies. The comparison between *6/*6 and *1/*6 + *1/*1 was based on 984 patients from 7 studies.
Neutropenia was defined as neutropenia grade 3-4 or neutropenia grade 4.
A fixed-effects model was used for the meta-analyses, because there was no significant heterogeneity between the studies (p>0.1). This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data extraction was standardised.
The authors indicate that the quality of the included studies was assessed, but do not present the assessment results.
Publication bias analyses were performed for all comparisons.

Results:
Neutropenia risk compared to either *1/*28 + *1/*6 + *1/*1 or *1/*6 + *1/*1:
*28/*28 + *6/*28 + *6/*6: OR=3.28 (95% CI: 2.15–4.98) (S)
*6/*6: OR=3.28 (95% CI: 1.89–5.69) (S)
*6/*6: The risk was also increased for *6/*6 + *1/*6 compared to *1/*1: OR=1.54 (95% CI: 1.18–2.04) (S) (9 studies with in total 994 patients)
There was no statistically significant heterogeneity between the studies.
There were no indications for publication bias. However, for the comparison of *6/*6 with *1/*6 + *1/*1, the OR was influenced by leaving individual studies out.</td><td>Authors' conclusion: 'In conclusion, the UGT1A1*6 and UGT1A1*6/*28 genotypes were associated with an increased risk of irinotecan-induced neutropenia in Asian cancer patients.'</td></tr>
<tr><td>ref. 8</td><td>Level of evidence</td><td>Meta-analysis of 18 clinical trials with in total 1303 Asian patients treated with irinotecan. Irinotecan</td><td>Authors' conclusion:</td></tr>
</table>

<table>
<tr>
<td>Chen YJ et al.
The association of UGT1A1*6 and UGT1A1*28 with irinotecan-induced neutropenia in Asians: a meta-analysis.
Biomarkers 2014;19:56-62.
PubMed PMID: 24308720.</td>
<td>score 3

*28 + *6: CTC-AE 4</td>
<td>doses in the studies varied from 30 to 350 mg/m^2.
Of the 18 studies included in the meta-analysis, 1 was also included separately in this risk analysis (Minami 2007).
Of the 18 studies in this meta-analysis, none were included in earlier meta-analyses. The meta-analyses of Liu 2014, Hu 2010 Clin Cancer Res and Hoskins 2007 did not investigate Asian patients. The meta-analyses of Liu 2013, Dias 2012, Hu 2010 Eur J Cancer did not investigate neutropenia risk.
The comparison for *28 + *6 was based on 886 patients from 13 studies, of which 335 *1/*28 + *1/*6 and 97 *28/*28 + *6/*28 + *6/*6. The comparison for *28 was based on 658 patients from 6 studies, of which 133 *1/*28 and 15 *28/*28. The comparison for *6 was based on 652 patients from 5 studies, of which 217 *1/*6 and 31 *6/*6.
A model-free generalized odds ratio ORG was calculated. ORG was defined such that ORG >1 if patients with neutropenia grade 3-4 have a higher gene variant load than patients without neutropenia grade 3-4.
A random-effects model was used for the meta-analyses, but prospective registration of the protocol was not mentioned. The search and selection strategy was transparent and the data extraction was standardised.
Quality of the included studies was not judged.
Publication bias analyses were performed for all comparisons.

Results:
<table>
<tr><td colspan="4">Prevalence of neutropenia per genotype/genotype group and effect of gene variants on neutropenia risk (OR_G):</td></tr>
<tr><td></td><td>*28/*28 and/or *6/*28 and/or *6/*6</td><td>*1/*28 and/or *1/*6</td><td>% of *1/*1 with neutropenia</td></tr>
<tr><td rowspan="2">*28 + *6</td><td>x 2.5</td><td>x 1.4</td><td rowspan="2">24%</td></tr>
<tr><td colspan="2">OR_G=2.55 (95% CI: 1.82–3.68) (S).
An OR_G of 2.55 indicates that patients with neutropenia grade 3-4 have a 155% higher gene variant load than patients without neutropenia grade 3-4.</td></tr>
<tr><td rowspan="2">*28</td><td>x 2.1</td><td>x 1.3</td><td rowspan="2">25%</td></tr>
<tr><td colspan="2">Trend for OR_G>1 (95% CI: 0.94–2.97) (NS).</td></tr>
<tr><td rowspan="2">*6</td><td>x 1.8</td><td>x 1.5</td><td rowspan="2">23%</td></tr>
<tr><td colspan="2">Trend for OR_G>1 (95% CI: 0.97–3.04) (NS).
However, this trend becomes much weaker (95% CI: 0.85–2.35) after reducing heterogeneity to non-significant by removal of one of the studies (Onoue 2009) from the meta-analysis.</td></tr>
<tr><td colspan="4">For *28 + *6 and for *28, the heterogeneity between the studies was not significant.</td></tr>
</table>
</td>
<td>‘In Asians, a combination test of UGT1A1*6 and UGT1A1*28 might be a potential bio-marker of irinotecan-induced neutropenia, an observation that will need additional trials for confirmation.’</td>
</tr>
</table>

Supplementary Table S3.4 continues on next page.

Supplementary Table S3.4: ***Continued***

		For *6, the heterogeneity between the studies was mode-rate and statistically significant. There were no indications for publication bias. For *28 + *6, the width of the 95% confidence interval of the OR_G of each study decreased with the study publication year. From 2008 on, the OR_G per publication year differed less than 20% with the OR_G of the subsequent publication year. The number of studies per publication year was maximally 3.	
ref. 9 Liu X et al. Association of UGT1A1*28 polymorphisms with irinotecan-induced toxicities in colorectal cancer: a meta-analysis in Caucasians. Pharmacogenomics J 2014;14:120-9. PubMed PMID: 23529007.	Level of evidence score: 3 *1/*28: CTC-AE 4 *28/*28: CTC-AE 4	A meta-analysis of 16 studies including a total of 2,328 mainly White patients with colorectal cancer. Of the 16 studies included in the meta-analysis, 7 were also included separately in this risk analysis (Marcuello, 2004; Rouits, 2004; Carlini, 2005; Massacesi, 2006; Toffoli, 2006; Côté, 2007 and Kweekel, 2008). A later publication of one study is also included in the meta-analysis (McLeod, 2006). The outcome measure was grade 3-4 toxicity. A random-effects model was used for the meta-analysis in case of significant heterogeneity ($p<0.1$). Otherwise, a fixed-effects model was used. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data extraction was standardised. The authors indicate that the quality of the included studies was evaluated based on study design, the detection method of the polymorphisms, chemotherapy regimens, and grading systems for toxicity, but do not present quality scores for the studies. Publication bias analyses were performed for all comparisons and for all subgroups. In case of publication bias, a trim and fill method was carried out for adjusting. *1/*28 versus *1/*1: - Increased risk of neutropenia (OR=1.90; 95% CI: 1.44–2.51) (S). Similar results were found after correction for publication bias and in the subgroups using irinotecan doses exceeding 150 mg/m^2 and irinotecan doses lower than 150 mg/m^2. There were insufficient studies using therapy without fluo-rouracil to compare therapy with and without fluorouracil. - No increased risk of diarrhoea (NS). There was a trend towards a higher risk of diarrhoea in the subgroup using irinotecan doses exceeding 150 mg/m^2. *28/*28 versus *1/*1: - Increased risk of neutropenia (OR=4.79; 95% CI: 3.28–7.01) (S). Similar results were found in the subgroups using therapy without fluorouracil and in those using fluorouracil-based therapy and in the subgroups using irinotecan doses exceeding 150 mg/m^2 (OR=4.64) and irinotecan doses lower than 150 mg/m^2 (OR=6.37). - Increased risk of diarrhoea (OR=1.84; 95% CI: 1.24–2.72) (S). The increased risk of diarrhoea was only observed in studies investigating irinotecan doses exceeding	Authors' conclusion: 'This meta-analysis provided evidence for the association between the UGT1A1*28 polymorphism and an increased risk of irinotecan-induced neutropenia and diarrhoea in colorectal cancer. Associations with significant neutropenia were consistent and strong. In contrast, associations with diarrhoea were weaker, and primarily seen when higher doses of irinotecan were administrated.'

		150 mg/m^2 (OR=2.37; 95% CI: 1.39–4.04 (S)) or in combination with fluorouracil (OR=1.78; 95% CI: 1.16–2.75 (S)). Non-fluorouracil-based therapy gave a higher OR than fluorouracil-based therapy, but the increase was not significant. *28/*28 versus (*1/*1+*1/*28): - Increased risk of neutropenia (OR=3.44; 95% CI: 2.45–4.82) (S). Similar results were found in the subgroup using non-fluorouracil-based therapy and the subgroup using fluorouracil-based therapy and in the subgroups using irinotecan doses exceeding 150 mg/m^2 (OR=3.34) and irinotecan doses lower than 150 mg/m^2 (OR=3.63). - Increased risk of diarrhoea (OR=1.71; 95% CI: 1.18–2.47) (S). The increased risk of diarrhoea was only observed in studies investigating irinotecan doses exceeding 150 mg/m^2 (OR=2.04; 95% CI: 1.23–3.38 (S)) or in combination with fluorouracil (OR=1.67; 95% CI: 1.11–2.52 (S)). Non-fluorouracil-based therapy gave a higher OR than fluorouracil-based therapy, but the increase was not significant. N.B.1: *28 is the most common allele variant in the White population. N.B.2: The most common irinotecan doses used in the Netherlands exceed 150 mg/m^2.	
ref. 10, kinetics Goetz MP et al. UGT1A1 genotype-guided phase I study of irinotecan, oxaliplatin, and capecitabine. Invest New Drugs 2013;31:1559-67. PubMed PMID: 24114122.	Level of evidence score: 3 *1/*28: Clinical Relevance Score AA *28/*28: Clinical Relevance Score AA	24 patients were treated with the maximum tolerated dose of irinotecan once every 3 weeks in combination with oxaliplatin and capecitabine. The maximum tolerated dose was 150 mg/m^2 for *1/*1 and *1/*28 and 75 or 100 mg/m^2 (both n=3) for *28/*28. Relevant co-medication was not excluded (although antiretroviral therapy was) Genotyping: - 9x *1/*1 - 9x *1/*28 - 6x *28/*28 *1/*28 versus *1/*1: - Dose-corrected SN-38 AUC increased by 4.6% (NS; from 2.33 to 2.44 ng.hour/mL per mg/m^2) *28/*28 versus *1/*1: - Dose-corrected SN-38 AUC increased by 71% (NS; from 2.33 to 3.99 ng.hour/mL per mg/m^2)	Authors' conclusion: 'UGT1A1 genotype affects the dose and pharmacokinetics of the CAPIRINOX regimen.' Dose-corrected SN-38 AUC versus *1/*1: *1/*28: 105% *28/*28: 171% Dose-corrected SN-38 AUC versus all genotypes: *1/*28: 88% *28/*28: 143%
ref. 11 Liu X et al.	Level of evidence score: 3	A meta-analysis of 12 studies including a total of 1,896 mainly White patients with colorectal cancer. Of the 12 studies included in the meta-analysis, 3 were also included separately in this risk analysis (Carlini,	Authors' conclusion: 'UGT1A1*28 polymorphism cannot

Supplementary Table S3.4 continues on next page.

Supplementary Table S3.4: ***Continued***

Association be-tween UGT1A1 *28 polymorphisms and clinical outcomes of irinotecan-based chemotherapies in colorectal cancer: a meta-analysis in Caucasians. PLoS One 2013;8:e58489. PubMed PMID: 23516488.	*1/*28: Clinical Relevance Score AA *28/*28: Clinical Relevance Score AA	2005; Toffoli, 2006 en Kweekel, 2008). A later publication of one study is also included in the meta-analysis (McLeod, 2006). Therapeutic response was defined as partial or complete response. A fixed-effects model was initially used for the meta-analysis, and confirmatory analyses with a random-effects model were performed in case of potential heterogeneity. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data extraction was standardised. The authors indicate that the quality of the included studies was evaluated based on study design, polymorphism detection method, combination regimens, line of therapy, and grading systems for response, but do not present quality scores for the studies. Publication bias analyses were performed for all comparisons and for all subgroups. In case of publication bias, a trim and fill method was carried out for adjusting. *1/*28 versus *1/*1: - No difference in therapeutic response, progression-free survival and death (NS). The same results were found in the subgroups using irinotecan doses exceeding 150 mg/m^2 and irinotecan doses lower than 150 mg/m^2. *28/*28 versus *1/*1: - No difference in therapeutic response, progression-free survival and death (NS). The same results were found on therapeutic response and progression-free survival in the subgroups using irinotecan doses exceeding 150 mg/m^2 and irinotecan doses lower than 150 mg/m^2. An increased mortality rate was found in the subgroup using irinotecan doses lower than 150 mg/m^2 (HR=1.48; 95% CI: 1.06–2.07) (S). However, these results were only based on two studies, of which only the largest found an effect. *28/*28 versus (*1/*1+*1/*28): - No difference in therapeutic response (NS). The same results were found in the subgroups using irinotecan doses exceeding 150 mg/m^2 and irinotecan doses lower than 150 mg/m^2. N.B.1: *28 is the most common allele variant in the White population. N.B.2: The most common irinotecan doses used in the Netherlands exceed 150 mg/m^2.	be considered as a reliable predictor of therapeutic response and progression-free survival in colorectal cancer patients treated with irinotecan-based chemotherapy. The overall survival relationship with UGT 1A1*28 in the patients with lower-dose irinotecan chemotherapy requires further validation.'
ref. 12 Dias MM et al. Impact of the UGT1A1*28 allele on response to irinotecan:	Level of evidence score: 4	A meta-analysis of 12 studies including a total of 1,898 patients. Of the 12 studies included in the meta-analysis, 5 were also included separately in this risk analysis (Carlini, 2005; Han, 2006; Toffoli, 2006; Kweekel, 2008 and Liu, 2008). A later publication of one study was also included in the meta-analysis (McLeod, 2006). Eight of the twelve studies were also included in the meta-analysis by Liu 2013. Response was defined as partial or complete response.	Authors' conclusion: 'An individual's response to irinotecan is unlikely to be

a systematic review and meta-analysis. Pharmacogenomics 2012;13:889-99. PubMed PMID: 22676194.	*1/*28: Clinical Relevance Score AA *28/*28: Clinical Relevance Score AA	A random-effects model was used for the meta-analyses, but prospective registration of the protocol was not mentioned. The search and selection strategy was transparent and the data extraction was standardised. The authors reported which of the included studies confirmed to each of 45 quality criteria. Publication bias was analysed for all comparisons, but not for the subgroups. *1/*28 versus *1/*1: - No difference in response (NS). *28/*28 versus *1/*1: - No difference in response (NS). (*28/*28+*1/*28) versus *1/*1: - No difference in response (NS). Similar results were found in the subgroups using irinotecan doses ≥250 mg/m^2, 150–250 mg/m^2 or <150 mg/m^2 and in the subgroups of patients with colorectal cancer and lung cancer.	affected by UGT1A1 *28 status.'
ref. 13 Hu ZY et al. Dose-dependent association between UGT1A1 *28 genotype and irinotecan-induced neutropenia: low doses also increase risk. Clin Cancer Res 2010;16:3832-42. PubMed PMID: 20562211.	Level of evidence score: 4 *1/*28: CTC-AE 4	A meta-analysis of 15 studies including a total of 1,998 mainly White patients. Of the fifteen studies included in the meta-analysis, eight were also included separately in this risk analysis (Marcuello, 2004; Rouits, 2004; Carlini, 2005; Massacesi, 2006; McLeod, 2006; Toffoli, 2006; Côté, 2007 and Kweekel, 2008). Ten of the fifteen studies in this meta-analysis were also included in the meta-analysis by Liu 2014. The meta-analysis of the relative extent of glucuronidation covered 9 studies including a total of 581 patients, of which two studies were performed among Asian patients. Meta-analyses were performed with a fixed-effects model. Since, this is only allowed in the absence of significant heterogeneity, this indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data extraction was standardised. The authors reported which of the included studies confirmed to each of 28 (neutropenia) or 30 (extent of glucuronidation) quality criteria. Publication bias was analysed for all comparisons, but not for the subgroups, except for neutropenia and dose <250 mg/m^2 and for neutropenia and dose 150–250 mg/m^2, which were the only subgroups with 8 or more studies. *1/*28 versus *1/*1: - Increased risk of grade 3-4 neutropenia (RR=1.43; 95% CI: 1.16–1.77) (S). Similar results were found in the subgroups using irinotecan doses <150 mg/m^2 (RR=2.94) and 150–250	Authors' conclusion: 'The UGT1A1 *28/*28 genotype was associated with an increased risk of neutropenia not only at medium or high doses of irinotecan but also at low doses. The dose-dependent manner of SN-38 glucuronidation explained why the association between UGT1A1 *28 and neutropenia was dose dependent.'

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Supplementary Table S3.4: ***Continued***

		mg/m^2 (RR=1.29). The RR for irinotecan doses ≥250 mg/m^2 was based on two studies and was non-significant. - Decreased weighted mean difference (WMD) of the extent of SN-38 glucuronidation (WMD = -1,55; 95% CI: -0.87 to -2.23) (S). Similar results were found for irinotecan doses <250 mg/m^2 (WMD=-1.85), but the WMD was non-significant for doses ≥250 mg/m^2. There was no significant heterogeneity between the studies for any of the comparisons. Egger's test for publication bias was significant for neutropenia for all investigated dose ranges (all doses, doses <250 mg/m^2 and doses of 150–250 mg/m^2), but Begg's test was not. There was no indication for publication bias for the extent of glucuronidation (only investigated for all doses).	
	*28/*28: CTC-AE 4	*28/*28 versus (*1/*1+*1/*28): - Increased risk of grade 3–4 neutropenia (RR=2.20; 95% CI: 1.82–2.66) (S). Similar results were found in the subgroups using irinotecan doses <150 mg/m^2 (RR=2.43) and 150–250 mg/m^2 (RR=2.00). The risk was higher in the subgroup using irinotecan doses ≥250 mg/m^2 (RR=7.22) than in the subgroup using irinotecan doses <250 mg/m^2 (S). - Decreased weighted mean difference (WMD) of the extent of SN-38 glucuronidation (WMD = -2.44; 95% CI: -1.73 to -3.14) (S). The difference was greater in the subgroup using irinotecan doses ≥250 mg/m^2 (WMD=-3.08) than in the subgroup using irinotecan doses <250 mg/m^2 (WMD=-1.62). There was no significant heterogeneity between the studies for any of the comparisons. Egger's test for publication bias was significant for neutropenia and all doses, but Begg's test was not. There were no indications for publication bias for the investigated dose ranges (doses <250 mg/m^2 and doses of 150–250 mg/m^2). There was no indication for publication bias for the extent of glucuronidation (only investigated for all doses). N.B.1: *28 is the most common allele variant in the White population. N.B.2: The most common irinotecan doses used in the Netherlands range from 180 to 350 mg/m^2.	
ref. 14 Hu ZY et al. Dose-dependent association be-tween UGT1A1-*28 polymorphism and	Level of evidence score: 3	A meta-analysis of 20 studies including a total of 1,760 patients (1,263 mainly White, 497 Asian). Of the 20 studies included in the meta-analysis, thirteen were also included separately in this risk analysis (Iyer, 2002; Font, 2003; Innocenti, 2004; Marcuello, 2004; Rouits, 2004; Carlini, 2005; de Jong, 2006; Han, 2006; Massacesi, 2006; Toffoli, 2006; Côté, 2007; Kweekel, 2008 and Liu, 2008). Eight of the twenty studies in this meta-analysis were also included in the meta-analysis by Liu 2014. Meta-analyses were performed with a fixed-effects model. Since, this is only allowed in the absence of	Authors' conclusion: 'Patients carrying UGT1A1*28 allele(s) are at an increased risk of irinotecan-induced severe diarrhoea. This

irinotecan-induced diarrhoea: a meta-analysis. Eur J Cancer 2010;46:1856-65. PubMed PMID: 20335017.		significant heterogeneity, this indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data extraction was standardised. The authors indicate that the quality of the included studies was assessed based on study design, number of patients, source of population, mutation detection method, races, tumour types, chemotherapy regimens and grade criteria for diarrhoea, but do not present the assessment results. Publication bias was analysed for all comparisons for *28, but not for the subgroups, except for doses ≥ 125 mg/m^2 for all patients and for Whites, which were the only subgroups with 6 or more studies. Potential publication bias was evaluated by visual examination for possible skewness in funnel plots and Egger's test. The Duval and Tweedie nonparametric trim and fill procedure was performed to further assess the possible effect of publication bias in case of a significant Egger's test. Publication bias analysis was not performed for *6 (only 4 studies).	increased risk is only apparent in those who are administrated with medium or high irinotecan doses.'
	*1/*28: CTC-AE 4	*1/*28 versus *1/*1: - Increased risk of grade 3-4 diarrhoea (OR=1.73; 95% CI: 1.25–-2.40) (S). Similar results were found in the subgroup using irinotecan doses ≥125 mg/m^2 (OR=1.92; 95% CI: 1.31–2.82). The OR was significant at this dose in the subgroup of White patients, but not in the subgroup of Asian patients (two studies only). No differences were found in the subgroups using irinotecan doses <125 mg/m^2 (NS). There was no significant heterogeneity between the studies for any of the comparisons. Egger's test showed significant publication bias for Whites and dose ≥125 mg/m^2, but adjustment for the likely effect of bias using trim and fill gave a pooled OR of 1.74 (95% CI: 1.16-2.59; S), which is only a slight change from the estimate of 1.87 (95% CI: 1.25–2.81; S) without trim and fill. There were no indications for publication bias for all patients and all doses and for all patients and doses ≥125 mg/m^2.	
	*28/*28: CTC-AE 4	*28/*28 versus *1/*1: - Increased risk of grade 3–4 diarrhoea (OR=2.23; 95% CI: 1.31–3.81) (S). Similar results were found in the subgroup using irinotecan doses ≥125 mg/m^2 (OR=3.69; 95% CI: 2.00–6.83). No differences were found in the subgroup using irinotecan doses <125 mg/m^2 (NS). There were no studies investigating *28/*28 versus *1/*1 in Asian patients. Meta-regression analysis of the dependence of the OR on the dose found that the OR increased by 4.30 when the dose increased by 100 mg/m^2. This would give rise to an OR of almost 5 at a dose of 180 mg/m^2 and an OR of more than 13 at a dose of 350 mg/m^2. This linear relationship was only found for *28/*28 versus *1/*1. There was no significant heterogeneity between the studies for any of the comparisons. There were no indications for publication bias for the two investigated comparisons (all doses and doses ≥125 mg/m^2). Because all studies concerned Whites, there were no ethnicity subgroups.	

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	PM: CTC-AE 4	*28/*28 versus (*1/*1+*1/*28): - Increased risk of grade 3-4 diarrhoea at a dose ≥125 mg/m^2 (OR=2.49; 95% CI: 1.42–4.36) (S). The OR was non-significant when all doses were included (NS). No differences were found in the subgroup using irinotecan doses <125 mg/m^2 (NS). There was no significant heterogeneity between the studies for any of the comparisons. There were no indications for publication bias for the two investigated comparisons (all doses and doses ≥125 mg/m^2). Because all studies concerned Whites, there were no ethnicity subgroups. *6/*6 versus (*1/*1+*1/*6): - Increased risk of grade 3–4 diarrhoea (OR=3.54; 95% CI: 1.16–10.77) (S). The data were derived from four Asian studies. Analysis of heterogeneity between the studies was not reported. Publication bias analysis was not performed. (*1/*28+*28/*28) versus *1/*1: - Increased risk of grade 3–4 diarrhoea (OR=1.81; 95% CI: 1.38–2.39) (S). Similar results were found in the subgroups using irinotecan doses ≥125 mg/m^2 (all patients, White patients and Asian patients). No differences were found in the subgroups using irinotecan doses < 125 mg/m^2 (NS). There was no significant heterogeneity between the studies for any of the comparisons. Egger's test showed significant publication bias for Whites and dose ≥125 mg/m^2, but adjustment for the likely effect of bias using trim and fill gave a pooled OR of 1.78 (95% CI: 1.28–2.49; S), which also indicates a significantly increased risk of toxicity (OR without trim and fill was 1.93 (95% CI: 1.38–2.70; S)). There were no indications for publication bias for all patients and all doses and for all patients and doses ≥125 mg/m^2. NOTE1: *28 is the most common allele variant in the White population. *6 is relatively common in Asian patients. N.B.2: The most common irinotecan doses used in the Netherlands range from 180 to 350 mg/m^2.	
ref. 15, kinetics Denlinger CS et al. Pharmacokinetic analysis of irinotecan plus bevacizumab in patients with advanced solid tumors.	Level of evidence score: 4	29 patients were treated with irinotecan 180 mg/m^2 once every two weeks in combination with fluorouracil and folinic acid. Co-medication was excluded. Genotyping: - 9x *1/*1 - 15x *1/*28 - 5x *28/*28	Authors' conclusion: 'UGT1A1 polymorphisms were associated with variability in irinotecan pharmacokinetics.'

Cancer Chemother Pharmacol 2009;65:97-105. PubMed PMID: 19415281.	*1/*28: Clinical Relevance Score A *28/*28: Clinical Relevance Score A	*1/*28 versus *1/*1: - Dose-corrected SN-38 AUC0-48h increased by 4.8% (S; from 1.65 to 1.73 ng.hour/mL per mg/m^2) *28/*28 versus *1/*1: - Dose-corrected SN-38 AUC0-48h increased by 109% (S; from 1.65 to 3.45 ng.hour/mL per mg/m^2)	Dose-corrected SN-38 AUC versus *1/*1: *1/*28: 105% *28/*28: 209% Dose-corrected SN-38 AUC versus all genotypes: *1/*28: 86% *28/*28: 172%
ref. 16 Kweekel DM et al. UGT1A1*28 genotype and irinotecan dosage in patients with metastatic colorectal cancer: a Dutch Colorectal Cancer Group study. Br J Cancer 2008;99:275-82.	Level of evidence score: 3 *28/*28: CTC-AE 4 *1/*28: CTC-AE 4	218 patients, 80 (3x *28/*28, 31x *1/*28, 46x *1/*1) received irinotecan 350 mg/m^2 every three weeks, 138 (11x *28/*28, 62x *1/*28, 65x *1/*1) received irinotecan 350 mg/m^2 every three weeks plus capecitabine, chemotherapy regimens were fully known, but other co-medication was not, tumour evaluation was performed after every three cycles; *clinical endpoints* *1/*1 versus *1/*28 versus *28/*28: - Increased prevalence of febrile neutropenia for both monotherapy and combination therapy (S; 2.2% versus 19.4% versus 0% and 1.5% versus 6.5% versus 18.2% respectively). - No significant differences in the prevalence of grade 3–4 diarrhoea and the prevalence of all grade 3–4 toxicity for monotherapy or combination therapy. - No significant differences in the prevalence of dose reduction after cycle 1, dose per cycle and total dose for mono-therapy or combination therapy. (The dose was mainly reduced in cycles 2 and 3 and 89% was due to gastro-intestinal toxicity). - No significant differences in the prevalence of complete and partial response for monotherapy or combination therapy. - No significant differences in the prevalence of patients without disease progression for monotherapy or combination therapy.	Authors' conclusion: 'We observed that the UGT1A1*28 genotype is associated with an enhanced risk of febrile neutropenia but not with IRI dose reductions. However, upfront dose reduction may result in a lower incidence of febrile neutropenia in these patients.'
ref. 17 Liu CY et al. UGT1A1*28 polymorphism predicts irinotecan-induced severe toxicities without affecting treatment outcome and survival in patients with	Level of evidence score: 3 (*28/*28 + *1/*28): CTC-AE 4	128 patients, 6x *28/*28, 20x *1/*28, 102x *1/*1, received irinotecan 180 mg/m^2 every two weeks for 12 cycles as part of first-line therapy with IFLa, other co-medication not known, median follow-up was 18 months, tumour evaluation was performed after every fourth cycle; *clinical endpoints* (*28/*28 + *1/*28) versus *1/*1: - Prevalence of grade 3-4 neutropenia increased by 998% (S; from 4.9% to 53.8%). - Prevalence of febrile neutropenia increased by 887% (S; from 3.9% to 38.5%).	Authors' conclusion: 'The current data suggested that the UGT1A1*28 polymorphism may be a key determinant for predicting irinotecan-induced severe

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metastatic colorectal carcinoma. Cancer 2008;112:1932-40.		- Prevalence of diarrhoea increased by 356% (S; from 5.9% to 26.9%). - Prevalence of hospitalisation for febrile neutropenia or grade 3-4 diarrhoea increased by 468% (S; from 8.8% to 50%). - Prevalence of treatment-related mortality increased by 475% (S; from 2% to 11.5%). - Prevalence of elevated bilirubin levels before the treatment increased by 163% (S; from 8.8% to 23.1%). - Need for dose reduction increased by 233% (S; from 12.7% to 42.3% of the patients). Dose reduction was equally as often due to febrile neutropenia as due to into-lerable diarrhoea. - No significant differences in the response rate, progression-free survival and overall survival. N.B.: No genotyping was performed for the *6 allele, which is common among Asian populations.	toxicities without affecting treatment outcome for patients with metastatic colorectal cancer.'
ref. 18 Lankisch TO et al. Gilbert's Syndrome and irinotecan toxicity: combination with UDP-glucuronosyl-transferase 1A7 variants increases risk. Cancer Epidemiol Biomarkers Prev 2008;17:695-701.	Level of evidence score: 3 (*28/*28 + *1/*28): Clinical Relevance Score AA	96 patients without clinical diagnoses of Gilbert's syndrome, 58x (*28/*28 + *1/*28), 38x *1/*1, received irinotecan 80 mg/m^2 every week for 12 weeks in combination with either oxaliplatin or 5-fluorouracil and folic acid, other co-medication not known; *clinical endpoints* No significant association of the *28 allele with diarrhoea, anaemia, thrombocytopenia, leukopenia, loss of body weight and irinotecan dose reduction.	Authors' conclusion: 'Our data derived from one of the largest pharmacogenomic study cohorts of irinotecan-treated individuals to date corroborate data from different studies that have failed to find hematologic or gastrointestinal drug toxicity in patients carrying the UGT1A1*28 allele and suggest that additional risk factors may play a permissive role.'
ref. 19 Hoskins JM et al. UGT1A1*28 genotype and irinotecan-induced neutropenia: dose matters. J Natl Cancer Inst	Level of evidence score: 3	Meta-analysis of nine studies, of which eight have also been included in this risk analysis, 821 patients, 84x *28/*28, 737x (*1/*28 + *1/*1), irinotecan doses ranged from 80 mg/m^2 per week to 350 mg/m^2 every three weeks. Meta-analyses were performed with a random-effects model, but preregistration of the protocol (including the statistical analysis) was not mentioned. The search and selection strategy and the method of data extraction were not mentioned either. Assessment of the quality of the included studies was not reported.	Authors' conclusion: 'The risk of experiencing irinotecan-induced hematologic toxicity for patients with a UGT1A1 *28/*28 genotype thus appears

2007;99:1290-5.	*28/*28: CTC-AE 4 *28/*28: Clinical Relevance Score AA	Publication bias was analysed by funnel plot only and for haematological toxicity and all doses only, *Clinical endpoints* *28/*28 versus (*1/*28 + *1/*1): - Increased risk of grade 3–4 haematological toxicity at high doses (>250 mg/m^2) (S; OR=27.8 (95% CI 4.0–195)). - Increased risk of grade 3–4 haematological toxicity at medium doses (150–250 mg/m^2) (S; OR=3.22 (95% CI 1.52–6.81)). - No significantly increased risk of grade 3-4 haematological toxicity at low doses (<150 mg/m^2) (NS). - No significantly increased risk of grade 4 diarrhoea independent of dose (NS). There was no heterogeneity between the studies (most probably only tested for grade 3–4 haematological toxicity and all doses). There was no evidence for publication bias for the only investigated comparison: grade 3–4 haematological toxicity and all doses.	to be a function of the dose of irinotecan administered.'
ref. 20 Minami H et al. Irinotecan pharmacokinetics/ pharmacodynamics and UGT1A genetic polymorphisms in Japanese: roles of UGT1A1*6 and *28. Pharmacogenet Genomics 2007;17:497-504.	Level of evidence score: 3 IM: CTC-AE 4 PM: CTC-AE 4	176 patients, 4x *28/*28, 26x *1/*28, 55x *1/*1, 5x *6/*6, 32x *1/*6, 7x *6/*28, 5x *60/*60, 25x *1/*60, 9x *6/*60, 8x *28/*60, received monotherapy (n=56) or combination therapy with irinotecan, doses of irinotecan ranged from 100 mg/m^2 per week to 150 mg/m^2 every three weeks. Association of genotype with AUC was determined for all patients, association with toxicity only for patients using monotherapy. The effect of *28 and *6 on AUC was similar. *clinical endpoints* 0 versus 1 versus 2 *28 or *6 alleles: - Increased incidence of grade 3-4 neutropenia (S; 14% versus 24% versus 80%). - No association with the incidence of diarrhoea. kinetic endpoints *1/*28 versus *1/*1: - Median SN-38G/SN-38 AUC ratio decreased by 40% (S; from 6.13 to 3.65). 1x (*28 or *6) versus *1/*1: - Dose-corrected SN-38 AUC increased by 40% (S; deter-mined from the slope of the regression line). *28/*28 versus *1/*1: - Median SN-38G/SN-38 AUC ratio decreased non-significantly by 40% (NS; from 6.13 to 3.65). 2x (*28 or *6) versus *1/*1: - Dose-corrected SN-38 AUC increased by 140% (S; determined from the slope of the regression line).	Authors' conclusion: 'The haplotypes significantly associated with reduced area under concentration curve ratios and neutropenia contained UGT1A1 *6 or *28, and both of them should be genotyped before irinotecan is given to Japanese and probably other Asian patients.'

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		*1/*1 versus *28/*1 versus *28/*28: - Significant gene-dose effect of the *28 allele on the median SN-38G/SN-38 AUC ratio (S). N.B.: Genotyping was performed for the most common alleles in Asian populations (*6, *28 and *60). The effect of *60 and *1 on metabolic ratio was not significantly different.	
ref. 21 Stewart CF et al. UGT1A1 promoter genotype correlates with SN-38 pharmacokinetics, but not severe toxicity in patients receiving low-dose irinotecan. J Clin Oncol 2007;25:2594-600.	Level of evidence score: 3 *28/*28: Clinical Relevance Score AA *1/*28: Clinical Relevance Score AA	72 paediatric patients, 9x *28/*28, 36x *1/*28, 27x *1/*1, received oral or intravenous irinotecan doses ranging from 15–75 mg/m^2 per day 5 days/week for two weeks as monotherapy or as combination therapy. *Clinical endpoints* - No association of *28 with the incidence of grade 3-4 neutropenia or diarrhoea. - Bilirubin levels before treatment were elevated in *28/*28 patients (S; from 0.3–0.4 to 0.6 mg/dL). *Kinetic endpoints* *1/*1 versus *28/*1 versus *28/*28: - Increased SN-38 AUC (NS). - Decreased SN-38G/SN-38 AUC ratios (NS).	Authors' conclusion: 'Severe toxicity was not increased in pediatric patients with the 7/7 genotype when treated with a low-dose protracted schedule of irinotecan. Therefore, UGT1A1 genotyping is not a useful prognostic indicator of severe toxicity for patients treated with this irinotecan dosage and schedule.'
ref. 22 Côté JF et al. UGT1A1 polymorphism can predict hematologic toxicity in patients treated with irinotecan. Clin Cancer Res 2007;13:3269-75.	Level of evidence score: 3 *28/*28: CTC-AE 4 *1/*28: CTC-AE 4	Prospective study, 89 patients, 8x *28/*28, 44x *1/*28, 37x *1/*1, received irinotecan 180 mg/m^2 every two weeks for twelve cycles in FOLFIRI[a] regimen. *clinical endpoints* *28/*28 versus *1/*1: - Increased incidence of grade 3-4 haematological toxicity by 209% (NS; from 16.2% to 50%). *28/28 versus *1/*28 versus *1/*1: - Increased incidence of grade 3–4 haematological toxicity (NS; 50% versus 25% versus 16.2%). - Increased incidence of grade 3–4 neutropenia (S; 50% versus 23% versus 13.5%). - No significant differences in the incidence of grade 3-4 gastrointestinal toxicity. - No differences in median dose. - Increased incidence of disease-free survival at 3 years (NS; 87% versus 52% versus 42%).	Authors' conclusion: 'This study supports the clinical utility of identification of UGT1A1 promoter polymorphisms before LV5FU2 + CPT-11 treatment to predict early hematologic toxicity. The -3156G>A polymorphism seems to be a better predictor than the UGT1A1 (TA)6TAA>(TA)7TAA polymorphism.'

ref. 23 Ramchandani RP et al. The role of SN-38 exposure, UGT1A1*28 polymorphism, and baseline bilirubin level in predicting severe irinotecan toxicity. J Clin Pharmacol 2007;47:78-86.	Level of evidence score: 3 *28/*28: CTC-AE 4 *1/*28: Clinical Relevance Score A	Pooled analysis of the data from Innocenti et al. and Iyer et al., 81 patients, 10x *28/*28, 32x *1/*28, 39x *1/*1, received irinotecan 300 or 350 mg/m2 every three weeks. Toxicity data from the 1st cycle were analysed. *Clinical endpoints* - A higher SN-38 AUC and the *28/*28 genotype were significantly associated with lower trough neutrophil counts (S). They both had significantly independent effects on trough neutrophil counts and together accounted for 49% of the variation. A model including the *28 allele only accounted for 22% of the variation. - An alternative model showed that elevated bilirubin levels before treatment and the *28/*28 genotype showed significant associations with lower trough neutrophil counts (S). Together they accounted for 31% of the variation. *Kinetic endpoints* - Increased dose-corrected SN-38 AUC for both *1/*28 and *28/*28 versus *1/*1 (S). The genotypes accounted for approximately 10% of the variation in SN-38 AUC.	Authors' conclusion: 'This model can be used to predict the magnitude of decrease in absolute neutrophil count, which can guide safer dosing regimens of irinotecan. However, we believe that the model could be further refined to have greater predictive power and better clinical utility.'
ref. 24 Zárate Romero R et al. Potential application of GSTT1-null genotype in predicting toxicity associated to 5-fluouracil irinotecan and leucovorin regimen in advanced stage colorectal cancer patients. Oncol Rep 2006;16:497-503.	Level of evidence score: 3 *1/*28: Clinical Relevance Score AA	51 patients, 26x *1/*28, 21x *1/*1, received irinotecan 180 mg/m2 every two weeks in combination with 5-fluorouracil and folinic acid for a median five cycles. *Clinical endpoints* - No association of the *28 allele with grade 3 haematological toxicity (NS). - No association of the *28 allele with grade 3 gastrointestinal toxicity (NS). Grade 4 toxicity was not found in this study, 78% of the grade 3 toxicity concerned gastrointestinal toxicity.	Authors' conclusion: 'Patients with the UGT1A1*28 allele may develop toxicity easily after irinotecan chemotherapy. In our treatment schedule, this relation was not observed.'
ref. 25 de Jong FA et al. Prophylaxis of irinotecan-induced diarrhea with neomycin and potential role for UGT1A1*28 genotype	Level of evidence score: 3	Prospective study, 52 patients, 3x *28/*28, 23x *1/*28, 26x *1/*1, received irinotecan 350 mg/m^2 every three weeks in combination with neomycin or placebo. Pharmacokinetic parameters were determined for 43 patients, 2x *28/*28, 19x *1/*28, 21x *1/*1. Relevant foods and CYP3A inhibitors or inducers were excluded, apart from prophylactic anti-emetics. Neomycin did not affect irinotecan toxicity or pharmacokinetics. *Clinical endpoints*	Authors' conclusion: 'It is suggested that the UGT1A1*28 genotype status could be used as a screening tool for a priori prevention of irinotecan-induced

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Supplementary Table S3.4: ***Continued***

screening: a double-blind, randomized, placebo-controlled study. Oncologist 2006;11:944-54.	(*28/*28 + *1/*28): CTC-AE 3	(*28/*28 + *1/*28) versus *1/*1: - The incidence of grade 2–3 diarrhoea increased by 100% (S; from 34.6% to 69.2%). - The incidence of grade 0–1 diarrhoea decreased by 53% (S; from 65.4% to 30.8%). - No difference in the incidence of grade 3–4 neutropenia (NS). - No significant decrease in trough neutrophil counts (NS). *Kinetic endpoints* *1/*1 versus *28/*1 versus *28/*28: - Decreased median SN-38 metabolic clearance (S; from 1268 to 804 to 489 L/h).	delayed-type diarrhea.' SN-38 clearance versus *1/*1: *1/*28: 63% *28/*28: 39% SN-38 clearance versus all genotypes: *1/*28: 79% *28/*28: 48%
ref. 26 Toffoli G et al. The role of UGT1A1*28 polymorphism in the pharmacodynamics and pharmacokinetics of irinotecan in patients with metastatic colorectal cancer. J Clin Oncol 2006;24:3061-8.	Level of evidence score: 3 *28/*28: CTC-AE 4 *28/*28: Clinical Relevance Score AA[#] *1/*28: Clinical Relevance Score A	Prospective study, 250 patients, 22x *28/*28, 114x *1/*28, 114x *1/*1, irinotecan 180 mg/m^2 every two weeks in FOLFIRI[a] regimen, other co-medication not known; *Clinical endpoints* - 1st cycle: significant association between *28 allele and grade 3–4 haematological toxicity, no association with non-haematological toxicity (including diarrhoea). - Entire treatment (dose adjusted to adverse events): no association between *28 allele and toxicity or dose reduction. - *28/*28: During 1st cycle: OR severe haematological toxicity versus *1/*1 was 8.63 (95% CI 1.31–56.55), non-haematological toxicity OR=4.10 (95% CI 0.86–19.55). Throughout entire treatment: haematological toxicity OR=1.97 (95% CI 0.56–6.99), non-haematological toxicity OR=1.41 (95% CI 0.45–4.47). No significant difference in dose reduction versus *1/*1 (from 17.5% to 18.2%). Significant decrease in the risk of progressive/stable disease and progression versus *1/*1, OR=0.32 (95% CI 0.12–0.86) and 0.19 (95% CI 0.04–0.89) respectively. There was no significant increase in overall survival. - *1/*28: During 1st cycle: OR severe haematological toxicity versus *1/*1 was 3.47 (95% CI 0.69–17.34), non-haematological toxicity OR=0.63 (95% CI 0.15–2.75). Throughout entire treatment: haematological toxicity OR=1.93 (95% CI 0.89–4.23), non-haematological toxicity OR=1.09 (95% CI 0.53–2.24). The incidence of dose reduction increased from 17.5% to 23.2% versus *1/*1 (NS by 33%). The risk of progressive/stable disease and progression decreased non-significantly versus *1/*1, OR=0.92 (95% CI 0.53–1.56) and 0.77 (95% CI 0.42–1.39) respectively. *Kinetic endpoints* Significant correlation between the *28 allele and a lower SN-38G/SN-38 AUC ratio or a higher irinotecan AUC x (SN-38/ SN-38G). These kinetic parameters also significantly differ between the group with and the group without serious toxicity.	Authors' conclusion: 'The results indicate that UGT1A1*28 polymorphism is of some relevance to toxicity; however, it is less important than discussed in previous smaller trials. In particular, the possibility of a dose reduction for irinotecan in patients with a UGT1A1*28 polymorphism is not supported by the result of this analysis.' 'The observed increased response rate in patients with lower GR and increased BI (indicative of a biochemical effect of a reduced UGT enzyme activity) and the trend towards increased

		N.B.: 5-FU dosed individually guided by adverse events.	tumor response and survival in *28/*28 patients suggest the need for careful consideration before irinotecan dose reduction in patients carrying the polymorphic *28 allele is recommended.'
ref. 27 Han JY et al. Comprehensive analysis of UGT1A polymorphisms predictive for pharmacokinetics and treatment outcome in patients with non-small-cell lung cancer treated with irinotecan and cisplatin. J Clin Oncol 2006;24:2237-44.	Level of evidence score: 3 *1/*28: Clinical Relevance Score AA *1/*6: Clinical Relevance Score A *6/*6: CTC-AE 4	81 patients, irinotecan 80 mg/m^2 on day 1 (+cisplatin) and day 8 of 3-weekly cycles, other co-medication not known; *28: Genotyping: 12x *28/*1, 69x *1/*1 *Kinetic endpoints* - *28/*1: SN-38G/SN-38 AUC ratio versus *1/*1 **increased** from 10.9 to 14.9 (NS by 37%). *Clinical endpoints* - *28/*1: no differences in tumour response, toxicity or dose versus *1/*1. *6: Genotyping: 6x *6/*6, 26x *1/*6, 49x *1/*1 *Kinetic endpoints* - *6/*6: SN-38 AUC increased from 113.9 to 200.4 ng.hour/mL versus *1/*1 (S by 76%). - *1/*6: SN-38 AUC increased from 113.9 to 126.7 ng.hour/mL versus *1/*1 (S by 11%). - *6/*6: no difference in the weekly irinotecan dose (in mg/m^2/week) versus (*1/*6+*1/*1) (NS) *Clinical endpoints (*6/*6 versus (*1/*6+*1/*1))* - The percentage of responders decreased from 50% to 0% (S) - Decreased progression-free survival (S) and overall survival (S) - The percentage of patients with grade 4 neutropenia increased from 24% to 67% (S by a factor 2.8) - No difference in the percentage of patients with grade 3 diarrhoea (NS)	
ref. 28 McLeod HL et al.	Level of evidence score: 3	520 patients, 212 received irinotecan 100–125 mg/m^2 once weekly, 109x in IFLa regimen (11x *28/*28, 54x *1/*28, 44x *1/*1), 103x in IROX[b] regimen, other co-medication not known;	

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Supplementary Table S3.4: ***Continued***

UGT1A1*28, toxicity and out-come in advanced colorectal cancer: results from Trial N9741. J Clin Oncol 2006;24 (suppl. abstr. 3520).	*28/*28: CTC-AE 4 *1/*28: CTC-AE 4	- *28/*28: the incidence of grade 4 neutropenia with IROX regimen increased significantly from 9.6% to 54.5% versus *1/*1 (S by 468% and OR 15.3, 95% CI 3–78); this increase was non-significant with the IFL regimen (from 6.8% to 18.2%, NS by 168%). - *1/*28: the incidence of grade 4 neutropenia with IROX regimen increased significantly from 9.6% to 15.0% versus *1/*1 (S by 56%); this increase was non-significant with the IFL regimen (from 6.8% to 11.1%, NS by 63%). UGT1A1 is not a predictor of incidence of diarrhoea, tumour response, time to progression or overall survival.	
ref. 29 Massacesi C et al. Uridine diphosphate glucuronosyl transferase 1A1 promoter polymorphism predicts the risk of gastrointestinal toxicity and fatigue induced by irinotecan-based chemotherapy. Cancer 2006;106:1007-16.	Level of evidence score: 3 *28/*28: CTC-AE 4 *1/*28: CTC-AE 5	56 patients, 7x *28/*28, 22x *1/*28, 27x *1/*1, irinotecan 80 mg/m2 weekly and raltitrexed every three weeks, other co-medication not known; - *28/*28 + *1/*28: significant increase versus *1/*1 in the incidence of diarrhoea, nausea and fatigue, no increase in neutropenia and liver toxicity. Genotype has no predictive power for response, time to disease progression or overall survival. - A patient with the *1/*28 genotype died of kidney failure due to severe diarrhoea and vomiting in combination with haematological toxicity.	
ref. 30 Wright MA et al. A phase I pharmacologic and pharmacogenetic trial of sequential 24-hour infusion of irinotecan followed by leucovorin and a 48-hour infusion of fluorouracil in adult patients with solid tumors.	Level of evidence score: 3 *28/*37: Clinical Relevance Score A *1/*28: Clinical Relevance Score A	32 patients, 30x genotyped, 3x *28/*37,18x *1/*28, 9x *1/*1, irinotecan 70-140 mg/m2 every two weeks, folinic acid and 5-FU, other co-medication not known; - *28/*37 + *1/*28: significantly increased SN-38/SN-38G AUC ratio versus *1/*1.	

Clin Cancer Res 2005;11:4144-50.			
ref. 31 Kweekel DM et al. Ondersteuning van de chemotherapiekeuze [Support for choice of chemotherapy]. Pharm Weekblad 2005;20:685-7.	Level of evidence score: 3 *28/*28: CTC-AE 3 *1/*28: CTC-AE 3	8 patients, 1x *28/*28, 2x *1/*28, 5x *1/*1, irinotecan+capecitabine doses not known, other co-medication not known; - *28/*28: no response, ≥ grade 3 toxicity. - *1/*28: 1 patient responded while another did not. Both < grade 3 toxicity. - *1/*1: response in 3 in 5 patients, 1 patient had ≥ grade 3 toxicity, other 4 < grade 3.	Subpopulation of the CAIRO study by Dutch Colorectal Cancer Group.
ref. 32 Steiner M et al. 5-fluorouracil/ irinotecan induced lethal toxicity as a result of a combined pharmacogenetic syndrome: report of a case. J Clin Pathol 2005;58:553-5.	Level of evidence score: 1 *1/*28: CTC-AE 5	Female patient received irinotecan 80 mg/m^2 weekly + 5-FU, folinic acid. The dose was reduced due to adverse events (grade 2 nausea, grade 1 leukopenia) after the second cycle. Severe diarrhoea and grade 4 neutropenia occurred. The patient developed sepsis and died. Genotyping: *1/*28 and heterozygous DPD*2A.	
ref. 33 Soepenberg O et al. Phase I pharmacokinetic, food effect, and pharmacogenetic study of oral irinotecan given as semisolid matrix capsules in patients with solid tumors. Clin Cancer Res 2005;11:1504-11.	Level of evidence score: 3 *28/*28: Clinical Relevance Score A *1/*28: Clinical Relevance Score A	25 patients of which 23 were genotyped, 1x *28/*28, 8x *1/*28, 13x *1/*1, 1x *36/*1, oral irinotecan 70–80 mg/m^2 on days 1 to 5 of three-weekly cycles, co-medication not known; *28 allele had a significant effect on SN-38 Cmax. No difference in toxicity.	
ref. 34 Zhou Q et al.	Level of evidence score: 3	29 patients, 11% *28, oral irinotecan 100 mg/m^2 weekly, co-medication not known;	

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Pharmacogenetic profiling across the irinotecan pathway in Asian patients with cancer. Br J Clin Pharmacol 2005;59:415-24.	*28/*28: Clinical Relevance Score AA *1/*28: Clinical Relevance Score AA	The UGT1A1 genotype did not have a significant effect on kinetic parameters of irinotecan, SN-38 or SN-38G. N.B.: No genotyping was performed for the *6 allele, which is common among Asian populations.	
ref. 35 Carlini LE et al. UGT1A7 and UGT1A9 polymorphisms predict response and toxicity in colorectal cancer patients treated with capecitabine/irinotecan. Clin Cancer Res 2005;11:1226-36.	Level of Evidence Score: 3 *28/*28: Clinical Relevance Score AA *28/*37: Clinical Relevance Score AA	67 patients, 1x *36/*1, 1x *36/*37, 28x *1/*1, 29x *1/*28, 1x *1/*37, 5x *28/*28, 1x *28/*37, irinotecan 100–125 mg/m^2 + capecitabine on days 1 and 8 of three-weekly cycles, other co-medication not known; - No significant association between genotype and tumour response, but there was a trend towards a better response in patients with low enzyme activity (*28/*28 and *28/*37) compared to those with high enzyme activity (*36/*1 and *1/*1), by 83% and 46% respectively. - No significant association between genotype and toxicity, none of the six patients with low enzyme activity had toxic adverse events.	
ref. 36 Kitagawa C et al. Genetic polymorphism in the phenobarbital-responsive enhancer module of the UDP-glucuronosyltransferase 1A1 gene and irinotecan toxicity. Pharmacogenet Genomics 2005;15:35-41.	Level of evidence score: 3 *28/*28: CTC-AE 4	119 patients, 7x *28/*28, 17x *1/*28, 95x *1/*1, irinotecan dose not known, co-medication not known; - *28/*28: significant association between genotype and the occurrence of severe toxicity, leukopenia and/or diarrhoea (OR 5.33, 95% CI 2.02–14.1). N.B.: No genotyping was performed for the *6 allele, which is common among Asian populations.	
ref. 37 Marcuello E et al. UGT1A1 gene variations and irinotecan treatment in patients with metastatic colorectal cancer.	Level of evidence score: 3 *28/*28: CTC-AE 4	95 patients 10x *28/*28, 45x *1/*28, 40x *1/*1, one of the following four regimens: irinotecan 350 mg/m^2 every three weeks, irinotecan 350 mg/m^2 every three weeks + raltitrexed, irinotecan 80 mg/m^2 once weekly + 5-FU, irinotecan 180 mg/m^2 every two weeks + 5-FU+levofolinic acid, other co-medication not known; - *28/*28: significant increase versus *1/*1 in the incidence of diarrhoea [from 17% to 70% (S by 312%)] and asthenia [from 25% to 70% (S by 180%)]. Non-significant increase in grade 3–4	

Br J Cancer 2004;91:678-82.	*1/*28: CTC-AE 4	haematological toxicity from 15% to 40% (NS by 167%). UGT1A1 genotype is the only variable associated with severe diarrhoea. No difference in overall survival. - *1/*28: significant increase versus *1/*1 in the incidence of diarrhoea [from 17% to 33% (S by 94%)] and asthenia [from 25% to 38% (S by 52%)]. Non-significant increase in grade 3–4 haematological toxicity from 15% to 27% (NS by 80%). No difference in overall survival.	
ref. 38 Rouits E et al. Relevance of different UGT1A1 polymorphisms in irinotecan-induced toxicity: a molecular and clinical study of 75 patients. Clin Cancer Res 2004;10:5151-9.	Level of evidence score: 3 *1/*28: CTC-AE 4 *28/*28: CTC-AE 5 (level of evidence score: 2)	75 patients, 7x *28/*28, 35x *1/*28, 31x *1/*1, 2x *36/*1 or *36/*28, irinotecan 85 mg/m^2 weekly + 5-FU + folinic acid or irinotecan 180 mg/m^2 every two weeks in FOLFIRI[a] regimen, other co-medication not known; - *28/*28: grade 3–4 neutropenia increased from 10% to 71% (S by 638%), grade 4 diarrhoea increased from 3% to 29% (NS by 793%). - *1/*28: grade 3–4 neutropenia increased from 10% to 40% (S by 313%), grade 4 diarrhoea increased from 3% to 6% (NS by 79%). One patient (*28/*28), who developed grade 4 diarrhoea with dehydration, fever and collapse, died. N.B.: 5-FU dosed individually guided by adverse events.	
ref. 39 Paoluzzi L et al. Influence of genetic variants in UGT1A1 and UGT1A9 on the in vivo glucuronidation of SN-38. J Clin Pharmacol 2004;44:854-60.	Level of evidence score: 3 *28/*28: Clinical Relevance Score A *1/*28: Clinical Relevance Score A	94 patients, 86x genotyped: 5x *28/*28, 37x *1/*28, 44x *1/*1, median irinotecan dose 600 mg, no relevant co-medication; *Kinetic endpoints* - *28/*28: SN-38G/SN-38 AUC ratio decreased from 7.00 to 2.51 versus *1/*1 (S by 64%). SN-38 AUC increased (S by 18%; from 508 to 600 ng.h/mL). No significant differences in irinotecan and SN-38G AUCs. - *1/*28: SN-38G/SN-38 AUC ratio decreased from 7.00 to 6.26 versus *1/*1 (S by 11%). SN-38 AUC increased (S by 18%; from 508 to 600 ng.h/mL). Other parameters differed NS from *1/*1. *Clinical endpoints* There was no significant association between the UGT1A1*28 genotype and the occurrence of grade 2-4 diarrhoea.	SN-38 AUC versus *1/*1: *1/*28: 118% *28/*28: 118% SN-38 AUC versus all genotypes: *1/*28: 109% *28/*28: 109%
ref. 40 Sai K et al. UGT1A1 haplotypes associated with reduced glucuronidation and increased serum	Level of evidence score: 3 *28/*28: Clinical Relevance Score A	195 patients, 85 with cancer, single dose of irinotecan 60–150 mg/m^2, other oncolytic drugs as co-medication. *28: Genotyping: 3x *28/*28, 15x *1/*28, 23x *1/*1. - *28/*28: SN-38G/SN-38 AUC ratio decreased from 6.36 to 3.57 versus *1/*1 (S by 44%).	

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bilirubin in irinotecan-administered Japanese patients with cancer. Clin Pharmacol Ther 2004;75:501-15.	*1/*28: Clinical Relevance Score A *6: Clinical Relevance Score A	- *28/*1: SN-38G/SN-38 AUC ratio decreased from 6.36 to 3.45 versus *1/*1 (S by 46%). - *28 haplotype had the greatest impact on AUC ratio. *6: Genotyping: 2x *6/*6, 14x *1/*6, 23x *1/*1. - *6/*6: SN-38G/SN-38 AUC ratio decreased from 6.36 to 4.27 versus *1/*1 (trend, NS by 33%). - *6/*1: SN-38G/SN-38 AUC ratio decreased from 6.36 to 4.23 versus *1/*1 (NS by 33%). - *6/*60: SN-38G/SN-38 AUC ratio decreased versus *1/*60 (trend, NS by 33%). - Significant association of *6 with decrease in SN-38G/SN-38 AUC in multiple regression analysis. NOTE: the factors gender, co-medication, irinotecan dose, tumour type and performance status did not affect the AUC ratio. Age did.	
ref. 41 Innocenti F et al. Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. J Clin Oncol 2004;22:1382-8.	Level of evidence score: 3 *28/*28: CTC-AE 4 *1/*28: CTC-AE 4	65 patients, 6x *28/*28, 25x *1/*28, 30x *1/*1, 2x *1/*37, 1x *36/*1, 1x *28/*37, irinotecan 350 mg/m^2 every three weeks, co-medication not known; *Clinical endpoints* - *28/*28: grade 4 neutropenia increased from 0% to 50% versus *1/*1 (S). Grade 3 diarrhoea in 1x *28/*28 versus 0x *1/*1. - *1/*28: grade 4 neutropenia increased from 0% to 12.5% versus *1/*1 (S). Grade 3 diarrhoea in 2x *1/*28 versus 0x *1/*1. *Kinetic endpoints* Significant correlation between SN-38 AUC, SN-38G/SN-38 AUC ratio and number of *28 alleles. SN-38 AUC: 336 versus 458 versus 542 ng.h/mL for *1/*1 versus *1/*28 versus *28/*28.	Authors' conclusion: 'There is no consistency across different studies on whether the AUC of irinotecan, SN-38, SN-38G, or a combination of these three parameters (the biliary index) is the strongest predictor of either severe neutropenia or diarrhea. Moreover, the safe dose of irinotecan in UGT1A1*28 homozygous patients has not been definitively identified yet, although it is likely to be approximately a 20% dose reduction given the relationship of genotype to SN-38 expo-sure.'

			SN-38 AUC versus *1/*1: *1/*28: 136% *28/*28: 161% SN-38 AUC versus (*1/*1 + *1/*28 + *28/*28): *1/*28: 112% *28/*28: 133%
ref. 42 Font A et al. Weekly regimen of irinotecan/docetaxel in previously treated non-small cell lung cancer patients and correlation with uridine diphosphate glucuronosyl-transferase 1A1 (UGT1A1) polymorphism. Invest New Drugs 2003;21:435-43.	Level of evidence score: 3 *28/*28: Clinical Relevance Score AA *1/*28: Clinical Relevance Score AA	47 patients, 7x *28/*28, 17x *1/*28, 23x *1/*1, irinotecan 70 mg/m^2 weekly + docetaxel, other co-medication not known; - *28/*28 + *1/*28: no difference in grade 3-4 toxicity versus *1/*1 (decreased from 43% to 41%, NS by 5%). Disease control increased from 34% to 54% (NS by 60%), progression-free survival increased by 33% from 3 to 4 months, survival increased by 27% from 8 to 11 months, 1-year survival increased by 95% from 21% to 41%.	Authors' conclusion: 'But we found no differences in toxicity according to UGT1A1 polymorphism. This patient population has been heavily pretreated and therefore could reduce the relevance of the UGT1A1 polymorphism as a genetic predictive marker, as compared to using first-line irinotecan-treated patients.'
ref. 43 Mathijssen RH et al. Irinotecan pathway genotype analysis to predict pharmacokinetics. Clin Cancer Res 2003;9:3246-53.	Level of evidence score: 3 *28/*28: Clinical Relevance Score AA *1/*28: Clinical Relevance Score AA	65 patients, 2x *28/*28, 19x *1/*28, 32x *1/*1, irinotecan 350 mg/m^2 every three weeks or 200–300 mg/m^2 every three weeks + cisplatin, co-medication not known; No significant differences in kinetic parameters between different UGT1A1*28 genotypes. There was a trend that the SN-38 AUC increases in the presence of allele variants.	
ref. 44 Iyer L et al.	Level of evidence score: 3	20 patients, 4x *28/*28, 7x *1/*28, 9x *1/*1, irinotecan 300 mg/m2 every three weeks, co-medication not known;	SN-38 AUC versus *1/*1:

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UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. Pharmacogenomics J 2002;2:43-7.	*28/*28: Clinical Relevance Score A *1/*28: Clinical Relevance Score A	*Clinical endpoints* Significant correlation between the absolute trough neutrophil count and genotype. Diarrhoea or grade 3–4 neutropenia only in *28/*28 and *1/*28. *Kinetic endpoints* - *28/*28: SN-38G/SN-38 AUC ratio decreased from 9.28 to 2.41 versus *1/*1 (S by 74%), SN-38 AUC0-24h increased from 205.13 to 513.37 ng.h/mL (S by 159%). - *1/*28: SN-38G/SN-38 AUC ratio decreased from 9.28 to 4.04 versus *1/*1 (S by 56%), SN-38 AUC0-24h increased from 205.13 to 288.61 ng.h/mL (S by 41%).	*1/*28: 141% *28/*28: 259% SN-38 AUC versus all genotypes: *1/*28: 96% *28/*28: 177%
ref. 45 Ando Y et al. Polymorphisms of UDP-glucuronosyl-transferase gene and irinotecan toxicity: a pharmacogenetic analysis. Cancer Res 2000;60:6921-6.	Level of evidence score: 3 *28/*28: CTC-AE 4 *1/*28: CTC-AE 4 *6/*6: Clinical Relevance Score AA *1/*6: Clinical Relevance Score AA	Case-control study including 26 cases (≥ grade 3 diarrhoea, ≥ grade 4 neutropenia on irinotecan) and 92 controls, 65% received various doses of weekly irinotecan, various oncolytic drugs as co-medication, other co-medication not known; *28: 15% of the cases were *28/*28, 31% *1/*28, while this was 3% and 11% respectively for the controls. The difference in *28 allele distribution between cases and controls was significant. *28 allele was a significant risk factor for occurrence of severe irinotecan toxicity, OR was 7.23 (95% CI 2.52–22.3). *6: 0% of the cases were *6/*6, 15% *1/*6, while this was 2% and 23% respectively for the controls. The difference in *6 allele distribution between cases and controls was not significant.	
ref. 46 Wasserman E et al. Severe CPT-11 toxicity in patients with Gilbert's syndrome: two case reports. Ann Oncol 1997;8:1049-51.	Level of evidence score: 2 Gilbert's syndrome: CTC-AE 4	Two patients (metastatic colon cancer) with Gilbert's syndrome (low UGT1A1 activity) developed severe diarrhoea and neutropenia on treatment with irinotecan; - Patient 1: 10 cycles of irinotecan 150 mg/m2 + oxaliplatin, serum bilirubin elevation and grade 4 neutropenia during each cycle. Grade 4 diarrhoea only developed during the first cycle. SN-38G/SN-38 AUC ratio was 1.8. - Patient 2: 2 cycles of irinotecan 200 mg/m2 + oxaliplatin, serum bilirubin elevation and grade 4 neutropenia during each cycle. Grade 4 diarrhoea only developed during the first cycle. SN-38G/SN-38 AUC ratio was 4.2.	
ref. 47 SmPC Campto (irinotecan hydrochloride trihydrate) 23-11-20.	Level of evidence score: 0	Pharmacodynamic data: Patients with Reduced UGT1A1 Activity: Uridine diphosphate-glucuronosyl transferase 1A1 (UGT1A1) is involved in the metabolic deactivation of SN-38, the active metabolite of irinotecan, to inactive SN-38 glucuronide (SN-38G). The UGT1A1 gene is highly polymorphic, resulting in highly variable metabolic capacities among individuals. One specific variation of the UGT1A1 gene includes a polymorphism in the promoter region known as the UGT1A1*28	

	*28/*28: CTC-AE 4	variant. This variant and other congenital deficiencies in UGT1A1 expression (such as Crigler-Najjar syndrome and Gilbert's syndrome) are associated with reduced activity of this enzyme. Data from a meta-analysis indicate that individuals with Crigler-Najjar syndrome (types 1 and 2) or those who are homozygous for the UGT1A1*28 allele (Gilbert's syndrome) are at increased risk of haematological toxicity (grade 3 to 4) following administration of irinotecan at moderate or high doses (>150 mg/m^2). A relationship between UGT1A1 genotype and the occurrence of irinotecan-induced diarrhoea was not established. Patients known to be homozygous for UGT1A1*28 should receive the normally indicated irinotecan starting dose. However, these patients should be monitored for haematological toxicities. A reduced irinotecan starting dose should be considered for patients who have experienced haematological toxicity with previous treatment. The exact reduction in starting dose in this patient population has not been established and any subsequent dose modifications should be based on a patient's tolerance of the treatment. There are at present insufficient data to conclude on clinical utility of UGT1A1 genotyping.	
ref. 48 SmPC Camptosar (irinotecan) 30-01-20 (USA).	Level of evidence score: 0 *28/*28: CTC-AE 4	Dosage in patients with reduced UGT1A1 Activity: When administered in combination with other agents, or as a single-agent, a reduction in the starting dose by at least one level of Camptosar should be considered for patients known to be homozygous for the UGT1A1*28 allele. However, the precise dose reduction in this patient population is not known, and subsequent dose modifications should be considered based on individual patient tolerance to treatment. Warning: Individuals who are homozygous for the UGT1A1*28 allele (UGT1A1 7/7 genotype) are at increased risk for neutropenia following initiation of Camptosar treatment. In a study of 66 patients who received single-agent Camptosar (350 mg/m^2 once-every-3-weeks), the incidence of grade 4 neutropenia in patients homozygous for the UGT1A1*28 allele was 50%, and in patients heterozygous for this allele (UGT1A1 6/7 genotype) the incidence was 12.5%. No grade 4 neutropenia was observed in patients homozygous for the wild-type allele (UGT1A1 6/6 genotype). In a prospective study (n=250) to investigate the role of UGT1A1*28 polymorphism in the development of toxicity in patients treated with Camptosar (180 mg/m^2) in combination with infusional 5-FU/LV, the incidence of grade 4 neutropenia in patients homozygous for the UGT1A1*28 allele was 4.5%, and in patients heterozygous for this allele the incidence was 5.3%. Grade 4 neutropenia was observed in 1.8% of patients homozygous for the wild-type allele. In another study in which 109 patients were treated with Camptosar (100–125 mg/m^2) in combination with bolus 5-FU/LV, the incidence of grade 4 neutropenia in patients homozygous for the UGT1A1*28 allele was 18.2%, and in patients heterozygous for this allele the incidence was 11.1%. Grade 4 neutropenia was observed in 6.8% of patients homozygous for the wild-type allele. When administered in combination with other agents or as a single-agent, a reduction in the starting	

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		dose by at least one level of Camptosar should be considered for patients known to be homozygous for the UGT1A1*28 allele. However, the precise dose reduction in this patient population is not known and subsequent dose modifications should be considered based on individual patient tolerance to treatment. A laboratory test is available to determine the UGT1A1 status of patients. Testing can detect the UGT1A1 6/6, 6/7 and 7/7 genotypes. Pharmacokinetics: UGT1A1 activity is reduced in individuals with genetic polymorphisms that lead to reduced enzyme activity such as the UGT1A1*28 polymorphism. Approximately 10% of the North American population is homozygous for the UGT1A1*28 allele (also referred to as UGT1A1 7/7 genotype). In a prospective study, in which irinotecan was administered as a single-agent (350 mg/m2) on a once-every-3-week schedule, patients with the UGT1A1 7/7 genotype had a higher exposure to SN-38 than patients with the wild-type UGT1A1 allele (UGT1A1 6/6 genotype).	

[a] FOLFIRI, IFL = irinotecan, fluorouracil and leucovorin (= folinic acid).

[b] IROX = irinotecan, oxaliplatin.

Abbreviations: *1/*28 = genotype leading to a reduced UGT1A1 activity, *28/*28 = genotype leading to a strongly reduced UGT1A1 activity, AUC = area under the concentration-time curve, CI = confidence interval, CTC-AE = Common Terminology Criteria for Adverse Events, DPD = dihydropyrimidine dehydrogenase, 5-FU = 5-fluorouracil, HR = hazard ratio, HRadj = adjusted hazard ratio, IM = IM other = intermediate metaboliser, genotype otherwise = *1 in combination with an allele with reduced activity other than *28 (e.g. *1/*6), NS = non-significant, OR = odds ratio, ORadj = adjusted odds ratio, PM = PM other = poor metaboliser, genotype otherwise = two alleles with reduced activity of which at least one other than *28 (e.g. *6/*28 or *6/*6), RR = relative risk, S = significant, SN-38 = active metabolite of irinotecan (7-ethyl-10-hydroxycamptothecin), SN-38G = 7-ethyl-10-hydroxy-camptothecin-glucuronide, UGT = uridine diphosphate glucuronosyltransferase

UGT1A1*1 = TA_6 = [$A(TA)_6TAA$] = wild-type, UGT1A1*28 = TA_7 = [$A(TA)_7TAA$] (reduced UGT1A1 activity), UGT1A1*36 = TA_5 = [$A(TA)_5TAA$] (increased UGT1A1 activity), UGT1A1*37 = TA_8 = [$A(TA)_8TAA$] (UGT1A1 activity more strongly reduced than for *28), UGT1A1*6 = gene variant in Asians, reduced activity, comparable to *28.

The clinical relevance score additionally includes the scores AA#, AA and A, since these do not exist in the CTC-AE. These regard “Positive clinical effect”, “No clinical or kinetic effect”, and “Significant kinetic effect or not clinically relevant effect”, respectively.

Supplementary Table S3.5: Dutch Pharmacogenetics Working Group (DPWG) Guideline for *UGT1A1* and irinotecan: the therapeutic recommendation and its rationale, and the kinetic and clinical consequences for each predicted UGT1A1 phenotype

Genotype/ predicted phenotype	Therapeutic recommendation	Rationale of the therapeutic recommendation	Kinetic consequences	Clinical consequences
*28/*28 [1–42]	**Start with 70% of the normal dose.** If the patient tolerates this initial dose, the dose can be increased, guided by the neutrophil count.	There is ample evidence for an increased risk of serious adverse events at normal doses (also when compared to all other genotypes/phenotypes), while convincing evidence for an increased efficacy is lacking. There is strong evidence that the *28 variant is associated with an increased frequency of serious adverse events. All 9 meta-analyses [1,5,6,11,13,24,39–41] investigating adverse events and 15 [2,8,9,12,15–20,25–27,30,34,43] of the 21 [10,14,21,23,28,31] studies found this increased risk. In addition, all 7 meta-analyses [5,6,11,13,24,39,41] and 2 studies [2,16] investigating the effect of *28/*28 and/or *6/*6 and/or *6/*28 compared with all other genotypes, found that this risk was also increased for *28/*28 and/or PM other patients compared to all other patients. Two [6,41] of the three [11] meta-analyses that investigated grade 3-4 neutropenia showed that the risk of neutropenia was also elevated at low doses. Two [5,41] of the three [11] meta-analyses that investigated grade 3–4 diarrhoea showed that the risk was elevated at high doses, but not at low doses (<150 or 125 mg/m^2). One meta-analysis also did not find an elevated diarrhoea risk at high doses [11]. For *28, one meta-analysis found the risk of severe toxicity (including neutropenia and diarrhoea) to be elevated at high doses (>150 mg/m^2), but not at low doses (<150 mg/m^2) [1]. The most common doses used in the Netherlands are	SN-38 AUC increased by 18–159%. SN-38 metabolic clearance decreased by 61%.	**Adverse event grade 3-4 neutropenia:** Three meta-analyses found an increased risk of neutropenia (OR=3.50–5.34), similar in one meta-analysis for doses higher and lower than 150 mg/m^2 (OR 4.64 and 6.37 respectively). One of the meta-analyses also found an increased risk in White patients (OR=5.39). This meta-analysis found the same for the risk compared to *1/*1 + *1/*28 (OR=4.12 for all patients, OR=3.39 for White patients). Three other meta-analyses also found an increased risk of neutropenia versus *1/*1 + *1/*28 (OR=3.44 or RR=2.20). Two of these meta-analyses showed this risk to be dose-dependent, while the third did not (OR=27.8 or RR=7.22 for doses ≥250 mg/m^2; OR=3.22 or RR=2.00 for doses of 150–250 mg/m^2; OR=NS or 3.34 or RR=2.43 for doses <150 mg/m^2; OR=3.63 for doses >150 mg/m^2). One meta-analysis found no increase in the risk of neutropenia compared to *1/*1 and compared to *1/*1+*1/*28. One meta-analysis found an increased risk for neutropenia for *28/*28+*6/*28+*6/*6 compared to *1/*1+*1/*28+*1/*6 (OR=3.28). One meta-analysis found a trend for an increased risk of neutropenia for *28 and an increased risk for *28+*6 (OR_G=2.55, which means that patients with neutropenia had a 155% higher frequency of *28 and *6 than patients without neutropenia). Results of separate studies ranged from no increase in the incidence of neutropenia to a significant increase (OR=1.97-15.3; OR_{corr}=2.89 for grade 3-4 neutropenia and 2.33 for grade 4 neutropenia (compared to *1/*1+*1/*28, increase by 50%-638%).

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		high doses (180 of 350 mg/m^2). Three [1,11,41] of the five [13,24] meta-analyses that investigated both neutropenia and diarrhoea showed that the risk of neutropenia increased more than the risk of diarrhoea. The fifth and second largest meta-analysis showed similar increased risk for diarrhoea and neutropenia for all patients, but in White patients only the risk for neutropenia was significantly increased [24]. Four [3,4,13,38] of the five [24] meta-analyses and eight [2,8,9,15,20,23,26,31] of the nine [18] studies did not show the *28 and/or *6 variants to be associated with increased effectiveness of the treatment. The fifth and largest meta-analysis found an increased efficacy for *1/*28+*28/*28 versus *1/*1 [24]. However, due to the *1/*28 and *28/*28 genotypes being analysed together, it is not clear whether this is also the case for *28/*28 separately. *1/*28 is the major group among White populations including the Dutch population. The standard irinotecan dose will therefore be based mainly on *1/*28. This is confirmed by one study showing that most *1/*28+*1/*1 tolerate the standard dose, while most *28/*28 do not [35]. Because development of severe adverse events results in temporary discontinuation of therapy, the effect of *28 on efficacy might be different in *1/*28 compared to *28/*28 (as suggested by [35]). Moreover, one meta-analysis found the increased efficacy for *1/*28+ *28/*28 versus *1/*1 only in 4 retrospective studies with in total 538 patients and not in 12 prospective studies with in total 1292 patients, suggesting the significance of the		**Adverse event grade 3–4 diarrhoea:** Five meta-analyses found an increased risk of diarrhoea (OR=1.69-5.93), but two only at doses exceeding 125 or 150 mg/m^2. Four meta-analyses found the same for the risk versus *1/*1 + *1/*28 (OR=2.04–6.25). One of the four meta-analyses found no increased risk in White patients versus *1/*1, but did find an increased risk compared to *1/*1 + *1/*28 (OR=1.62). One meta-analysis did not find an increased risk of grade 4 diarrhoea versus *1/*1 + *1/*28, not even at high doses. Results of individual studies ranged from no increase in the incidence of severe diarrhoea to a significant increase by 312%. There was one case where the patient died as a result of severe diarrhoea. **Severe toxicity (including grade 3–4 neutropenia and diarrhoea):** One meta-analysis found an increased risk of severe toxicity (OR=2.28–3.07), both for all patients (OR=2.28) and for White patients (OR=2.43), for all doses (OR=3.07) and at doses >150 mg/m^2 (OR=3.48), but not at doses <150 mg/m^2, for all tumour types (OR=2.76) and for tumours of the digestive system (OR=2.90), but not for tumours of the respiratory system. For patients with *6/*6, which leads to a comparable decrease in UGT1A1 activity, this same meta-analysis did find an effect at low doses and for tumours of the respiratory system. **Tumour response, survival:** Two meta-analyses found no difference in the therapeutic response, neither at doses exceeding 150 mg/m^2, nor at doses below 150 mg/m^2. Two meta-analyses found no difference in progression-free survival and death. The mortality rate was increased in the subgroup using doses <150 mg/m^2, but this was only based on one study and was not found in the second, larger meta-analysis. The larger meta-analysis found no

		result to be driven only by a small number of studies [24]. Finally, the ORs for all patients and for all White patients in this meta-analysis were small (1.20 and 1.23). For these reasons, the DPWG concludes that the evidence for an increased efficacy in *28/*28 patients is not convincing enough to refrain from recommending a dose reduction in these patients. The elevated frequency of serious adverse events in *28/*28 patients is consistent with the FDA advice in March 2005 (based on six studies) to add a passage to the Camptosar (irinotecan) SmPC that a reduction in the starting dose by at least one level of Camptosar should be considered for patients with the *28/*28 genotype [37]. Dose adjustments have been calculated on the basis of SN-38 AUC or clearance in studies: The calculation was based on 6 studies with a total of 28 patients with *28/*28 [7,17,28,30,33,42]. The weighted average of the calculated dose adjustment is a dose reduction to 58% (range 39–85%, median 53%) of the dose for *1/*1 and to 69% (range 48–92%, median 64%) of the dose for all patients. As the frequency of *1/*1 in Europe is less than 50%, and as caution should be exercised in reducing the dose, the calculated dose adjustment compared to all patients has been chosen. This was translated to 70% to be more achievable in		difference in the percentage of patients with one or more cycles with reduced irinotecan dose for *28/*28. One meta-analysis found no difference in tumour response for *1/*28+*28/*28. One meta-analysis found an improved tumour response for *1/*28+*28/*28 (OR=1.20 for all patients, OR=1.23 for White patients), but this difference was only significant in the 4 retrospective studies with a total of 538 patients, not in the 12 prospective studies with 1,292 patients. Results of individual studies ranged from no effect of genotype on tumour response, time to progression and overall survival to an association between genotype and improved tumour response. The risk of progression or progressive/stable disease was reduced (OR 0.19 and 0.32 respectively). One study involving 5 *28/*28 at 67% of the standard initial dose and 65 *1/*1+*1/*28 at the standard initial dose (180 mg/m^2) found that the incidence of clinical effects grade 3–4 was still 9.7 times higher for *28/*28, whilst the effectiveness was reduced (reduction in complete or partial response by 74%, reduction in the incidence of disease control (response or stable disease) by 57% and a reduction in the progression-free survival by the number of *28 alleles). However, one cannot rule out that this was caused by subsequently attempting a dose increase, with the maximum dose used for *28/*28 being 81% of the maximum dose for *1/*1 and 88% of the maximum dose for *1/*28. In the aforementioned study, the average maximum tolerated dose for *28/*28 was 76% of the dose for *1/*1+*1/*28 (156 versus 206 mg/m^2) and the maximum dose that was tolerated by the largest group of patients with the genotype (40%) for *28/*28 was 67% of the dose for *1/*1+*1/*28 (120 versus 180 mg/m^2).

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		clinical practice. Although the calculation leads to a broad range in outcomes and therefore does not strongly support a dose reduction, the eventual percentage is equivalent to the reduction used in practice if patients develop severe toxicity on irinotecan (20–30% reduction). In addition, one study confirmed that the maximum dose tolerated by the largest group (40%) of *28/*28 patients was 33% lower than the normal dose, while the maximum dose tolerated by the largest group (40%) of *1/*1+*1/*28 patients was the normal dose [35]. In this study, reduction of the initial dose with 33% for *28/*28 did not result in toxicity and efficacy that was comparable to those for *1/*1+*1/*28 on normal dose. This might however be due to the subsequent dose escalation with maximum doses for *28/*28 being less than 33% lower compared to the maximum doses for *1/*1 and *1/*28.		
*1/*28 [1–7,9,10, 12–22,24, 26–34,36, 38,40–46]	**NO action is needed for this gene-drug interaction.**	For *1/*28, a similar amount of evidence is present as for *28/*28. All seven meta-analyses that investigated the effect of *1/*28 and/or *1/*6 found an elevated frequency of serious adverse events for *1/*28 and/or *1/*6 versus *1/*1 [1,5,6,13,24,40,41]. However, *1/*28 is the major group among White populations including the Dutch population. This group is larger than the *1/*1 group. The standard irinotecan dose will therefore be based mainly on *1/*28. This is confirmed by a study showing that most *1/*28+*1/*1 tolerate the standard dose [35] and by the negligible dose adjustment calculated for *1/*28 compared to all	SN-38 AUC increased by 4.6–41%. SN-38 metabolic clearance decreased by 37%.	**Adverse event grade 3-4 neutropenia:** Four meta-analyses found an increased risk of neutropenia (RR=1.43, OR=1.71–1.91), two both at low and high doses and a third both for all patients (OR=1.71) and for White patients (OR=1.86). One meta-analysis found no increased risk of neutropenia. One meta-analysis found a trend for an increased risk of neutropenia for *28 and an increased risk for *28+*6 (OR_G=2.55, which means that patients with neutropenia had a 155% higher frequency of *28 and *6 than patients without neutropenia). Results of separate studies ranged from no increase in the incidence of neutropenia to a significant increase

		genotypes (see below). This means that dose reduction for *1/*28 would lead in suboptimal doses for this patient group. Therefore, the DPWG concludes that a gene-drug interaction is present, but that therapy adjustment is neither required nor advisable. Dose adjustments have been calculated on the basis of SN-38 AUC or clearance in studies: A total of 112 patients with *1/*28 were present in the 6 studies used for dose calculation [7,17,28,30,33,42]. The weighted average of the calculated dose adjustment is a dose reduction to 80% (range 63–96%, median 79%) of the dose for *1/*1 and to 95% (range 79–116%, median 98%) of the dose for all patients. As the frequency of *1/*1 in Europe is less than 50%, and as caution should be exercised in reducing the dose, the calculated dose adjustment compared to all patients has been chosen. This is equivalent to a dose reduction by 5% and is minor to the extent that it supports the choice not to advise therapy adjustment for *1/*28 patients at this time.		(OR=1.93–3.47; increase by 12.5–313%). **Adverse event grade 3-4 diarrhoea:** Three meta-analyses found an increased risk of diarrhoea (OR=1.45–1.73), but one only at doses ≥125 mg/m^2 (OR=1.92). The other meta-analysis did find an increased risk for all patients (OR=1.56), but not for White patients. Two other meta-analyses found no difference, one only a trend for a higher risk at doses >150 mg/m^2. Results of individual studies ranged from no increase in the incidence of diarrhoea to a significant increase by 94%. There were two cases where the patient died as a result of severe diarrhoea in combination with haematological toxicity. **Severe toxicity (including grade 3-4 neutropenia and diarrhoea):** One meta-analysis found an increased risk of severe toxicity (OR=1.60–1.77), both for all patients (OR=1.60) and for White patients (OR=1.59), for all doses (OR=1.77) and at doses >150 mg/m^2 (OR=1.81), but not at doses <150 mg/m^2, for all tumour types (OR=1.68) and for tumours of the digestive system (OR=1.73), but not for tumours of the respiratory system. **Tumour response, time to progression and overall survival**: four meta-analyses and individual studies found no difference. One of these four meta-analyses found a trend for an increase in the percentage of patients with one or more cycles of reduced irinotecan dose for *1/*28. One meta-analysis found an improved tumour response for *1/*28+*28/*28 (OR=1.20 for all patients, OR=1.23 for White patients), but this difference was only significant in the 4 retrospective studies with a total of 538 patients, not in the 12 prospective studies

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				with 1,292 patients.
PM other [1,5,7,12, 13,17,28–30,33,34, 39,40,42, 43]	**Start with 70% of the normal dose.** If the patient tolerates this initial dose, the dose can be increased, guided by the neutrophil count.	There is ample evidence for an increased risk of serious adverse events at normal doses (also when compared to all other genotypes/phenotypes), while convincing evidence for an increased efficacy is lacking. There is strong evidence that the *6 and/or *28 variants are associated with an increased frequency of serious adverse events. All 4 meta-analyses [1,5,13,39] investigating adverse events and 5 [12,17,30,34,43] of the 6 [28] studies found this increased risk. In addition, all 3 meta-analyses [5,13,39] and the only studies [43] investigating the effect of *6/*6 and/or *6/*28 and/or *28/*28 compared with all other genotypes, found that this risk was also increased for PM other and/or *28/*28 patients compared to all other patients. One meta-analysis that investigated grade 3–4 diarrhoea showed that the risk was elevated at high doses, but not at low doses (<150 or 125 mg/m^2) [5]. For *6, one meta-analysis found the risk of severe toxicity (including neutropenia and diarrhoea) to be increased at both high and low doses, with the ORs being higher at low doses [1]. The most common doses used in the Netherlands are high doses (180 of 350 mg/m^2). The only effectiveness meta-analysis [13] did not show the *6 variant to be associated with increased effectiveness of the treatment, but the only effectiveness study [43] suggested a decreased effectiveness for *6/*6. Taking into account the lack of sufficient evidence for *28, which has comparable kinetic and clinical	The information on kinetic consequences has mainly been derived from the *28/*28 genotype which encodes the PM phenotype but for which there are separate pharmacogenetic guidelines. ***28/*28:** SN-38 AUC increased by 18-159%. SN-38 metabolic clearance decreased by 61%. **PM + *28/*28 (*6/*6 + *6/*28 + *28/*28):** SN-38 AUC increased by 140%. ***6/*6:** SN-38 AUC increased by 76%.	**Adverse event grade 3-4 neutropenia:** *6/*6: Two meta-analyses found an increased risk of neutropenia (OR=3.03–3.28). Another meta-analysis found no increased risk of neutropenia compared to *1/*1, but did find an increased risk compared to *1/*1+*1/*6 (OR=5.00). A third meta-analysis found a trend towards an increased risk of neutropenia for *6. One study found a 2.8-fold increased percentage of patients with grade 4 neutropenia compared to *1/*1+*1/*6 (from 24% to 67%). PM + *28/*28 (*6/*6 + *6/*28 + *28/*28): One meta-analysis found an increased risk of neutropenia (OR=3.28). A second meta-analysis found an increased risk of neutropenia for *28+*6 (OR_G=2.55, which means that patients with neutropenia had a 155% higher frequency of *28 and *6 than patients without neutropenia). One study found a 5.7-fold increased incidence of neutropenia (from 14% to 80%). **Adverse event grade 3-4 diarrhoea:** *6/*6: Three meta-analyses found an increased risk of diarrhoea (OR=3.54–17.6). One of these meta-analyses also found an increased risk compared to *1/*1+*1/*6 (OR=5.26). One study found no association. PM + *28/*28: One study found no association with the incidence of diarrhoea. **Severe toxicity (including grade 3-4 neutropenia and diarrhoea):**

		effects as *6, the DPWG concludes that the evidence for an increased efficacy in PM other patients is not convincing enough to refrain from recommending a dose reduction in these patients. Because, the SN-38 glucuronide/SN-38 AUCs are almost the same for *28/*28 and *6/*6, suggestive of a similar effect on irinotecan metabolism, the same dose reduction as for *28/*28 is recommended for PM other [12].		*6/*6: One meta-analysis including only Asian studies found an increased risk of severe toxicity (OR=3.16–-3.21) for all doses (OR=3.17), at doses >150 mg/m^2 (OR=2.91) and at doses <150 mg/m^2 (OR=9.42), for all tumour types (OR=3.21), for tumours of the digestive system (OR=3.00) and for tumours of the respiratory system (OR=18.2; based on only 1 study). **Tumour response, survival:** *6/*6: One meta-analysis found no difference in tumour response for *1/*6+*6/*6. One study found that the percentage of responders decreased from 50% to 0% and also found decreased progression-free and overall survival compared to *1/*6+*6/*6.
IM other [1,7,12,13, 17,28–30, 33,34,40, 42,43]	**NO action is needed for this gene-drug interaction.**	For IM other and *1/*28, a similar amount of evidence is present as for PM other and *28/*28. All three meta-analyses that investigated the effect of *1/*6 and/or *1/*28 found an elevated frequency of serious adverse events for *1/*6 and/or *1/*28 versus *1/*1 [1,13,40]. However, *1/*28 is the major group among White populations including the Dutch population. The standard irinotecan dose will therefore be based mainly on *1/*28. This group is larger than the *1/*1 group. This is confirmed by a study showing that most *1/*28+*1/*1 tolerate the standard dose [35] and by the negligible dose adjustment calculated for *1/*28 compared to all genotypes (see the rationale for *1/*28). This means that dose reduction for *1/*28 would lead in suboptimal doses for this patient group. Because the kinetic and clinical effects of *6	The information on kinetic consequences has mainly been derived from the *1/*28 genotype which encodes the IM phenotype but for which there are separate pharmacogenetic guidelines. ***1/*28:** SN-38 AUC increased by 4.6-41%. SN-38 metabolic clearance	**Adverse event grade 3–4 neutropenia:** *1/*6: One meta-analysis found an increased risk of neutropenia (OR=1.95). Another meta-analysis found no increased risk. A third meta-analysis found a trend for an increased neutropenia risk for *6. IM + *1/*28 (*1/*6 + *1/*28): One meta-analysis found an increased risk of neutropenia for *28+*6 (OR_G=2.55, which means that patients with neutropenia had a 155% higher frequency of *28 and *6 than patients without neutropenia). One study found a 1.7-fold increased incidence of neutropenia (from 14% to 24%). **Adverse event grade 3–4 diarrhoea:** *1/*6: Two meta-analyses found an increased risk of diarrhoea (OR=1.98–4.36). IM + *1/*28 (*1/*6 + *1/*28): One study found no

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		and *28 are comparable, the same is true for IM other. Therefore, the DPWG concludes that a gene-drug interaction is present, but that therapy adjustment is neither required nor advisable.	decreased by 37%. **IM + *1/*28 (*1/*6 + *1/*28):** SN-38 AUC increased by 40%. ***1/*6:** SN-38 AUC increased by 11%.	association with the incidence of diarrhoea. **Severe toxicity (including grade 3-4 neutropenia and diarrhoea):** *1/*6: One meta-analysis including only Asian studies found an increased risk of severe toxicity (OR=1.75–2.08) for all doses (OR=2.08), at doses >150 mg/m^2 (OR=1.82) and at doses <150 mg/m^2 (OR=3.49), for tumours of all types (OR=1.75), for tumours of the digestive system (OR=1.66) and for tumours of the respiratory system (OR=12.0; based on only 1 study). **Tumour response, time to progression and overall survival:** One meta-analysis found no difference in tumour response for *1/*6+*6/*6.

*1/*28 = genotype leading to a reduced UGT1A1 activity, *28/*28 = genotype leading to a strongly reduced UGT1A1 activity, AUC = area under the concentration-time curve, IM = IM other = intermediate metaboliser, genotype otherwise = *1 in combination with an allele with reduced activity other than *28 (e.g. *1/*6), NS = non-significant, OR = odds ratio, PM = PM other = poor metaboliser, genotype otherwise = two alleles with reduced activity of which at least one other than *28 (e.g. *6/*28 or *6/*6), RR = relative risk, SN-38 = active metabolite of irinotecan (7-ethyl-10-hydroxycamptothecin), UGT = uridine diphosphate glucuronosyltransferase, UGT1A1*1 = TA6 = [A(TA)6TAA] = wild-type, UGT1A1*28 = TA7 = [A(TA)7TAA] (reduced UGT1A1 activity), UGT1A1*6 = gene variant in Asians, reduced activity, comparable to *28.

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Supplementary Table S3.6: Suggested clinical decision support texts for health care professionals for irinotecan

***UGT1A1 *28/*28*: IRINOTECAN**

Pharmacist and physician text
Serious, life-threatening adverse events occur more often in patients with this genetic variation. The genetic variation reduces conversion of irinotecan to inactive metabolites.

- Start with 70% of the normal dose
  If the patient tolerates this initial dose, the dose can be increased, guided by the neutrophil count.

Background information
Mechanism:
Irinotecan is a prodrug that is converted predominantly by carboxylesterases to the active metabolite SN-38, which has 100-1000-fold higher activity than irinotecan itself.

SN-38 is predominantly metabolised by UGT1A1 and otherwise by UGT1A6, UGT1A7, UGT1A9 and UGT1A10 to the inactive metabolite SN-38-glucuronide.

For more information about the UGT1A1 *28/*28 genotype, see the general background information about UGT1A1 on the KNMP Kennisbank (search for UGT1A1).

Clinical consequences:
Adverse event grade 3–4 neutropenia: Three meta-analyses found an increased risk of neutropenia (OR = 3.50–5.34), similar in one meta-analysis for doses higher and lower than 150 mg/m^2 (OR 4.64 and 6.37 respectively). One of the meta-analyses also found an increased risk in White patients (OR=5.39). This meta-analysis found the same for the risk compared to *1/*1 + *1/*28 (OR=4.12 for all patients, OR=3.39 for White patients). Three other meta-analyses also found an increased risk of neutropenia versus *1/*1 + *1/*28 (OR=3.44 or RR=2.20). Two of these meta-analyses showed this risk to be dose-dependent, while the third did not (OR=27.8 or RR=7.22 for doses ≥250 mg/m^2; OR=3.22 or RR=2.00 for doses of 150–250 mg/m^2; OR=NS or 3.34 or RR=2.43 for doses <150 mg/m^2; OR=3.63 for doses >150 mg/m^2). One meta-analysis found no increase in the risk of neutropenia compared to *1/*1 and compared to *1/*1+*1/*28. One meta-analysis found an increased risk for neutropenia for *28/*28+*6/*28+*6/*6 compared to *1/*1+*1/*28+*1/*6 (OR=3.28). One meta-analysis found a trend for an increased risk of neutropenia for *28 and an increased risk for *28+*6 (OR_G=2.55, which means that patients with neutropenia had a 155% higher frequency of *28 and *6 than patients without neutropenia). Results of separate studies ranged from no increase in the incidence of neutropenia to a significant increase (OR=1.97–15.3; OR_{corr}=2.89 for grade 3–4 neutropenia and 2.33 for grade 4 neutropenia (compared to *1/*1+*1/*28), increase by 50–638%).

Adverse event grade 3–4 diarrhoea: Five meta-analyses found an increased risk of diarrhoea (OR=1.69–5.93), but two only at doses exceeding 125 or 150 mg/m^2. Four meta-analyses found the same for the risk versus *1/*1 + *1/*28 (OR=2.04–6.25). One of the four meta-analyses found no increased risk in White patients versus *1/*1, but did find an increased risk compared to *1/*1 + *1/*28 (OR=1.62). One meta-analysis did not find an increased risk of grade 4 diarrhoea versus *1/*1 + *1/*28, not even at high doses. Results of individual studies ranged from no increase in the incidence of severe diarrhoea to a significant increase by 312%. There was one case where the patient died as a result of severe diarrhoea.

Severe toxicity (including grade 3–4 neutropenia and diarrhoea): One meta-analysis found an increased risk of severe toxicity (OR=2.28–3.07), both for all patients (OR=2.28) and for White patients (OR=2.43),

for all doses (OR=3.07) and at doses >150 mg/m^2 (OR=3.48), but not at doses <150 mg/m^2, for all tumour types (OR=2.76) and for tumours of the digestive system (OR=2.90), but not for tumours of the respiratory system. For patients with *6/*6, which leads to a comparable decrease in UGT1A1 activity, this same meta-analysis did find an effect at low doses and for tumours of the respiratory system.

Tumour response, survival etc.: Two meta-analyses found no difference in the therapeutic response, neither at doses exceeding 150 mg/m^2, nor at doses below 150 mg/m^2. Two meta-analyses found no difference in progression-free survival and death. The mortality rate was increased in the subgroup using doses <150 mg/m^2, but this was only based on one study and was not found in the second, larger meta-analysis. The larger meta-analysis found no difference in the percentage of patients with one or more cycles with reduced irinotecan dose for *28/*28. One meta-analysis found no difference in tumour response for *1/*28+*28/*28. One meta-analysis found an improved tumour response for *1/*28+*28/*28 (OR=1.20 for all patients, OR=1.23 for White patients), but this difference was only significant in the 4 retrospective studies with a total of 538 patients, not in the 12 prospective studies with 1,292 patients.

Results of individual studies ranged from no effect of genotype on tumour response, time to progression and overall survival to an association between genotype and improved tumour response. The risk of progression or progressive/stable disease was reduced (OR 0.19 and 0.32 respectively).

One study involving 5 *28/*28 at 67% of the standard initial dose and 65 *1/*1+*1/*28 at the standard initial dose (180 mg/m^2) found that the incidence of clinical effects grade 3–4 was still 9.7 times higher for *28/*28, whilst the effectiveness was reduced (reduction in complete or partial response by 74%, reduction in the incidence of disease control (response or stable disease) by 57% and a reduction in the progression-free survival by the number of *28 alleles). However, one cannot rule out that this was caused by subsequently attempting a dose increase, with the maximum dose used for *28/*28 being 81% of the maximum dose for *1/*1 and 88% of the maximum dose for *1/*28.

In the aforementioned study, the average maximum tolerated dose for *28/*28 was 76% of the dose for *1/*1+*1/*28 (156 versus 206 mg/m^2) and the maximum dose that was tolerated by the largest group of patients with the genotype (40%) for *28/*28 was 67% of the dose for *1/*1+*1/*28 (120 versus 180 mg/m^2).

Kinetic consequences:
SN-38 AUC increased by 18–159%.
SN-38 metabolic clearance decreased by 61%.

Literature

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42. Wasserman E, et al. Severe CPT-11 toxicity in patients with Gilbert's syndrome: two case reports. Ann Oncol 1997;8:1049–51.
43. SmPCs Campto (NL) and Camptosar (USA).

Preferably implemented as a lookup text only, not as a popup text:

***UGT1A1 *1/*28*: IRINOTECAN**

Pharmacist and physician text
NO action is needed for this gene-drug interaction.

This genetic variation (*1/*28) is more common in Western populations than the wild-type (*1/*1). This means that treatment is largely geared to patients with this genetic variation. Adjustment of the treatment is therefore not useful.

Background information
Mechanism:
Irinotecan is a prodrug that is converted predominantly by carboxylesterases to the active metabolite SN-38, which has 100-1000-fold higher activity than irinotecan itself.

SN-38 is predominantly metabolised by UGT1A1 and otherwise by UGT1A6, UGT1A7, UGT1A9 and UGT1A10 to the inactive metabolite SN-38-glucuronide.

For more information about the UGT1A1 *1/*28 genotype, see the general background information about UGT1A1 on the KNMP Kennisbank (search for UGT1A1).

Clinical consequences:
Adverse event grade 3–4 neutropenia: Four meta-analyses found an increased risk of neutropenia (RR=1.43, OR=1.71–1.91), two both at low and high doses and a third both for all patients (OR=1.71) and for White patients (OR=1.86). One meta-analysis found no increased risk of neutropenia. One meta-analysis found a trend for an increased risk of neutropenia for *28 and an increased risk for *28+*6 (OR_G=2.55, which means that patients with neutropenia had a 155% higher frequency of *28 and *6 than patients without neutropenia). Results of separate studies ranged from no increase in the incidence of neutropenia to a significant increase (OR=1.93–3.47; increase by 12.5–313%).

Adverse event grade 3–4 diarrhoea: Three meta-analyses found an increased risk of diarrhoea (OR=1.45–1.73), but one only at doses ≥125 mg/m^2 (OR=1.92). The other meta-analysis did find an increased risk for all patients (OR=1.56), but not for White patients. Two other meta-analyses found no difference, one only a trend for a higher risk at doses >150 mg/m^2. Results of individual studies ranged from no increase in the incidence of diarrhoea to a significant increase by 94%. There were two cases where the patient died as a result of severe diarrhoea in combination with haematological toxicity.

Severe toxicity (including grade 3–4 neutropenia and diarrhoea): One meta-analysis found an increased risk of severe toxicity (OR=1.60–1.77), both for all patients (OR=1.60) and for White patients (OR=1.59), for all doses (OR=1.77) and at doses >150 mg/m^2 (OR=1.81), but not at doses <150 mg/m^2, for all tumour types (OR=1.68) and for tumours of the digestive system (OR=1.73), but not for tumours of the respiratory system.

Tumour response, time to progression and overall survival: four meta-analyses and individual studies found no difference. One of these four meta-analyses found a trend for an increase in the percentage of patients with one or more cycles of reduced irinotecan dose for *1/*28. One meta-analysis found an improved tumour response for *1/*28+*28/*28 (OR=1.20 for all patients, OR=1.23 for White patients), but this difference was only significant in the 4 retrospective studies with a total of 538 patients, not in the 12 prospective studies with 1,292 patients.

Kinetic consequences:
SN-38 AUC increased by 4.6–41%.
SN-38 metabolic clearance decreased by 37%.

Literature

1. Yang Y, et al. UGT1A1*6 and UGT1A1*28 polymorphisms are correlated with irinotecan-induced toxicity: A meta-analysis. Asia Pac J Clin Oncol 2018;14:e479–e489.
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***UGT1A1* PM OTHER: IRINOTECAN**

Pharmacist and physician text
Serious, life-threatening adverse events occur more often in patients with this genetic variation. The genetic variation reduces conversion of irinotecan to inactive metabolites.

- Start with 70% of the normal dose
  If the patient tolerates this initial dose, the dose can be increased, guided by the neutrophil count.

Background information
Mechanism:
Irinotecan is a prodrug that is converted predominantly by carboxylesterases to the active metabolite SN-38, which has 100–1000-fold higher activity than irinotecan itself.

SN-38 is predominantly metabolised by UGT1A1 and otherwise by UGT1A6, UGT1A7, UGT1A9 and UGT1A10 to the inactive metabolite SN-38-glucuronide.

For more information about the UGT1A1 PM other phenotype, see the general background information about UGT1A1 on the KNMP Kennisbank (search for UGT1A1).

Clinical consequences:
Adverse event grade 3-4 neutropenia:
*6/*6: Two meta-analyses found an increased risk of neutropenia (OR=3.03–3.28). Another meta-analysis found no increased risk of neutropenia compared to *1/*1, but did find an increased risk compared to *1/*1+*1/*6 (OR=5.00). A third meta-analysis found a trend towards an increased risk of neutropenia for *6. One study found a 2.8-fold increased percentage of patients with grade 4 neutropenia compared to *1/*1+*1/*6 (from 24% to 67%).

PM + *28/*28 (*6/*6 + *6/*28 + *28/*28): One meta-analysis found an increased risk of neutropenia (OR=3.28). A second meta-analysis found an increased risk of neutropenia for *28+*6 (OR_G=2.55, which means that patients with neutropenia had a 155% higher frequency of *28 and *6 than patients without neutropenia). One study found a 5.7-fold increased incidence of neutropenia (from 14% to 80%).

Adverse event grade 3–4 diarrhoea:
*6/*6: Three meta-analyses found an increased risk of diarrhoea (OR=3.54–17.6). One of these meta-analyses also found an increased risk compared to *1/*1+*1/*6 (OR=5.26). One study found no association. PM + *28/*28: One study found no association with the incidence of diarrhoea.

Severe toxicity (including grade 3–4 neutropenia and diarrhoea):
*6/*6: One meta-analysis including only Asian studies found an increased risk of severe toxicity (OR=3.16–3.21) for all doses (OR=3.17), at doses >150 mg/m^2 (OR=2.91) and at doses <150 mg/m^2 (OR=9.42), for all tumour types (OR=3.21), for tumours of the digestive system (OR = 3.00) and for tumours of the respiratory system (OR=18.2; based on only 1 study).

Tumour response, survival etc.:
*6/*6: One meta-analysis found no difference in tumour response for *1/*6+*6/*6.
ne study found that the percentage of responders decreased from 50% to 0% and also found decreased progression-free and overall survival compared to *1/*6+*6/*6.

Kinetic consequences:
The information on kinetic consequences has mainly been derived from the *28/*28 genotype which encodes the PM phenotype but for which there are separate pharmacogenetic guidelines.

*28/*28: SN-38 AUC increased by 18–159%. SN-38 metabolic clearance decreased by 61%.
PM + *28/*28 (*6/*6 + *6/*28 + *28/*28): SN-38 AUC increased by 140%.
*6/*6: SN-38 AUC increased by 76%.

Literature

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2. Chen X, et al. UGT1A1 polymorphisms with irinotecan-induced toxicities and treatment outcome in Asians with lung cancer: a meta-analysis. Cancer Chemother Pharmacol 2017;79:1109–17.
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6. Hu ZY, et al. Dose-dependent association between UGT1A1*28 polymorphism and irinotecan-induced diarrhoea: a meta-analysis. Eur J Cancer 2010;46:1856–65.
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11. Paoluzzi L, et al. Influence of genetic variants in UGT1A1 and UGT1A9 on the in vivo glucuronidation of SN-38. J Clin Pharmacol 2004;44:854–60.
12. Sai K, et al. UGT1A1 haplotypes associated with reduced glucuronidation and increased serum bilirubin in irinotecan-administered Japanese patients with cancer. Clin Pharmacol Ther 2004;75:501–15.
13. Innocenti F, et al. Genetic variants in the UDP-glucuronosyl-transferase 1A1 gene predict the risk of severe neutropenia of irinotecan. J Clin Oncol 2004;22:1382–8.
14. Iyer L, et al. UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. Pharmacogenomics J 2002;2:43–7.
15. Ando Y, et al. Polymorphisms of UDP-glucuronosyl-transferase gene and irinotecan toxicity: a pharmacogenetic analysis. Cancer Res 2000;60:6921–6.

Preferably implemented as a lookup text only, not as a popup text:

UGT1A1 IM OTHER: IRINOTECAN

Pharmacist and physician text

NO action is needed for this gene-drug interaction.

This genetic variation (IM) is more common in Western populations than the wild-type (*1/*1). This means that treatment is largely geared to patients with this genetic variation. Adjustment of the treatment is therefore not useful.

Background information

Mechanism:

Irinotecan is a prodrug that is converted predominantly by carboxylesterases to the active metabolite SN-38, which has 100–1000-fold higher activity than irinotecan itself.

SN-38 is predominantly metabolised by UGT1A1 and otherwise by UGT1A6, UGT1A7, UGT1A9 and UGT1A10 to the inactive metabolite SN-38-glucuronide.

For more information about the UGT1A1 IM other phenotype, see the general background information about UGT1A1 on the KNMP Kennisbank (search for UGT1A1).

Clinical consequences:

Adverse event grade 3-4 neutropenia:

*1/*6: One meta-analysis found an increased risk of neutropenia (OR=1.95). Another meta-analysis found no increased risk. A third meta-analysis found a trend for an increased neutropenia risk for *6.

IM + *1/*28 (*1/*6 + *1/*28): One meta-analysis found an increased risk of neutropenia for *28+*6 (OR_G=2.55, which means that patients with neutropenia had a 155% higher frequency of *28 and *6 than patients without neutropenia). One study found a 1.7-fold increased incidence of neutropenia (from 14% to 24%).

Adverse event grade 3–4 diarrhoea:

*1/*6: Two meta-analyses found an increased risk of diarrhoea (OR=1.98–4.36).

IM + *1/*28 (*1/*6 + *1/*28): One study found no association with the incidence of diarrhoea.

Severe toxicity (including grade 3–4 neutropenia and diarrhoea):

*1/*6: One meta-analysis including only Asian studies found an increased risk of severe toxicity (OR=1.75–2.08) for all doses (OR=2.08), at doses >150 mg/m^2 (OR=1.82) and at doses <150 mg/m^2 (OR=3.49), for tumours of all types (OR=1.75), for tumours of the digestive system (OR=1.66) and for tumours of the respiratory system (OR=12.0; based on only 1 study).

Tumour response, time to progression and overall survival:

One meta-analysis found no difference in tumour response for *1/*6+*6/*6.

Kinetic consequences:

The information on kinetic consequences has mainly been derived from the *1/*28 genotype which encodes the IM phenotype but for which there are separate pharmacogenetic guidelines.

*1/*28: SN-38 AUC increased by 4.6–41%. SN-38 metabolic clearance decreased by 37%.

IM + *1/*28 (*1/*6 + *1/*28): SN-38 AUC increased by 40%.

*1/*6: SN-38 AUC increased by 11%.

Literature

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2. Chen X, et al. UGT1A1 polymorphisms with irinotecan-induced toxicities and treatment outcome in Asians with lung cancer: a meta-analysis. Cancer Chemother Pharmacol 2017;79:1109–17.
3. Chen YJ, et al. The association of UGT1A1*6 and UGT1A1*28 with irinotecan-induced neutropenia in Asians: a meta-analysis. Biomarkers. 2014;19:56–62.
4. Goetz MP, et al. UGT1A1 genotype-guided phase I study of irinotecan, oxaliplatin, and capecitabine. Invest New Drugs 2013;31:1559–67.
5. Denlinger CS, et al. Pharmacokinetic analysis of irinotecan plus bevacizumab in patients with advanced solid tumors. Cancer Chemother Pharmacol 2009;65:97–105.
6. Minami H, et al. Irinotecan pharmacokinetics/pharmacodynamics and UGT1A genetic polymorphisms in Japanese: roles of UGT1A1*6 and *28. Pharmacogenet Genomics 2007;17:497–504.
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13. Ando Y, et al. Polymorphisms of UDP-glucuronosyl-transferase gene and irinotecan toxicity: a pharmacogenetic analysis. Cancer Res 2000;60:6921–6.

Supplementary Table S3.7: Based on the criteria and corresponding scores given by the Dutch Pharmacogenetics Working Group (DPWG), the Clinical Implication Score for irinotecan is "Essential"

Table S3.7a: Definitions of the available Clinical Implication Scores

Potentially beneficial	PGx testing for this gene-drug pair is potentially beneficial. Genotyping can be considered on an individual patient basis. If, however, the genotype is available, the DPWG recommends adhering to the gene-drug guideline	0–2 +
Beneficial	PGx testing for this gene-drug pair is beneficial. It is advised to consider genotyping the patient before (or directly after) drug therapy has been initiated to guide drug and dose selection	3–5 +
Essential	PGx testing for this gene-drug pair is essential for drug safety or efficacy. Genotyping must be performed before drug therapy has been initiated to guide drug and dose selection	6–10 +

Table S3.7b: Criteria on which the attribution of Clinical Implication Score is based

Clinical Implication Score Criteria	Possible score	Given score
Clinical effect associated with gene-drug interaction (drug- or diminished efficacy-induced)		
• CTCAE Grade 3 or 4 (clinical effect score D or E)	+	
• CTCAE Grade 5 (clinical effect score F)	++	++[1]
Level of evidence supporting the associated clinical effect grade ≥3		
• One study with level of evidence score ≥3	+	
• Two studies with level of evidence score ≥3	++	
• Three or more studies with level of evidence score ≥3	+++	+++[2]
Number needed to genotype (NNG) in the Dutch population to prevent one clinical effect grade ≥3		
• 100<NNG≤1000	+	
• 10<NNG≤100	++	++[3]
• NNG≤10	+++	
PGx information in the Summary of Product Characteristics (SmPC)		
• At least one genotype/phenotype mentioned	+	+[4]
OR		
• Recommendation to genotype	++	
OR		
• At least one genotype/phenotype mentioned as a contra-indication in the corresponding section	++	
Total Score:	10+	8+
Corresponding Clinical Implication Score:		Essential

[1] The risk of serious life-threatening toxicity is increased for patients with a genotype resulting in diminished UGT1A1 enzyme activity (*28/*28 and PM). This toxicity can be fatal (grade 5) (Rouits 2004). This results in the maximum score of 2 points for the first criterion of the clinical implication score, the clinical effect associated with the gene-drug interaction (2 points for CTCAE grade 5).

[2] The increased risk for serious life-threatening toxicity (code E corresponding to grade 4) has been shown in 14 studies and 9 meta-analyses. This results in the maximum score of 3 points for the second criterion of the clinical implication score, the level of evidence supporting the associated clinical effect grade ≥3 (3 points for three or more publications with level of evidence score ≥3).

[3] The number needed to genotype was deduced to be 41, using the data on Whites in the second largest meta-analysis (Liu 2017) and the prevalence of *28/*28 in the Dutch population. For White patients, Liu 2017 found only the risk for severe neutropenia to be increased for *28/*28 compared to *1/*1+*1/*28, not the risk for severe diarrhoea. In the 12 studies with Caucasian patients in this meta-analysis, the incidence of neutropenia grade 3–4 was 38% for *28/*28 and 11% for *1/*1+*1/*28. Thus, dose adjustment for *28/*28 leading to similar SN-38 concentrations as in *1/*1+ *1/*28 on normal dose, would prevent neutropenia grade 3–4 in 27% of *28/*28. With a prevalence of *28/*28 in the Dutch population of 9%, this would amount to 2.4% of all Dutch patients, i.e. a number needed to genotype of 41. The calculated number needed to genotype of 41 results in 2 out of the maximum of 3 points for the third criterion of the clinical implication score, the number needed to genotype (NNG) in the Dutch population to prevent one clinical effect grade ≥3 (2 points for 10<NNG≤100).

[4] The Dutch Summary of Product Characteristics (SmPC) indicates that *28/*28 patients are at increased risk of haematological toxicity (grade 3 to 4) following administration of irinotecan at moderate or high doses (>150 mg/m^2). This results in 1 out of the maximum of 2 points for the fourth and last criterion of the clinical implication score, the pharmacogenetics information in the SmPC (1 point for at least one genotype/phenotype mentioned in the SmPC, but not mentioned as a contra-indication and no recommendation to genotype).

CHAPTER 4

UGT1A1 genotype-guided dosing of irinotecan: A prospective safety and cost analysis in poor metaboliser patients

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ABSTRACT

Aim

To determine the safety, feasibility, pharmacokinetics, and cost of *UGT1A1* genotype-guided dosing of irinotecan.

Patients and methods

In this prospective, multicentre, non-randomised study, patients intended for treatment with irinotecan were pre-therapeutically genotyped for *UGT1A1*28* and *UGT1A1*93*. Homozygous variant carriers (UGT1A1 poor metabolisers; PMs) received an initial 30% dose reduction. The primary endpoint was incidence of febrile neutropenia in the first two cycles of treatment. Toxicity in UGT1A1 PMs was compared to a historical cohort of UGT1A1 PMs treated with full dose therapy, and to UGT1A1 non-PMs treated with full dose therapy in the current study. Secondary endpoints were pharmacokinetics, feasibility, and costs.

Results

Of the 350 evaluable patients, 31 (8.9%) patients were UGT1A1 PM and received a median 30% dose reduction. The incidence of febrile neutropenia in this group was 6.5% compared to 24% in historical UGT1A1 PMs (p=0.04) and was comparable to the incidence in UGT1A1 non-PMs treated with full dose therapy. Systemic exposure of SN-38 of reduced dosing in UGT1A1 PMs was still slightly higher compared to a standard-dosed irinotecan patient cohort (difference: +32%). Cost analysis showed that genotype-guided dosing was cost-saving with a cost reduction of €183 per patient).

Conclusion

UGT1A1 genotype-guided dosing significantly reduces the incidence of febrile neutropenia in UGT1A1 PM patients treated with irinotecan, results in a therapeutically effective systemic drug exposure, and is cost-saving. Therefore, *UGT1A1* genotype-guided dosing of irinotecan should be considered standard of care in order to improve individual patient safety.

INTRODUCTION

Irinotecan is a commonly prescribed anticancer drug for the treatment of advanced colorectal and pancreatic cancer. However, treatment with irinotecan is often complicated by severe adverse events (AEs) such as febrile neutropenia and diarrhea [1]. This may lead to hospitalisation, loss of quality of life, treatment delay and even treatment discontinuation. Irinotecan is a prodrug that is activated via carboxylesterases in the liver and blood to SN-38 [1]. SN-38 in turn is inactivated in the liver and intestines into SN-38-glucuronide by UDP-glucuronosyltransferase 1A1 (UGT1A1). UGT1A1 is the main enzyme responsible for the inactivation of SN-38 [2].

Several genetic variants within the *UGT1A1* gene are known to be associated with a higher exposure to SN-38 and, therefore, with an increased risk for irinotecan-associated severe AEs [3]. Two highly prevalent and clinically relevant genetic variants in *UGT1A1* in the Caucasian population are *UGT1A1*28* and *UGT1A1*93* [4, 5]. *UGT1A1*28* is a tandem repeat polymorphism in the promotor region of the *UGT1A1* gene that leads to reduced enzyme activity [6, 7]. Homozygous carriers of this variant have a decreased *UGT1A1* gene expression of up to 70% and are considered UGT1A1 poor metabolisers (UGT1A1 PM) [7]. *UGT1A1*93* is in partial linkage disequilibrium (LD) with *UGT1A1*28* (r^2=0.83; https://ldlink.nci.nih.gov/).

A considerable amount of case reports and genetic association studies has been published on the increased risk for irinotecan-associated AEs in homozygous *UGT1A1*28* variant allele carriers [8–13]. Moreover, multiple meta-analyses have confirmed this association [14–18]. Besides the *UGT1A1*28* polymorphism, a replication study confirmed that *UGT1A1*93* is also strongly associated with an increased risk of irinotecan-induced neutropenia [4].

Despite these compelling results, prospective clinical studies have still not been conducted and *UGT1A1* genotyping is not being routinely applied. The main reason for this is that no alternative dose is available for UGT1A1 PM patients. We hypothesised that *UGT1A1* genotype-guided dosing of irinotecan reduces the risk of severe AEs, and thereby improves the individual patient safety. Therefore, to the best of our knowledge, for the first time the safety, feasibility, pharmacokinetics and costs of *UGT1A1* genotype-guided dosing of irinotecan was studied in UGT1A1 PM patients.

METHODS

Study design

This was a prospective, multicentre, nonrandomised clinical implementation study conducted in two large teaching hospitals and two academic centres in the Netherlands. The primary endpoint was febrile neutropenia during the first two cycles of treatment with irinotecan. Secondary endpoints were: other toxicities, treatment delay due to *UGT1A1* genotyping (feasibility), pharmacokinetics of irinotecan and SN-38 in UGT1A1 PMs, cost of *UGT1A1* genotype-guided dosing of irinotecan, conjugated bilirubin and total bilirubin plasma concentrations.

Toxicity in UGT1A1 PMs was compared to historical control patients, i.e. patients homozygous polymorphic for *UGT1A1*28* and/or *UGT1A1*93* treated with full dose therapy identified from systematic literature search. In addition, toxicity in UGT1A1 PMs was compared to UGT1A1 non-PMs treated with full dose in the current study, under the assumption that these groups would experience comparable degrees of toxicity. The study was approved by a central medical ethical review board, the Medical Research Ethics Committees United, and approval from the board of directors of each individual hospital was obtained for all participating centres. The study was registered at the Netherlands Trial Register (www.trialregister.nl study-number NL6270).

Patient selection

Patients were included if they were aged 18 years or older with a pathologically confirmed malignancy intended to be treated with irinotecan at a dose of ≥180 mg/m^2 or 450–600 mg flat dose. For further inclusion and exclusion criteria see **Supplementary Methods**.

Historical controls were selected from published studies identified from a systematic literature search in which unselected cohorts of irinotecan-treated Caucasian patients with pancreatic or colorectal cancer were genotyped for *UGT1A1*28*. Further selection criteria are described in the **Supplementary Methods**.

Procedures

Prior to start of treatment with irinotecan, patients were genotyped for *UGT1A1*28* and *UGT1A1*93*. Genotypes were converted to phenotypes in the following manner: homozygous carriers of *UGT1A1*28* and/or *UGT1A1*93* were defined UGT1A1 PM, heterozygous carriers of *UGT1A1*28* and/or *UGT1A1*93* were defined UGT1A1 intermediate metaboliser (UGT1A1 IM) and *UGT1A1* wild type individuals were defined UGT1A1 extensive metaboliser, i.e. normal metaboliser (UGT1A1 EM).

UGT1A1 PMs were given an initial irinotecan dose reduction of 30% in cycle 1 based on previous pharmacokinetic and clinical evidence [19–22]. Thereafter, the dose was further individualized based on ANC and clinical tolerance. **Supplementary Figure S4.1** and **S4.2** depict the dosing nomogram for UGT1A1 PMs.

Toxicity was graded according to NCI CTCAE version 4.03 [23].

In order to determine adequate drug exposure of reduced dosing in UGT1A1 PMs the pharmacokinetics of irinotecan and SN-38 was determined by use of a limited sampling strategy (**Supplementary Methods**) [24]. Both irinotecan and SN-38 concentrations at 2.5h and 49.5 h as well as systemic SN-38 exposure (area under the concentration-time curve, $AUC_{0\text{-}500h}$) of reduced dosing in the UGT1A1 PM cohort were analysed and compared to a standard dosed irinotecan patient cohort [25]. Given the various irinotecan treatment schedules included in the study all irinotecan and SN-38 concentrations and SN-38 AUCs were dose-normalised for UGT1A1 PM to 126 mg/m^2 (corresponding to 70% dose intensity) and for the standard dosed cohort to 180 mg/m^2 (100% dose intensity).

A cost analysis was conducted from a health care perspective. Direct health care costs were calculated, based on costs of screening and subsequent drug treatment and treatment for toxicity. The impact of parameter uncertainty on model outcomes was analysed using one-way sensitivity analyses in which each of the parameters were individually varied by ±20%. Further details are provided in the **Supplementary Methods**.

Statistical analysis

Based on prior data we assumed that the intervention reduces the incidence of febrile neutropenia from 26% to 3%, similar to the incidence of febrile neutropenia in wild type and heterozygous carriers [26–28]. Based on this assumption, a total of 31 UGT1A1 PMs and 47 historical control were needed to demonstrate the abovementioned reduction in incidence of febrile neutropenia with a power of 80% and a 2-sided test with an alpha of 0.05.

Patients were considered evaluable if they received at least one dose of irinotecan. Treatment outcomes were analysed using the Chi-square test or Fisher's exact test, where applicable. Pharmacokinetic data was presented as geometric mean and the coefficient of variation. The relative difference between UGT1A1 PMs and the standard dosed cohort was calculated and was analysed using an unpaired t-test. All statistical tests were 2-sided and were performed using a 5% significance level. All statistical analyses were performed with SPSS for Windows (version 25.0; IBM Corp., Armonk, NY).

Additional details of the study methods and results, including subgroup analysis, genotype frequencies and bilirubin plasma concentrations, are provided in the **Supplementary Methods** and **Supplementary Results**.

RESULTS

Between August 2017 and December 2020, a total of 404 consecutive patients were pre-therapeutically genotyped for the selected *UGT1A1* polymorphisms and enrolled in this study. In total, 54 patients were excluded either because of screen failures, refrain from chemotherapy or loss to follow-up (**Figure 4.1**).

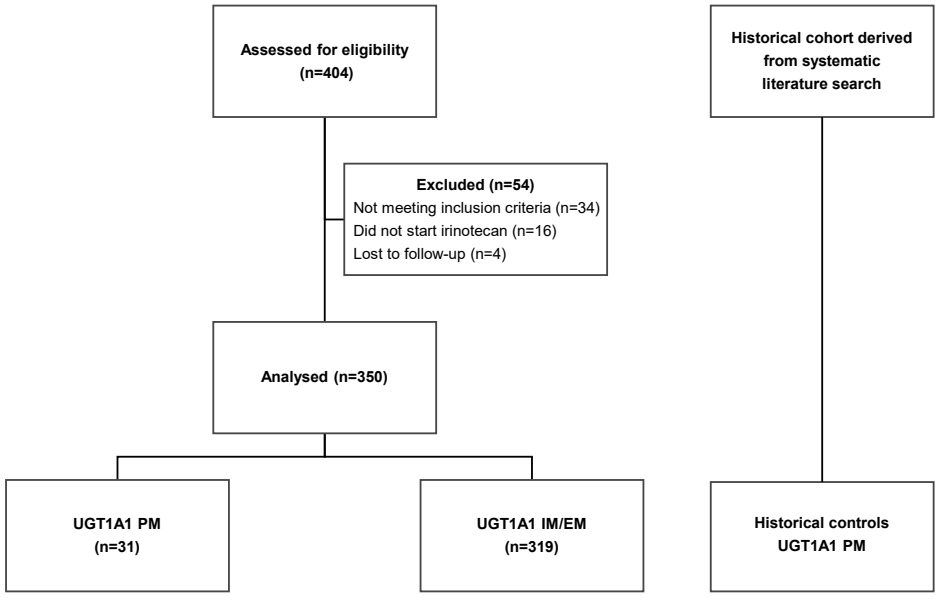

Figure 4.1: Consort diagram of the study.
Abbreviations: UGT1A1 PM = UGT1A1 poor metaboliser; UGT1A1 IM = UGT1A1 intermediate metaboliser; UGT1A1 EM = UGT1A1 extensive metaboliser.

Overall patient and treatment characteristics

Of the 350 evaluable patients, a total of 8.9% (N=31) was UGT1A1 PM, individual genotype frequencies are reported in **Supplementary Table S4.1**. **Table 4.1** presents an overview of the baseline characteristics of all patients. Given the heterogeneous patient population with regard to line of treatment, we tested whether the incidences of febrile neutropenia and neutropenia grade ≥3 differed between pre-treated and first-line treatment patients,

Table 4.1: Baseline characteristics

	UGT1A1 PM N=31	UGT1A1 IM N=158	UGT1A1 EM N=161	All patients N=350
Sex, No. (%)				
Male	19 (61)	89 (56)	81 (50)	189 (54)
Female	12 (39)	69 (44)	80 (50)	161 (46)
Age, median (IQR), years	61 (58–67)	63 (57–69)	63 (56–71)	63 (57–69)
Ethnic origin, No. (%)				
Caucasian	29 (94)	155 (98)	159 (98)	343 (98)
African descent	2 (6)	2 (1)	1 (1)	5 (1)
Hispanic	0 (0.0)	1 (1)	1 (1)	2 (1)
Cancer type, No. (%)				
Pancreatic cancer	14 (45)	82 (52)	78 (48)	174 (50)
Colorectal cancer	13 (42)	63 (40)	74 (46)	150 (43)
Other[a]	4 (13)	13 (8)	9 (6)	26 (7)
Cancer stage, No. (%)				
Local	1 (3)	17 (11)	16 (10)	34 (10)
Locally advanced	10 (32)	32 (20)	41 (25)	83 (24)
Metastatic	20 (65)	109 (69)	104 (65)	233 (66)
Previous treatment with chemotherapy[b], No. (%)	16 (52)	81 (51)	88 (55)	185 (53)

Table 4.1 continues on next page.

Table 4.1: ***Continued***

	UGT1A1 PM N=31	UGT1A1 IM N=158	UGT1A1 EM N=161	All patients N=350
Treatment regimen, No. (%)				
FOLFIRINOX q2w[c]	14 (45)	79 (50)	80 (50)	173 (50)
FOLFIRI q2w[c]	5 (16)	32 (20)	27 (17)	64 (18)
FOLFIRI q2w + targeted agent[b]	3 (9)	12 (8)	17 (11)	32 (9)
Irinotecan q3w[d]	7 (23)	18 (11)	19 (11)	44 (13)
Other	2 (7)	17 (11)	18 (11)	37 (11)
WHO performance status, No. (%)				
0	6 (19)	37 (23)	40 (25)	83 (24)
1	13 (42)	54 (34)	58 (36)	125 (36)
2	1 (3)	3 (2)	3 (2)	7 (2)
0–2; not specified[e]	11 (36)	64 (41)	60 (37)	135 (38)
BSA, median (IQR), m^2	1.80 (1.70–2.01)	1.93 (1.79–2.06)	1.89 (1.74–2.04)	1.90 (1.75–2.04)

[a] Cancer type: esophagus (n=9), biliary tract (n=4), gastric (n=4), unknown origin (n=3), duodenum (n=2), liposarcoma (n=1), lung (n=1), neuroendocrine sigmoid (n=1), urothelial (n=1).

[b] Chemotherapy not containing irinotecan, previous irinotecan treatment was an exclusion criterion

[c] Irinotecan dose 180 mg/m^2.

[d] Irinotecan flat dose 600 mg.

[e] WHO performance status (WHO PS) not further specified. However, WHO PS was always 0–2 as it was properly evaluated as inclusion criterion.

Abbreviations: UGT1A1 PM = UGT1A1 poor metaboliser; UGT1A1 IM = UGT1A1 intermediate metaboliser; UGT1A1 EM = UGT1A1 extensive metaboliser.

however, this was not the case (data not shown). UGT1A1 PMs were treated with a median dose intensity of irinotecan in the first cycle of 70% (IQR 67.8–70.6%) compared to 99% (IQR 97–101%) in UGT1A1 non-PMs. The overall median dose intensity was 69% (IQR: 60–71%) versus 96% (IQR: 81–100%), respectively. Furthermore, pre-therapeutic genotyping showed to be feasible: in only one (0.3%) of all 350 evaluable patients genotype results were delayed and treatment with irinotecan was initiated before genotyping results were available.

Toxicity of *UGT1A1* genotype-guided dosing

Systematic literature research revealed a total of nine studies that fulfilled the selection criteria for the historical cohort (**Supplementary Table S4.2**) [8, 16, 26–32]. In this study, the incidence of febrile neutropenia in the first two cycles in UGT1A1 PMs treated with *UGT1A1* genotype-guided dosing was 7% and was significantly lower compared to 24% in historical controls treated with full dose therapy (p=0.04; **Figure 4.2**). Besides the primary endpoint, the incidence of grade ≥4 neutropenia (13% versus 56%; p<0.01) and chemotherapy-related hospital admissions (13% versus 42%; p<0.01) was also significantly lower in UGT1A1 PMs than in historical controls. The incidence of grade ≥3 diarrhea was also reduced, but not statistically significant (10% versus 22%; p=0.14).

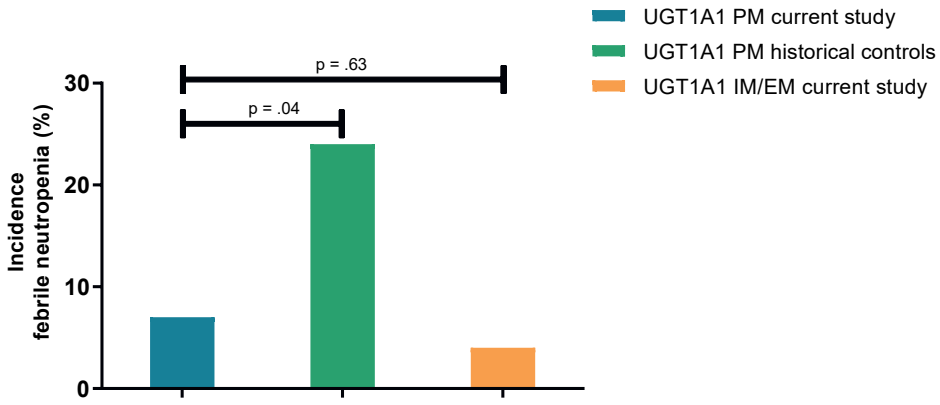

Figure 4.2: Incidence of febrile neutropenia.
The incidence (%) of febrile neutropenia in the first two cycles in; UGT1A1 PMs included in this study and treated with *UGT1A1* genotype-guided dosing, UGT1A1 PM historical controls treated with the full dose therapy, and UGT1A1 IM/EMs (non-PMs) treated with fill dose therapy in this study. Abbreviations: UGT1A1 PM = UGT1A1 poor metabolisers, UGT1A1 IM/EM = UGT1A1 intermediate and extensive metabolisers.

Table 4.2: Treatment outcomes for patients included in this study and historical controls

	UGT1A1 PM current study	UGT1A1 PM historical controls				UGT1A1 IM/EM current study	
	(N=31)		(N)	Reference[a]	P-value	(N=319)	P-value
Dose intensity 1st cycle, median (IQR)	70.0% (67.8–70.6%)	100%[b]	n.a.	n.a.	n.a.	99.4% (97.2–100.6%)	n.a.
Dose intensity all cycles, median (IQR)	69.4% (60.0–71.2%)	unknown	n.a.	n.a.	n.a.	95.6% (81.0–100.0%)	n.a.
Hematologic toxicity, No. (%)							
Febrile neutropenia in the first 2 cycles	2 (7)	12 (24)[c]	50	[26–28]	0.04	13 (4)	0.63
Febrile neutropenia	3 (10)	12 (24)	50	[26–28]	0.11	15 (5)	0.21
Grade ≥3 neutropenia	12 (39)	43 (35)	122	[16]	0.72	53 (17)	<0.01
Grade ≥4 neutropenia	4 (13)	14 (56)	25	[8, 27, 29]	<0.01	28 (13)	0.51
Grade ≥3 leukopenia	5 (16)	8 (32)	25	[26]	0.16	38 (12)	0.56
Grade ≥3 thrombocytopenia	0 (0.0)	unknown	n.a.	n.a.	n.a.	4 (1)	1.00
Non-hematologic toxicity, No. (%)							
Grade ≥3 diarrhea	3 (10)	25 (22)	116	[16]	0.14	51 (16)	0.44
Grade ≥3 nausea	1 (3)	unknown	n.a.	n.a.	n.a.	18 (6)	1.00
Grade ≥3 anorexia	2 (7)	unknown	n.a.	n.a.	n.a.	12 (4)	0.36
Grade ≥3 other toxicity	4 (13)	unknown	n.a.	n.a.	n.a.	34 (11)	0.76
Overall grade ≥3 toxicity	18 (58)	39 (52)	75	[28, 30, 31]	0.57	112 (35)	0.01
Chemotherapy-related hospital admissions[d], No. (%)	4 (13)	22 (42)	53	[8, 26, 32]	<0.01	72 (23)	0.21
Early treatment withdrawal due to toxicity, No. (%)	3 (10)	unknown	n.a.	n.a.	n.a.	32 (10)	1.00
Treatment delay due to toxicity, No. (%)	12 (39)	unknown	n.a.	n.a.	n.a.	85 (27)	0.15

[a] Historical controls were selected per treatment outcome, therefore different publications per outcome are reported.

[b] Dose intensity in cycle 1 was not always reported, based on the inclusion criteria for the historical cohort it was assumed that no dose reductions were performed since the intention was to treat patients with full dose.

[c] From 2 historical control cohorts (N=36) the incidence of febrile neutropenia was only available for all cycles [26, 27].

[d] Hospital admissions due to irinotecan alone, or due to combination of chemotherapy.

Abbreviations: UGT1A1 PM = UGT1A1 poor metabolisers; UGT1A1 IM/EM = UGT1A1 intermediate and extensive metabolisers; n.a. = not applicable.

In comparison to UGT1A1 IM/EMs treated with full dose therapy in this study, the incidence of febrile neutropenia of genotype-guided dosing in UGT1A1 PMs was in line with the hypothesis and comparable with the incidence of 4.1% in UGT1A1 non-PMs treated with full dose therapy (p=0.63; **Figure 4.2)**. Despite initial 30% dose reduction, UGT1A1 PMs experienced more grade ≥3 neutropenia (39% versus 17%; p<0.01) and overall grade ≥3 toxicity (58% versus 35%; p=0.01). An overview of all toxicity outcomes of UGT1A1 genotype-guided dosing is presented in **Table 4.2**.

In subgroup analysis, the incidence of grade ≥3 neutropenia (22% versus 11%; p<0.01) and grade ≥4 neutropenia (12% versus 6%; p=0.04) was higher in UGT1A1 IMs compared to UGT1A1 EMs, respectively. **Supplementary Table S4.3** lists all results of the subgroup analyses.

Pharmacokinetics

The initial 30% dose reduction in UGT1A1 PMs was reflected in significantly lower (-30%, p<0.01) mean irinotecan plasma concentration compared to the standard dosed control cohort. Of interest, whilst applying the 30% dose reduction, the systemic exposure of the active metabolite SN-38 of reduced dosing in UGT1A1 PMs (N=17) was slightly higher compared to the standard dosed irinotecan patient cohort (N=46) with a borderline significant relative difference in SN-38 AUC0-500h of +32% (95% CI: -0.5% to 75.8%) and a geometric mean (CV) of 391 ng*h/mL (43.7%) versus 296 ng*h/mL (75.3%), respectively. All pharmacokinetic data are provided in **Figure 4.3** and **Table 4.3**.

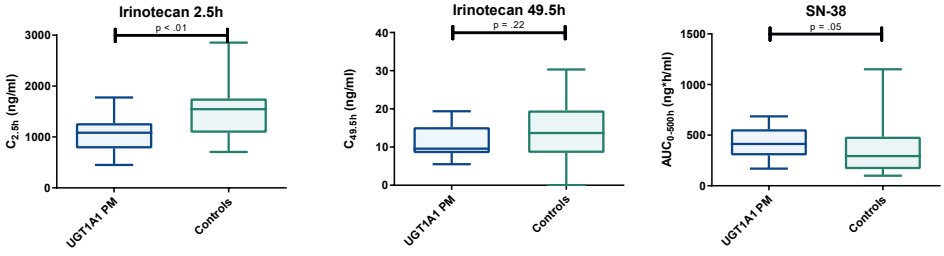

Figure 4.3: Plasma concentrations of irinotecan and AUCs of its active metabolite SN-38 of reduced dosing in UGT1A1 PMs versus standard dosed irinotecan patient cohort.
All irinotecan plasma concentrations and SN-38 AUCs were dose-normalised for UGT1A1 PM to 126 mg/m^2 (corresponding with 70% dose intensity) and for the standard dosed cohort to 180 mg/m^2 (100% dose intensity) to correct for the various irinotecan treatment regimens included in this study.
Abbreviations: C = concentration; UGT1A1 PM = UGT1A1 poor metaboliser; UGT1A1 IM/EM = UGT1A1 intermediate and extensive metabolisers; AUC = area under the curve.

Table 4.3: Pharmacokinetics of irinotecan and its active metabolite SN-38 in UGT1A1 PMs versus standard dosed irinotecan patient cohort

PK parameters	UGT1A1 PM (Geometric mean, CV)	n[a]	UGT1A1 IM/EM (Geometric mean, CV)	n[a]	Relative difference UGT1A1 PM vs. UGT1A1 IM/EM (95% CI)	P-value
Plasma irinotecan[b]						
$C_{2.5h,}$ ng/mL	999 (34.3%)	17	1419 (32.7%)	45	-29.6% (-41.4% to -15.4%)	<0.01
$C_{49.5h}$, ng/mL	10.7 (37.1%)	17	12.8 (60.7%)	44	-16.5% (-37.7% to 12.0%)	0.22
Plasma SN-38[b]						
$C_{2.5h,}$ ng/mL	18.3 (47.3%)	17	20.0 (60.4%)	46	-8.3% (-32.2% to 24.0%)	0.57
$C_{49.5h}$, ng/mL	1.65 (61.0%)	17	0.89 (123.5%)	46	+84.8% (24.4% to 174.5%)	<0.01
$AUC_{0\text{-}500h}$, ng*h/mL	391 (43.7%)	17	296 (75.3%)	46	+32.2% (-0.50% to 75.8%)	0.05

Pharmacokinetic data of UGT1A1 PMs with a reduced dose of irinotecan and UGT1A1 IM/EMs with a standard dose of irinotecan.
[a] Not all UGT1A1 PMs (14/31) participated in the PK part of the study because not all patients consented or due to logistic reasons blood sampling was not possible.
[b] All data are dose-normalised for UGT1A1 PM to 126 mg/m^2 (corresponding with 70% dose intensity) and for the standard dosed cohort to 180 mg/m^2 to be able to compare all different dosing levels in this study.
Abbreviations: UGT1A1 PM = UGT1A1 poor metaboliser; UGT1A1 IM/EM = UGT1A1 intermediate and extensive metabolisers; CV = coefficient of variation; CI = confidence interval.

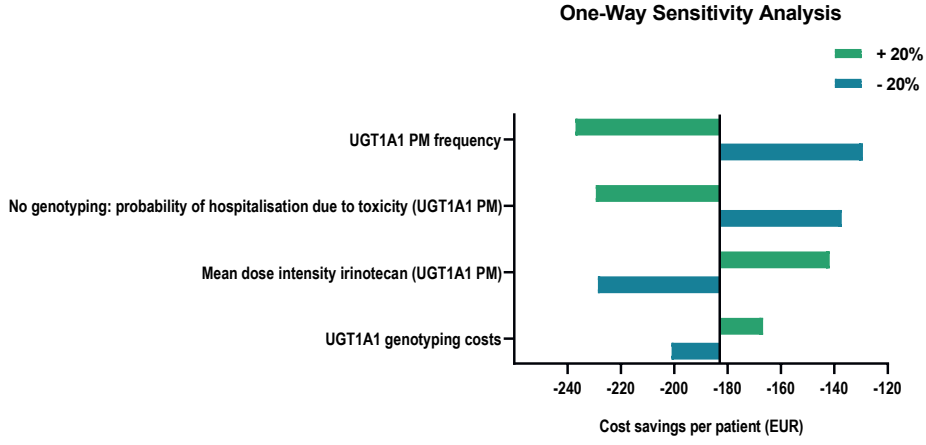

Figure 4.4: One-way sensitivity analysis of *UGT1A1* genotype-guided dosing of irinotecan versus conventional dosing of irinotecan.
Abbreviations: UGT1A1 PM = UGT1A1 poor metaboliser.

Cost analysis

Supplementary Figure S4.3 and **Supplementary Table S4.4** provide the decision tree and the parameter estimates used in the cost analysis, respectively. The expected total treatment costs of *UGT1A1* genotype-guided dosing were €7,232 per patient compared to €7,415 per

patient for conventional dosing. Thereby, genotype-guided dosing resulted in a total cost reduction of €183 per patient, outweighing screening costs (**Supplementary Table S4.5**). The tornado diagram (**Figure 4.4**) shows the effect on the cost reduction of *UGT1A1* genotyping when all model parameters were individually varied by 20%. The model proved to be most sensitive to UGT1A1 PM frequency.

DISCUSSION

This is the first prospective clinical study providing evidence of *UGT1A1* genotype-guided dosing of irinotecan in all UGT1A1 phenotypes. We demonstrated that genotype-guided dosing significantly reduced the incidence of febrile neutropenia and chemotherapy-related hospital admissions in UGT1A1 PMs. Nonetheless, systemic drug exposure of the active metabolite SN-38 remained adequate and was even slightly higher. This shows that *UGT1A1* genotype-guided dosing of irinotecan significantly improves patient safety without a risk of underdosing. As a result, *UGT1A1* genotype-guided dosing was successfully implemented in four hospitals in the Netherlands.

UGT1A1 genotype-guided dosing of irinotecan proved to be feasible in daily practice as there was no delay in start of treatment. Moreover, it proved to be cost-saving compared to non-screening. Three previously published cost analyses suggested *UGT1A1* genotype-guided dosing resulted in cost reductions ranging from €112 up to €596 per patient, however, this was calculated retrospectively [33–35]. In this cost study, conducted alongside the clinical trial, we confirm a total saving of €183 per patient.

The overall dose intensity in UGT1A1 PMs was 69% (IQR 60–70%), which confirms the previously reported maximum tolerated dose intensities in three dose-finding studies. These studies reported dose intensities ranging from 43% to 72%, indicating that a 30% dose reduction in UGT1A1 PMs is adequate [20, 21, 36]. In addition, a French proof of concept trial demonstrated that *UGT1A1* genotype-guided dosing of FOLFIRI with irinotecan dose intensification based on tolerance, resulted in a mean irinotecan dose in UGT1A1 PMs after 3 months of 140 mg/m^2 (dose intensity 78%) [37]. Our pharmacokinetic analysis shows a slightly higher exposure to SN-38 in UGT1A1 PMs of reduced dosing versus controls. Notwithstanding, incidences of grade 4 neutropenia, febrile neutropenia and diarrhea in UGT1A1 PMs was comparable to the incidences in UGT1A1 non-PMs. In addition, the overall median dose intensity was 69%, demonstrating good tolerance over time. Therefore, a starting dose reduction of 30% in UGT1A1 PMs is sufficient, and should not necessarily be further reduced despite slightly higher SN-38 systemic exposure.

Hereby, we believe this study adds important data. Similarly to pre-therapeutic genotyping of dihydropyrimidine dehydrogenase (DPD; *DPYD*) in patients treated with capecitabine or 5-fluorouracil, it must be noted that the same efforts were carried out before *DPYD* genotyping in fluoropyrimidine treatment was accepted and implemented by most cancer societies.

There are some potential drawbacks associated with our study. First, the ideal study design would have been a randomised controlled trial with a *UGT1A1* genotype-guided dosing arm and a conventional dosing arm that is also powered to assess survival outcomes. However, such a trial is hardly feasible, since at least 6,000 patients need to be prospectively screened for inclusion. More important, with the available evidence favoring genotype-guided dosing and the known risk of overexposure with standard dosing, it is rather unethical to randomise patients. Nonetheless, the 30% dose reduction of irinotecan in UGT1A1 PMs resulted in a slightly higher systemic exposure to SN-38 as in a standard dosed patient cohort. Therefore, we consider it unlikely that *UGT1A1* genotype-guided dosing will negatively affect overall survival.

Second, patients and historical controls on different treatment schedules of irinotecan, with different cancer types and different lines of therapy were included in this study. Nevertheless, by dose normalisation of the pharmacokinetic data, and by reporting only adverse events related to irinotecan treatment, the different treatment schedules did not affect study results. In addition, previous treatment with chemotherapy was not related to the incidence of febrile neutropenia nor grade ≥3 neutropenia in this cohort. Third, while G-CSF use was allowed in this study, this information could not be retrieved for most of the historical controls. G-CSF use might influence the risk of febrile neutropenia. Nonetheless, the incidence of febrile neutropenia in our UGT1A1 PMs was comparable with the incidence in our UGT1A1 non-PMs treated with full dose therapy. Moreover, in comparison to UGT1A1 non-PMs treated with full dose therapy in this study, UGT1A1 PMs experienced more grade ≥3 neutropenia (39% versus 17%) indicating that neutropenia still occurred in our study despite allowance of G-CSF use.

Based on the lower incidence of grade ≥3 neutropenia in UGT1A1 EMs compared to UGT1A1 IMs in our subgroup analysis and on several dose-finding studies of irinotecan in UGT1A1 IM and EM patients, further research into optimising the safety of irinotecan treatment in UGT1A1 IM patients is of great interest [38, 39]. It should be noted that the results of our study are not directly applicable for the Asian population, but could potentially be extrapolated. However, since in Asian patients the *UGT1A1*6* rather than *UGT1A1*28* is of major importance, Asians were excluded in our study [40].

Conclusion

In conclusion, the results of this prospective study show that *UGT1A1* genotype-guided dosing significantly reduces the incidence of febrile neutropenia in UGT1A1 PM patients treated with irinotecan, is feasible in daily practice and is cost-saving. In addition, systemic drug exposure of the active metabolite remained at least adequate with applying a 30% dose reduction. Therefore, *UGT1A1* genotype-guided dosing of irinotecan should be considered new standard of care in order to improve the individual patient safety.

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SUPPLEMENTARY MATERIAL

Supplementary Methods

Inclusion and exclusion criteria

Patients were included if they were aged 18 years or older with a pathologically confirmed malignancy intended to be treated with irinotecan at a dose of ≥180 mg/m^2 or 450–600 mg flat dose, written informed consent, a WHO performance status of 0, 1 or 2 and acceptable safety laboratory values.

The following laboratory values were defined: absolute neutrophil count (ANC) ≥1.5 x 10^9 /L, platelet count ≥100 x 10^9 /L, serum bilirubin ≤1.5 x upper limit of normal (ULN), ALT and AST ≤2.5 x ULN; in case of liver metastases ALT and AST ≤5 x ULN, and renal function (eGFR) ≥50 ml/min or creatinine ≤ 1.5 x ULN.

Exclusion criteria were prior treatment with irinotecan, known substance abuse, psychotic disorders or other diseases expected to interfere with the study or the patient's safety, Asian origin and the use of (over the counter) medication or (herbal) supplements that were known to interact with irinotecan (e.g. by induction of inhibition of CYP3A4).

Selection of historical controls by systematic literature search

A literature search was conducted to identify all available historical controls. We searched PubMed until December 2020 without any limitations on publication year using the following search terms: "irinotecan", "CPT-11", "UGT1A1". Reference lists in original articles and review articles were manually searched to identify additional potentially relevant publications.

Controls had to be treated with irinotecan monotherapy or combination therapy, in a comparable dose as the patients in the current study, i.e. ≥75 mg/m^2 for weekly schedules or ≥150 mg/m^2 for 2- or 3-weekly schedules. To avoid selection bias, patients described in case reports, case-control studies, review articles, and studies without patients homozygous polymorphic for *UGT1A1*28* and *UGT1A1*93* were excluded. In addition, studies that had to lower the starting dose of irinotecan during the trial due to excessive toxicity were excluded because these results may lead to overestimation of toxicity. For the primary endpoint studies that did not report on febrile neutropenia by genotype were excluded.

Supplementary procedures: Genotyping

Prior to start of treatment with irinotecan, patients were genotyped for two genetic variants in UGT1A1, i.e. UGT1A1*28 (TA repeat; NC_000002.12:g.233760233CAT>CATAT) and UGT1A1*93 (-3156G>A). DNA for genotyping was isolated from 200µl of whole EDTA blood that was obtained prior to start of therapy. Genotyping was conducted using validated real-time PCR reactions and included appropriate wild type, heterozygous and homozygous controls in every run. Genotyping was performed three times per week to minimise delay in start of treatment. *UGT1A1* genotyping was conducted in the local clinical laboratories of three of the participating centers and for one hospital in one of the other participating centers. Genotype frequencies were tested for Hardy-Weinberg equilibrium (HWE), a p-value >0.05 was considered consistent with HWE.

As described in the main article, genotypes were converted to phenotypes in the following manner: homozygous carriers of *UGT1A1*28* and/or *UGT1A1*93* were defined UGT1A1 PM, heterozygous carriers of *UGT1A1*28* and/or *UGT1A1*93* were defined UGT1A1 intermediate metaboliser (UGT1A1 IM) and UGT1A1 wild type individuals were defined UGT1A1 extensive metaboliser, i.e. normal metaboliser (UGT1A1 EM). Of note, given the known high LD of *UGT1A1*28* and *UGT1A1*93* of 0.83, heterozygous carriers were considered cis-compound heterozygous and therefore defined as IM phenotype [1].

Patients co-treated with fluoropyrimidines as concomitant chemotherapy were additionally genotyped for DPD deficiency as routine standard of care in line with current recommendations and treated accordingly [2].

Supplementary procedures: Toxicity assessments

Standard laboratory and clinical assessments were performed before the start of treatment and before each subsequent cycle according to standard practice.

Patients were monitored for toxicity during the first six cycles of 2-weekly treatment regimens and during the first four cycles of 3-weekly treatment regimens. Only AEs that were possibly, probably or definitely related to irinotecan treatment were considered treatment-related.

Supplementary procedures: Irinotecan dosing nomogram in UGT1A1 PMs

UGT1A1 PMs, i.e. homozygous variant allele carriers, were given an initial dose reduction of 30% in cycle 1. In order to optimise the dose for the individual patient, dosing of irinotecan was further based on absolute neutrophil count (ANC). ANC was determined one or two days prior to, or on the day of the following cycle of treatment. In case of febrile neutropenia before

standard ANC measurement dose reduction and/or delay was applied at the discretion of the treating physician. Dose escalation was only allowed provided that the first two cycles were fully completed, no other grade ≥2 toxicity occurred and was left to the discretion of the treating physician. In patients with mild (NCI-CTCAE grade 0 or 1) neutropenia, a 10–20% dose escalation was allowed with the opportunity to a further 10–20% dose escalation in the subsequent cycles (up to a maximum of 100% of the maximum indicated dose). In patients with grade 2 or 3 neutropenia, no further dose escalation was performed. In case of grade 4 neutropenia, further treatment was continued according to standard practice left to the discretion of the treating physician. **Supplementary Figures S4.1** and **S4.2** provide the genotype-guided and neutrophil-guided irinotecan dosing nomogram for UGT1A1 PMs.

The irinotecan starting doses in UGT1A1 IMs and EMs were left unchanged, as well as the doses of other concomitant chemotherapy.

Granulocyte colony-stimulating factor (G-CSF) use was allowed and left to the discretion of the treating physician.

Supplementary procedures: Pharmacokinetics

In order to demonstrate adequate drug exposure in UGT1A1 PMs, the pharmacokinetics of irinotecan and SN-38 was determined in the first cycle of treatment by use of a limited sampling strategy [3]. Additional informed consent was obtained for this part of the study. Two blood samples were obtained at 2.5 h and 49.5 h after start of the infusion. Blood samples were collected in lithium heparinised tubes and within 30 minutes after blood withdrawal centrifuged during 10 minutes at 1500 g at 4°C. Plasma was collected and stored at -20°C until analysis. Irinotecan and SN-38 plasma concentrations were analysed using a validated reversed-phase high-performance liquid chromatographic method with fluorescence detection as described by de Bruijn et al. [4] Irinotecan and SN-38 concentrations at 2.5h and 49.5 h and systemic SN-38 exposure ($AUC_{0\text{-}500h}$) of reduced dosing in the UGT1A1 PM cohort were compared to a general patient cohort treated with irinotecan-based chemotherapy [5]. Patient samples in the pharmacokinetic control cohort were processed and analysed precisely similar and at the same laboratory as the patient samples from the current study. The irinotecan and SN-38 concentrations and the AUC of SN-38 were presented as geometric means plus CV. To be able to compare all different dosing levels in this study, all irinotecan and SN-38 concentrations and SN-38 AUCs were dose-normalised, for UGT1A1 PM to 126 mg/m^2 (corresponding to a 70% dose intensity) and for the standard dosed cohort to 180 mg/m^2 (corresponding with 100% dose intensity). Since irinotecan shows dose-linear pharmacokinetics, dose-normalisation is allowed [6].

Supplementary procedures: Cost analysis

A cost analysis was conducted using a decision analytical model from a health care perspective and was restricted to direct medical costs only. The model was built according to the principles of good practice for decision analytical modeling [7], and compared the conventional, standard-dose treatment strategy with the *UGT1A1* genotype-guided dosing strategy. Costs of both strategies were calculated, based on costs of screening and subsequent drug treatment. Parameter estimations incorporated in the model were derived from data from our trial and relevant literature (when available). Events, as considered in the model, were multiplied with unit costs per event. Cost estimates for the various parameters were derived from the Dutch guide of pharmacotherapy and the Dutch guide for health-economic research [7,8]. Costs are given in Euros and are based on the year 2020. **Supplementary Table S4.4** provides all parameter estimates. In order to evaluate the impact of parameter uncertainty on model outcome a one-way sensitivity analysis was conducted. Here-in, each parameter was varied individually with ±20% of the base case value and the outcomes that led to the highest variation were plotted in a Tornado-diagram.

Feasibility of UGT1A1 genotype-guided dosing

Feasibility of *UGT1A1* genotype-guided dosing of irinotecan was defined as no treatment delay of more than two days due to prospective screening of *UGT1A1* genotype. Prospective screening of *UGT1A1* genotype was considered feasible when ≥95% of the screened patients had no treatment delay.

Conjugated bilirubin / unconjugated bilirubin

Under the hypothesis that UGT1A1 PM patients have a lower conjugated bilirubin / unconjugated bilirubin ratio than UGT1A1 non-PMs due to a lower glucuronidation rate, the predictive value of this ratio for UGT1A1 phenotype was analysed. Both sensitivity and specificity of the conjugated bilirubin / unconjugated bilirubin concentration ratio as a marker for UGT1A1 phenotype was calculated at various discrimination thresholds. Results were plotted in a receiver operating characteristics curve (ROC curve).

Supplementary Results

Genotype frequencies

The observed minor allele frequencies of *UGT1A1*28* and *UGT1A1*93* were respectively, 0.32 and 0.28, these were all consistent with HWE (**Supplementary Table S4.1**).

Conjugated bilirubin / unconjugated bilirubin

The conjugated bilirubin / unconjugated bilirubin concentration ratio was available for 265 patients at baseline. This ratio did not show any correlation with UGT1A1 phenotype, the AUC of the ROC curve was 0.43 (**Supplementary Figure S4.4**).

Supplementary References

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Supplementary Table S4.1: Genotype frequencies

Genotype status	N=350	(%)	HWE p-value	MAF
*UGT1A1*28*			0.34	0.32
WT[a]	160	46		
HET	159	45		
HOM	31	9		
*UGT1A1*93*			0.42	0.28
WT	176	51		
HET	148	42		
HOM	25	7		
Missing	1			

[a] One patient was a heterozygous carrier of *UGT1A1*36* (TA5/TA6 tandem repeat), this translates into an increased enzyme activity. Therefore this patient was combined with the wild type group.
Abbreviations: HWE = Hardy Weinberg Equilibrium, MAF = minor allele frequency, WT = wild type, HET = heterozygous, HOM = homozygous.

Supplementary Table S4.2: Overview historical controls

Author/year	n UGT1A1 PM	Type of cancer	Treatment schedule	Irinotecan dose	Frequency
Rouits et al. 2004 [9]	7	Colorectal	IRIFUFOL FOLFIRI	85 mg/m^2 180 mg/m^2	q1w d1 q2w d1
Toffoli et al. 2006 [10]	22	Colorectal	FOLFIRI	180 mg/m^2	q2w d1
Kweekel et al. 2008 [11]	14	Colorectal	CAPIRI monotherapy	250 mg/m^2 350 mg/m^2	q3w d1 q3w d1
Parodi et al. 2008 [12]	11	Colorectal	(m)IFL FOLFIRI CAPIRI	125 mg/m^2 180 mg/m^2 250 mg/m^2	q3w d1d8 q2w d1 q3w d1
Braun et al. 2009 [13]	29	Colorectal	FOLFIRI monotherapy	180 mg/m^2 350 mg/m^2	q2w d1 q3w d1
McLeod et al. 2010[a] [14]	11	Colorectal	IROX	200 mg/m^2	q3w d1
Shulman et al. 2011 [15]	25	Colorectal	IFL FOLFIRI XELIRI TEGAFIRI	125 mg/m^2 180 mg/m^2 200 mg/m^2 250 mg/m^2	q6w d1d8d15d22 q2w d1 q3w d1 q3w d1
Liu et al. 2014 [16]	122	Colorectal	N.a.[b]	≥150 mg/m^2	n.a. [b]
Roncato et al. 2017[c] [17]	22	Colorectal	FOLFIRI	180 mg/m^2	q2w d1

[a] 1 cohort within this study was not included because the starting the starting dose of irinotecan was lowered during the trial due to excessive toxicity.
[b] Meta-analysis.
[c] Same cohort as Toffoli et al. 2006, additional information on hospitalisation.

Supplementary Table S4.3: Subgroup analysis

Table S4.3A: Treatment outcomes of irinotecan monotherapy versus combination therapy in UGT1A1 PM with reduced dose

	UGT1A1 PM	UGT1A1 PM	
	Monotherapy ± targeted agent (N=9)	Combination therapy (N=22)	P-value
Hematologic toxicity			
Febrile neutropenia in the first 2 cycles	1 (11%)	1 (5%)	0.50
Grade ≥3 neutropenia	5 (56%)	7 (32%)	0.25
Grade ≥4 neutropenia	2 (22%)	2 (9%)	0.56
Non-hematologic toxicity			
Grade ≥3 diarrhea	0 (0%)	3 (14%)	0.54
Chemotherapy-related hospital admissions	1 (11%)	3 (14%)	1.00

Data are provided as N (%).
Abbreviations: UGT1A1 PM = UGT1A1 poor metaboliser.

Table S4.3B: Treatment outcomes of UGT1A1 IM with full dose versus UGT1A1 EM with full dose

	UGT1A1 IM	UGT1A1 EM	
	(N=158)	(N=161)	P-value
Hematologic toxicity			
Febrile neutropenia in the first 2 cycles	8 (5%)	5 (3%)	0.38
Grade ≥3 neutropenia	35 (22%)	18 (11%)	<0.01
Grade ≥4 neutropenia	19 (12%)	9 (6%)	0.04
Non-hematologic toxicity			
Grade ≥3 diarrhea	31 (20%)	20 (12%)	0.08
Chemotherapy-related hospital admissions	41 (26%)	31 (19%)	0.15

Data are provided as N (%).
Abbreviations: UGT1A1 IM = UGT1A1 intermediate metaboliser, UGT1A1 EM = UGT1A1 extensive metaboliser.

Table S4.3C: Treatment outcomes of UGT1A1 PM with reduced dose versus UGT1A1 EM with full dose

	UGT1A1 PM	UGT1A1 EM	
	(N=31)	(N=161)	P-value
Hematologic toxicity			
Febrile neutropenia in the first 2 cycles	2 (7%)	5 (3%)	0.31
Grade ≥3 neutropenia	12 (39%)	18 (11%)	<0.01
Grade ≥4 neutropenia	4 (13%)	9 (6%)	0.23
Non-hematologic toxicity			
Grade ≥3 diarrhea	3 (10%)	20 (12%)	1.00
Chemotherapy-related hospital admissions	4 (13%)	31 (19%)	0.40

Data are provided as N (%).
Abbreviations: UGT1A1 PM = UGT1A1 poor metaboliser, UGT1A1 EM = UGT1A1 extensive metaboliser.

Table S4.3D: Treatment outcomes of UGT1A1 PM with reduced dose versus UGT1A1 IM with full dose

	UGT1A1 PM	UGT1A1 IM	
	(N=31)	(N=158)	P-value
Hematologic toxicity			
Febrile neutropenia in the first 2 cycles	2 (7%)	8 (5%)	0.75
Grade ≥3 neutropenia	12 (39%)	35 (22%)	0.05
Grade ≥4 neutropenia	4 (13%)	19 (12%)	1.00
Non-hematologic toxicity			
Grade ≥3 diarrhea	3 (10%)	31 (20%)	0.19
Chemotherapy-related hospital admissions	4 (13%)	41 (26%)	0.12

Data are provided as N (%).
Abbreviations: UGT1A1 PM = UGT1A1 poor metaboliser, UGT1A1 IM = UGT1A1 intermediate metaboliser.

Supplementary Table S4.4: Cost and probability parameters used in the cost analysis

Variable	Baseline value	One way-sensitivity range	Reference
Probabilities and other parameters			
Frequency UGT1A1 phenotype			
UGT1A1 IM/EM	0.903	0.722–1.084	This study + [18]
UGT1A1 PM	0.097	0.078–0.116	This study + [18]
Risk of grade ≥3 toxicity			
UGT1A1 IM/EM	0.351	0.281–0.421	This study
UGT1A1 PM, reduced dose	0.581	0.465–0.697	This study
UGT1A1 PM, standard dose	0.520	0.416–0.624	Historical controls [10, 11, 13]
UGT1A1 IM/EM			
Hospitalisation nursing ward	0.226	0.181–0.271	This study
Mean duration (days)	6.9	5.5–8.3	This study
UGT1A1 PM, reduced dose			
Hospitalisation nursing ward	0.129	0.103–0.155	This study
Mean duration (days)	6.0	4.8–7.2	This study
UGT1A1 PM, standard dose			
Hospitalisation nursing ward	0.415	0.332–0.498	Historical controls [9, 15, 17]
Mean duration (days)	8.0	6.4–9.6	Assumption
Mean number of cycles	6.2	Fixed	This study
Mean dose intensity for UGT1A1 PM	0.694	0.555–0.833	This study
Cost parameters (€)			
UGT1A1 genotyping costs	83	66–99	National Healthcare Authority [19]
Hospitalisation nursing ward (per day)	712	Fixed	Guideline [8]
Additional costs for interventions related to toxicity (except hospitalisation)			
Grade <3	87	Fixed	[20]
Grade ≥3	238	Fixed	[20]
Treatment costs irinotecan per cycle			
Irinotecan medication[a]	519	415–623	This study + price info drugs [21]
Administration at day care	309	Fixed	Guideline [8]
Medical doctor visit	148	Fixed	Guideline [8]

All prices are indexed to the year 2020.

[a] Based on a mean irinotecan dose of 333 mg per cycle as determined in this study.

Abbreviations: UGT1A1 PM = UGT1A1 poor metaboliser, UGT1A1 IM = UGT1A1 intermediate metaboliser, UGT1A1 EM = UGT1A1 extensive metaboliser.

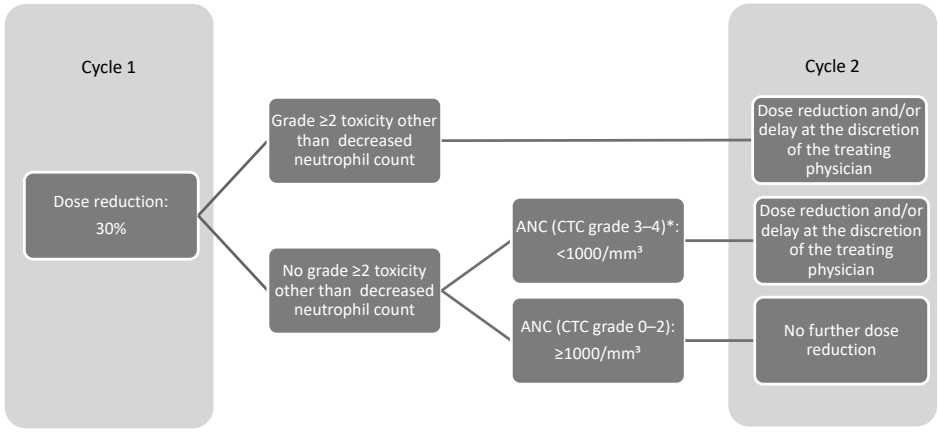
Supplementary Table S4.5: Costs of UGT1A1 genotype-guided dosing of irinotecan versus conventional dosing of irinotecan

Branch	Probability	Genotyping costs	Total costs	Expected costs
UGT1A1 genotyping				
A	0.097	83	5,887	573
B	0.903	83	7,373	6,656
Extra costs[a]	n.a.	n.a.	n.a.	3
Total costs	n.a.	n.a.	n.a.	7,232
No genotyping				
C	0.097	0.00	8,571	834
D	0.903	0.00	7,290	6,581
Total costs	n.a.	n.a.	n.a.	7,415
UGT1A1 genotyping versus no genotyping				-183[b]

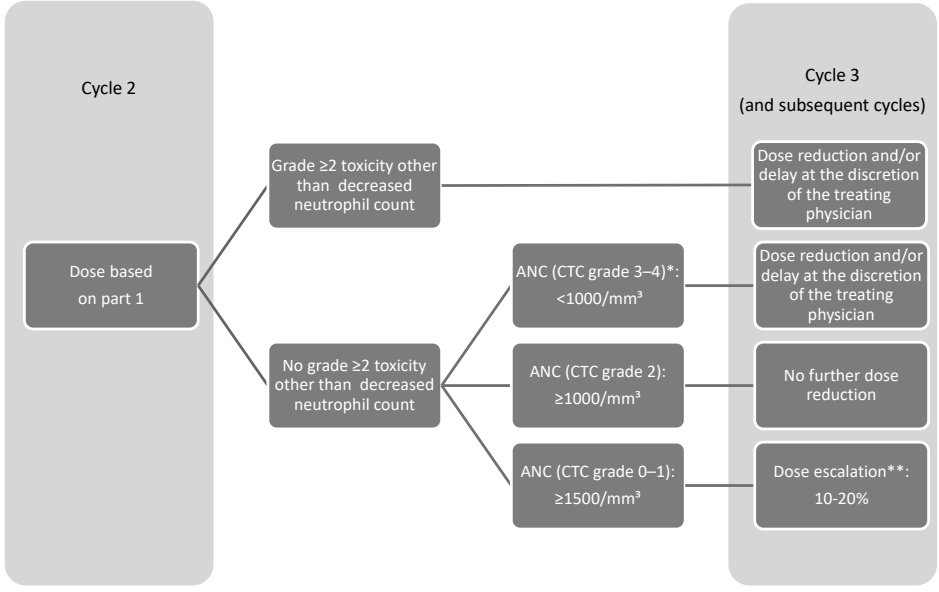
All costs are in €.

[a] Average costs per patient made for screening of patients that were screened, but not treated with irinotecan.

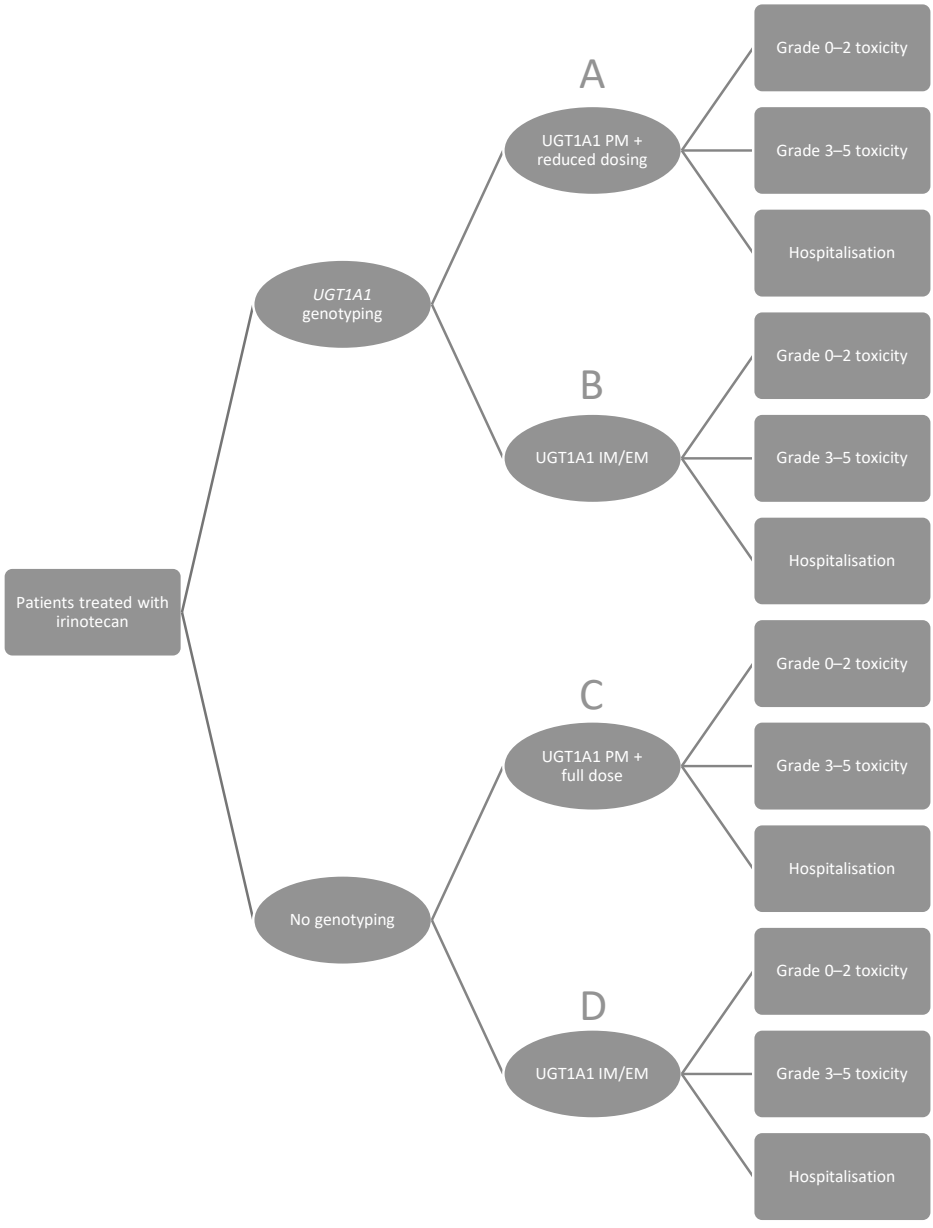
[b] Costs saved per patient by *UGT1A1* genotyping versus no genotyping.



Supplementary Figure S4.1: Genotype-guided and neutrophil-guided dosing nomogram UGT1A1 PMs – cycle 1 and 2.
* In case of febrile neutropenia a further 10–20% dose reduction in the next cycle is indicated.
Abbreviation: ANC = absolute neutrophil count.

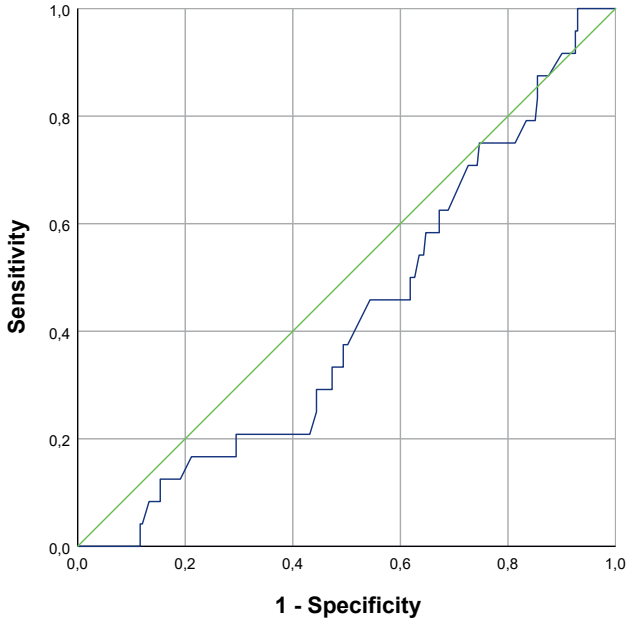


Supplementary Figure S4.2: Genotype-guided and neutrophil-guided dosing nomogram for UGT1A1 PMs – cycle 3 and subsequent cycles.
* In case of febrile neutropenia a further 10–20% dose reduction in the next cycle is indicated.
** Dose escalation up to a maximum of the indicated dose of 100%.
Abbreviation: ANC = absolute neutrophil count.



Supplementary Figure S4.3: Decision tree *UGT1A1* genotype-guided dosing of irinotecan used in the cost analysis.

Abbreviations: UGT1A1 PM = UGT1A1 poor metaboliser, UGT1A1 IM = UGT1A1 intermediate metaboliser, UGT1A1 EM = UGT1A1 extensive metaboliser.



Supplementary Figure S4.4: ROC curve of the conjugated bilirubin / unconjugated bilirubin concentration ratio as a marker for *UGT1A1* phenotype.

PART II

Discovery and validation of genetic biomarkers for hyperthermic intraperitoneal chemotherapy (HIPEC)

CHAPTER 5

Genetic variants in DNA repair pathways as potential biomarkers in predicting treatment outcome of intraperitoneal chemotherapy in patients with colorectal peritoneal metastasis: A systematic review

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ABSTRACT

Background

The introduction of cytoreductive surgery (CRS) followed by hyperthermic intraperitoneal chemotherapy (HIPEC) with either oxaliplatin or mitomycin C for patients with colorectal peritoneal metastasis (CPM) has resulted in a major increase in overall survival. Nonetheless, despite critical patient selection, the majority of patients will develop recurrent disease within one year following CRS+HIPEC. Therefore, improvement of patient and treatment selection is needed and may be achieved by the incorporation of genetic biomarkers. This systematic review aims to provide an overview of genetic biomarkers in the DNA repair pathway that are potentially predictive for treatment outcome of patients with colorectal peritoneal metastases treated with CRS+HIPEC with oxaliplatin or mitomycin C.

Methods

A systematic review was conducted according to the PRISMA guidelines. Given the limited number of genetic association studies of intraperitoneal mitomycin C and oxaliplatin in patients with CPM, we expanded the review and extrapolated the data from biomarker studies conducted in colorectal cancer patients treated with systemic mitomycin C and oxaliplatin-based chemotherapy

Results

In total, 43 papers were included in this review. No study reported potential pharmacogenomic biomarkers in patients with colorectal cancer undergoing mitomycin c-based chemotherapy. For oxaliplatin-based chemotherapy, a total of 26 genetic biomarkers within 14 genes were identified that were significantly associated with treatment outcome. The most promising genetic biomarkers were *ERCC1* rs11615, *XPC* rs1043953, *XPD* rs13181, *XPG* rs17655, *MNAT* rs3783819/ rs973063/rs4151330, MMR status, ATM protein expression, *HIC1* tandem repeat D17S5 and *PIN1* rs2233678.

Conclusion

Several genetic biomarkers have proven predictive value for the treatment outcome of systemically administered oxaliplatin. By extrapolation, these genetic biomarkers may also be predictive for the efficacy of intraperitoneal oxaliplatin. This should be the subject of further investigation

INTRODUCTION

Colorectal peritoneal metastasis (CPM) is associated with a poor prognosis and affects approximately 10–20% of colorectal cancer patients [1–4]. The introduction of cytoreductive surgery (CRS) followed by hyperthermic intraperitoneal chemotherapy (HIPEC) with either oxaliplatin or mitomycin C for patients with isolated CPM has led to a major increase in overall survival and even cure in up to 15% of patients [5, 6]. Therefore CRS + HIPEC is at present considered standard of care for patients with limited peritoneal metastases. Currently, patient selection for CRS + HIPEC is mainly based on the peritoneal carcinomatosis index (PCI) and performance status [7–9]. In addition, several clinical and pathological prognostic biomarkers have been identified, including completeness of cytoreduction, locoregional lymph node status and signet ring cell differentiation [10]. Nonetheless, despite critical patient selection, the majority of patients will develop recurrent disease within one year following CRS + HIPEC [11, 12]. In addition, post-operative surgical complications following CRS + HIPEC are frequent, including mortality in about 1–2% of patients [13].

Knowledge of genetic biomarkers that are predictive or prognostic for treatment outcome may be of additional value in patient and treatment selection, allowing further improvement of treatment outcome for the individual patient. In contrast to thousands of pharmacogenetic association studies that have been conducted in cancer patients treated with systemic chemotherapy, almost no data exist of genetic biomarkers in patients treated with intraperitoneal chemotherapy. Following intraperitoneal administration, oxaliplatin and mitomycin exert their anti-tumor effect locally at the tumor site. Both drugs share a comparable mechanism of action in that they both interfere with DNA synthesis and repair. Thereby, genetic variation in genes involved in DNA repair may reduce the functional activity of certain DNA repair genes, making tumor cells more susceptible for drug-induced DNA damage and hence increased drug efficacy [14, 15]. The DNA repair system is divided into six major DNA repair pathways, i.e. base-excision repair (BER), nucleotide-excision repair (NER), mismatch repair (MMR), homologous recombination (HR), nonhomologous end joining (NHEJ), and translesion DNA synthesis (TLS). In addition, pathways on damage response and DNA synthesis exist [15].

Notwithstanding the in general increasingly applied knowledge of genetic biomarkers in cancer therapy as a proven tool for patient and treatment selection, almost no predictive or prognostic data of genetic biomarkers for treatment outcome exist in patients with CPM treated with intraperitoneal chemotherapy. Therefore, we conducted a systematic review to provide an overview of genetic biomarkers in the DNA repair pathway that are potentially

predictive for treatment outcome of patients with colorectal peritoneal metastases treated with CRS + HIPEC with oxaliplatin or mitomycin C.

METHODS

A systematic literature review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [16].

Of the studies on the use of mitomycin C and oxaliplatin in HIPEC treatment, only two studies were found that have reported biomarkers related to DNA repair [17, 18]. Data obtained from genetic association studies conducted in other than CPM patients treated with oxaliplatin or mitomycin C may potentially be extrapolated to patients with CPM. Therefore, we expanded this review with studies investigating the association between genetic biomarkers related to DNA repair and treatment outcome in patients with colorectal cancer undergoing mitomycin C and oxaliplatin-based chemotherapy.

We searched PubMed until February 2020 without any limitations on publication year using the following search terms: “biomarker”, “oxaliplatin”, “mitomycin C”, “colorectal cancer” and “treatment outcome”. The full search string is provided in the supplementary material. In addition, reference lists in original articles and review articles were manually searched to identify additional potentially relevant publications. Literature was reviewed by two independent reviewers (LL and EH). In case of inconsistencies, results were discussed with a third reviewer (MD).

All publications were screened on title and abstract. Only studies that included patients with colorectal cancer were included and studies that were retracted, studies that did not provide original data or case-reports were excluded. The remaining publications were assessed based on screening of the full text. Only studies that reported on the association between genetic biomarkers related to DNA repair and treatment outcome undergoing mitomycin C- and oxaliplatin-based chemotherapy were included. To provide a total overview of the available evidence we included studies on various types of genetic biomarkers including genetic polymorphism, mRNA expression and protein expression. Treatment outcome had to be reported as overall survival (OS), progression-free survival (PFS), disease-free survival (DFS).

Risk of bias assessment was performed and adapted from the Q-genie tool and was based on the following bias items: clear phenotype and outcome definition, and correct nomenclature of genotype. We decided not to exclude studies because of small sample size, ethnic differ-

ences, differences in treatment regimens or type of biomarker, or no correction for covariates affecting treatment outcome due to scarcity of data.

All identified genetic biomarkers were subdivided into either one of the six described major DNA-repair pathways [19], i.e. NER, BER, MMR, HR, NHEJ, TLS, or otherwise into a category of genes involved in DNA damage response and DNA synthesis [15]. Results were summarized and presented per gene including a mechanistic background for the drug-gene interaction. The following information per study or genetic biomarker was reported: sample size, CRC type, treatment schedule, biomarker, type of sample, type of assay, rs number (if applicable), reference group and comparator group, and treatment outcome. Treatment outcomes were expressed as hazard ratios, relative risks or differences in median survival with 95% confidence intervals and p-values whichever was available.

The most promising genetic biomarkers were extracted from the results and summarized in a table. Evidence for these biomarkers had to meet the following 2 criteria: 1] no or almost none conflicting data and 2] an association with treatment outcome was reported in at least 2 studies or in one study with sufficient power (arbitrarily defined in this review as a minimum number of 300 patients), or the study included a control group with non-oxaliplatin based-chemotherapy in which no association or an association in the opposite direction was seen compared to the group with oxaliplatin-based chemotherapy.

RESULTS

Study selection

The search string in the PubMed database resulted in a total of 346 identified articles. **Figure 5.1** provides the selection procedure of relevant articles. An additional 17 studies were added that were identified from meta-analyses. After screening the title and abstract, 122 studies were excluded leaving 241 articles for further evaluation. After reviewing the full-text, 198 articles were excluded, resulting in a total of 43 studies that were included in this systematic review. The percent agreement between the two reviewers was 97% and Cohen's kappa was 0.87.

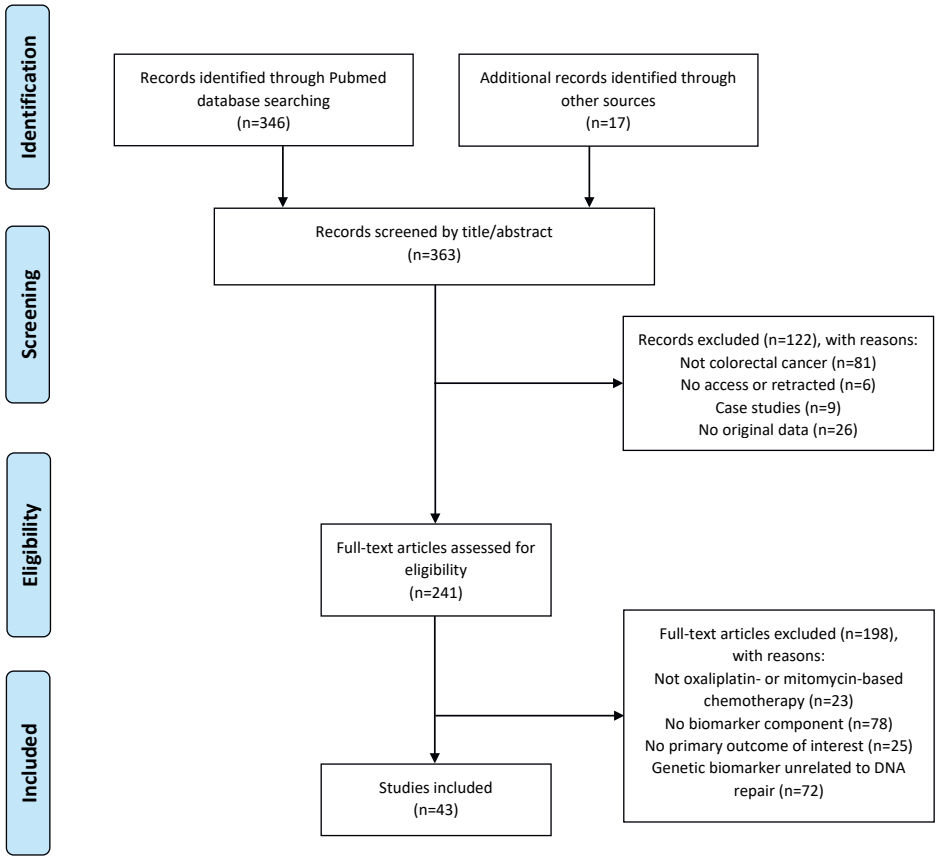

Figure 5.1: Flow chart of selection procedure literature.

Main results

The identified potential genetic biomarkers for treatment outcome of oxaliplatin-based chemotherapy could be divided over 4 out of the 6 major DNA-repair pathways, i.e. NER, BER, MMR and HR or were involved in DNA damage response or DNA synthesis, respectively. No studies were identified that reported on the association between genetic biomarkers and treatment outcome of mitomycin C-based chemotherapy in CRC patients. From all eligible studies, a total of 26 genetic biomarkers within 14 genes were identified in which at least one study had reported a significant association with treatment outcome. The most promising genetic biomarkers belonged to the NER, MMR or DNA damage response pathway and are summarized in **Table 5.1** and explained in more detail below; in contrast to biomarkers that belong to the BER, HR or DNA synthesis pathway which seem less promising due to lack of evidence or conflicting results The results from all included studies are summarized in

Figure 5.2, discussed per gene below and reported in detail in the **Supplementary material – Table S5.1-S5.10**.

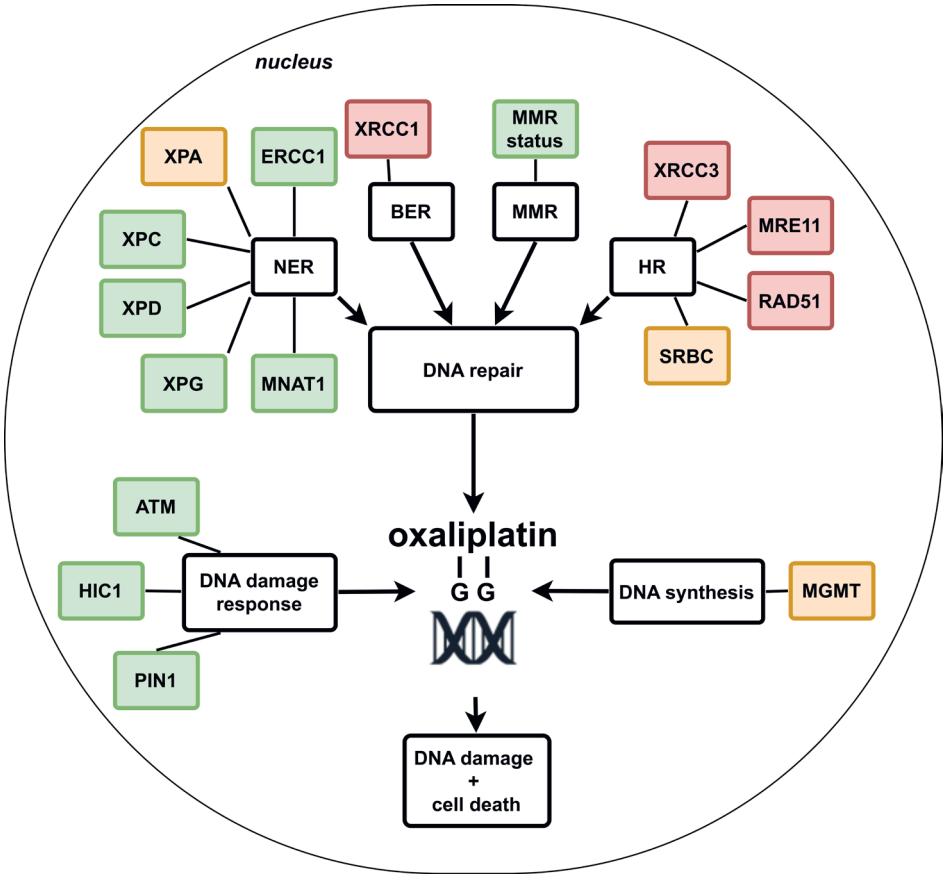

Figure 5.2: Schematic overview of potential genetic biomarkers within DNA repair pathways for treatment outcome of systemic oxaliplatin in colorectal cancer patients.
Green: no or almost none conflicting results and significant association with treatment outcome in ≥2 studies, or in 1 study with a sample size of ≥300, or inclusion of a non-oxaliplatin-based chemotherapy control group in which no association or an association in the opposite direction was seen compared to the group with oxaliplatin-based chemotherapy.
Orange: significant association with treatment outcome in 1 study.
Red: conflicting results or no significant association with treatment outcome.

NER pathway

ERCC1

Oxaliplatin DNA-adducts are mainly removed by the NER pathway [20].Excision repair cross-complementation group 1 (ERCC1) is a key protein in the NER pathway that is encoded by the *ERCC1* gene. Together with xeroderma pigmentosum complementation group F (XPF),

ERCC1 forms a heterodimer complex that can incise damaged DNA strands at the 5' side of the lesion [21]. In addition to their involvement in the NER pathway, the XPF/ERCC1 complex is also involved in double strand break repair (DSBR) [22]. Therefore, the expression of *ERCC1* is potentially associated with treatment outcome of oxaliplatin in CRC patients.

In two preclinical studies, elevated ERCC1 protein level was suggested to correlate with oxaliplatin-resistance in cells [23, 24]. Alteration in single nucleotide polymorphisms (SNPs) are expected to have an effect in gene expression level and function. Several *ERCC1* SNPs have been evaluated for their association with treatment outcome of oxaliplatin in CRC patients (**Supplementary material – Table S5.1**). The most commonly investigated nucleotide polymorphism is rs11615 [25–42]. A total of 10 studies showed a significant association between this polymorphism and treatment outcome [25–27, 31, 33–36, 39, 40]. Most studies, six out of 10, reported the mutant CC genotype to be the favorable genotype, with significantly better DFS, PFS, and OS [25, 27, 31, 35, 36, 39]. However, a few studies showed contradictory results. Three studies [26, 33, 34] reported that patients with the CC genotype had a worse treatment outcome in terms of PFS and OS. Another contradicting result was reported by Ruzzo et al. [40] where the rs11615 TT genotype was associated with prolonged PFS in univariate analysis and shorter PFS in multivariate analyses.

Two other reported polymorphisms of *ERCC1* are at codon 259 and 504 [38, 43]. Both polymorphisms showed no significant association with the treatment outcome. Moreover, 2 [26, 44] out of 5 [45–47] studies based on mRNA or protein expression level of ERCC1 showed a significant association between low ERCC1 expression and prolonged PFS and OS.

XPC

Xeroderma pigmentosum group C (XPC), located at chromosome 3p25, encodes for another important protein in the early steps of the NER pathway. XPC binds to RAD23B to form the heterodimeric complex, which is the first NER factor to facilitate the recognition of DNA damage and the initiation of DNA repair [48]. As DNA damage recognition is the rate-limiting step in the NER pathway, the XPC protein plays a critical role in proper DNA repair. Therefore, genetic biomarkers in *XPC* may have potential value in predicting response for oxaliplatin-based chemotherapy.

In **Table S5.3** (Supplementary material), three SNPs in the *XPC* gene that are potentially predictive of treatment response to oxaliplatin-based therapy in CRC patients are reported [49–51]. Only one SNP was significantly associated with survival. In the study by Kap et al. [50], patients carrying the variant allele rs1043953 had a longer OS after treatment with

oxaliplatin-based chemotherapy compared to non-carriers after adjusting for multiple testing, while the opposite association was found in patients who were treated with non-oxaliplatin based-chemotherapy.

XPD/ERCC2

The *xeroderma pigmentosum group D (XPD)*, or *excision repair cross complementation group 2* (*ERCC2*) gene, encodes for a helicase protein of 761 amino acids located on chromosome 19q13.3 [52]. The XPD protein is a part of the general transcription factor IIH complex, which is involved in the NER pathway by opening DNA double helix after damage recognition by XPC-RAD23B [53]. SNPs in *XPD* gene can alter the efficiency of DNA repair capacity and could thus be used as a predictive factor for oxaliplatin-based chemotherapy.

SNPs affecting codons 156, 312, and 751 (rs238406, rs1799793 and rs13181, respectively) proved to be extensively studied for their predictive value in CRC treatment (**Supplementary material – Table S5.4**). *XPD* rs238406 SNP was significantly associated with treatment outcome in one [54] out of three studies [31, 55]. The second SNP, rs1799793, was also significantly associated with treatment outcome in one [56] out of three studies [40, 55]. The wild type GG genotype seemed to be the favorable genotype. Sixteen studies assessed the predictive value of *XPD* rs13181polymorphism. In most studies a worse treatment outcome was observed in C allele carriers [31, 33, 36, 40, 42, 55, 57, 58]. Le Morvan et al. compared oxaliplatin treatment with irinotecan treatment and reported that the CC genotype was associated with a lower OS in patients treated with oxaliplatin, in contrast this was not observed in the same patient category treated with irinotecan [57]. However, the opposite association was observed in three studies [27, 28, 59], and five studies did not find significant associations with treatment outcome [25, 34, 41, 43, 60]. Lastly, one study assessed mRNA expression level of *XPD* for its association with treatment outcome, no significant association was observed [44].

XPG/ERCC5

The *xeroderma pigmentosum group G* (*XPG*) gene, also known as *ERCC5* (*Excision repair cross complementation group 5*), is one of the eight core functional genes in the NER pathway. The *XPG* gene, located at chromosome 13q32-33, encodes for a structure specific endonuclease protein that cleaves the 3' side of the damaged nucleotide during NER [61]. The low expression level of *XPG* has been shown to be associated with response to platinum-based chemotherapy in ovarian cancer [62, 63].

Four studies reported on the association between four different SNPs in the *XPG* gene and treatment outcome of oxaliplatin-based chemotherapy in CRC patients (**Supplementary material – Table S5.5**). The -763A>G and +25A>G polymorphisms in the promoter region of *ERCC5* were significantly associated with PFS and OS in patients treated with oxaliplatin [64]. Also, SNPs in rs1047768 and rs17655 were significantly associated with treatment outcome [43, 49, 65].

MNAT1

The *MNAT1* gene encodes for the ménage à trois-1 (MAT1) enzyme that is involved in the assembly of the cyclin dependent kinase-activating kinase (CAK) complex. Together with XPD and other subunits, the CAK-complex forms the TFIIH complex that is involved in the NER pathway [66].

Kap et al. [50] found three predictive SNPs, rs3783819, rs973063 and rs4151330 of the *MNAT1* gene for OS in CRC patients treated with oxaliplatin-based chemotherapy compared to CRC patients with non-oxaliplatin based chemotherapy (**Supplementary material – Table S5.6**). All three SNPs are in high linkage disequilibrium and p-values were corrected for multiple testing. Compared to non-carriers, carriership of these genetic variants was associated with longer OS, but not in patients who received non-oxaliplatin-based chemotherapy.

MMR pathway

MMR status

The DNA mismatch repair (MMR) system recognizes and repairs genetic mismatches that occur during DNA replication and DNA damage. MMR status is defined as deficient (dMMR) when one or more MMR protein (*MLH1*, *MSH2*, *PMS2*, and *MSH6*) expression is lost [67]. Germline mutations in MMR genes were found to be the driving mechanism for Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer (HNPCC) [68]. A defective MMR system will result in DNA replication errors, particularly in the short tandem repeat of DNA sequences of the genome referred to as microsatellites, which may lead to microsatellite instability (MSI). It has been suggested that MSI positively affects the clinical outcome of CRC. Mechanistically, oxaliplatin treatment is expected to be more effective in patients with defective MMR protein status as platinum adducts formed by oxaliplatin cannot be repaired.

A total of three studies, evaluating the predictive ability of MMR status in relation to oxaliplatin-based treatment, are included in **Table S5.9** (Supplementary material). In two out of three studies, OS was significantly higher in multivariate analysis in dMMR patients treated with

oxaliplatin-based therapy [46, 69]. In contrast, Kim et al. did not find an association between dMMR and treatment outcome of oxaliplatin-based chemotherapy [70].

DNA damage response

ATM

Ataxia telangiectasia mutated (ATM) is a serine/threonine protein kinase that is recruited and activated by the MRN complex during DNA DSBR [71]. The activation of the *ATM* gene leads to the phosphorylation of several key proteins that mediates the effect of ATM protein on DNA repair, cell cycle arrest or apoptosis [72]. Loss of *ATM* in preclinical models seems to increase sensitivity to DNA damaging agents, including platinum-based chemotherapy and ATM inhibitors [73].

Two studies reported a significant association of *ATM* with treatment outcome of oxaliplatin in CRC patients (**Supplementary material – Table S5.10**) [65, 74]. Sundar et al. [74] reported that loss of ATM protein expression in CRC resulted in favorable OS when treated with first line oxaliplatin chemotherapy (49 vs. 32 months; HR: 2.52 [1.00–6.37]). Important to note, loss of ATM expression did not result in favorable OS among patients treated with first line irinotecan-based therapy (24 vs. 33 months; HR: 0.72 [0.28–1.84]). In addition, the explorative study by Kweekel et al. [65] found a significantly shorter PFS for homozygous carriers of the *ATM* rs1801516 SNP, for OS no differences were found.

HIC1

The hypermethylated in cancer 1 (HIC1) protein plays an important role in the DNA repair through its direct binding to the Sirtuin 1 (SIRT1) promoter, thereby suppressing its transcription. SIRT1 is a deacetylase of XPA protein, a component of the NER pathway [75]. Since the variable number of tandem repeats near the promoter lesion of HIC1, which is associated with *HIC1* gene expression, there is a potential value of *HIC1* as a predictive biomarker for oxaliplatin efficacy.

In a study by Okazaki et al. [76], shown in **Table S5.10** (Supplementary material), patients treated with oxaliplatin-based chemotherapy with at least 5 tandem repeats of *HIC1*, in both alleles of the *HIC1* promoter region, had a significantly shorter PFS. In a control group who received irinotecan-based chemotherapy this difference in PFS was not seen. However, no significant association with OS was found.

PIN1

Peptidyl-prolyl cis/trans isomerase NIMA-interacting 1 (PIN1) is an enzyme encoded by the *PIN1* gene. It interacts with prominent DSBR factors and is involved in the regulation of HR and non-homologous end-joining (NHEJ) of DNA DSBR. Previous study showed that the overexpression of PIN1 suppresses HR and its depletion reduces NHEJ, by promoting CtIP polyubiquitylation and subsequent proteasomal degradation [77].

A study by Suenaga et al. [78], shown in **Table S5.10** (Supplementary material), reported that genetic polymorphism in *PIN1* was associated with treatment outcome of oxaliplatin. Patients treated with oxaliplatin-based chemotherapy carrying the *PIN1* rs2233678 C allele had a shorter PFS and OS compared to wild type patients. For OS this was replicated in a validation cohort. In contrast, in a control group treated with non-oxaliplatin-based chemotherapy patients with a C allele had longer median PFS than wild type patients.

Miscellaneous

Following our selection criteria, for XPA in the NER pathway [31, 43, 51], SRBC in the HR pathway [79] and MGMT in the DNA synthesis pathway [80] results remain inconclusive because the observed associations have not yet been replicated and the studies itself were relatively small (<300 patients).

For XRCC1 in the BER pathway a total of nine studies were identified that assessed the association between the *XRCC1* gene and treatment outcome of oxaliplatin-based chemotherapy in CRC patients, and showed conflicting results [25, 28, 30, 32, 34, 39, 59, 81]. All nine studies investigated the *1196A>G* polymorphism, and three studies showed a significant association [25, 59, 81]. However, two out of three studies [25, 81] found a significantly longer OS for the GG genotype, whereas the other study [59] a longer OS for the AA genotype.

For XRCC3 [34, 40], MRE11 [82] and RAD51 [82] in the HR pathway no significant associations with treatment outcome were reported.

DISCUSSION

The majority of patients with peritoneal metastases of colorectal cancer treated with CRS + HIPEC will develop recurrent disease despite critical patient selection. Therefore, improvement of patient and treatment selection is needed and further investigation of genetic biomarkers that are predictive or prognostic for treatment outcome may be of aid herein. We conducted

a systematic review to provide an overview of genetic biomarkers in the DNA repair pathway that are potentially predictive for treatment outcome of patients with colorectal peritoneal metastases treated with CRS + HIPEC with oxaliplatin or mitomycin C.

We expanded our review with studies investigating the association between genetic biomarkers related to DNA repair and treatment outcome in patients with colorectal cancer undergoing systemic chemotherapy, because only two studies could be retrieved that investigated the association of biomarkers related to DNA repair and intraperitoneally administered mitomycin C or oxaliplatin. The most promising genetic biomarkers were *ERCC1* rs11615, *XPC* rs1043953, *XPD* rs13181, *XPG* rs17655, *MNAT* rs3783819/ rs973063/rs4151330, MMR status, ATM protein expression, *HIC1* tandem repeat D17S5 and *PIN1* rs2233678. Combination studies of two DNA repair genes have also been studied and showed significant associations with treatment outcome.

Our findings for *ERCC1* rs11615 and *XPD* rs13181 are supported in 4 meta-analyses [83–86]. The other biomarkers have not been studied as extensively. To our knowledge the current review is the first to summarize the available evidence for these markers.

Our results showed that genetic biomarkers in the DNA repair pathway seem of added value in predicting oxaliplatin treatment outcome in colorectal cancer patients. Since the mechanism of action of oxaliplatin is irrespective of the route of administration, it is assumed very reasonable to extrapolate these associations to patients with colorectal peritoneal metastases treated with CRS + HIPEC. In our opinion, single genetic biomarkers within DNA repair should be incorporated into a polygenic risk profile because the effect of a single gene polymorphism may be partially overcome by compensation mechanisms. Comparable to the study by Kap et al., in which the predictive value of the model significantly improved by including more genetic variants [50]. Moreover, besides DNA repair, other pathways may also be of relevance in predicting treatment outcome, such as genetic variation in pharmacokinetic genes [87].

For some genetic biomarkers conflicting results were reported. This might partially be explained by ethnic discrepancy as has been suggested [83, 86]. In addition, studies with small sample sizes and differences in treatment regimens between studies may also attribute to these conflicting results. However, for the selection of the most promising genetic biomarkers we only selected biomarkers for which no or almost none conflicting data existed and results had to be replicated in at least 2 studies or in one study with sufficient power (>300 patients) or the study had to have a control group with non-oxaliplatin based chemotherapy.

Moreover, genetic variants in the DNA repair pathway seem to affect cancer susceptibility, prognosis and treatment outcome [88]. Therefore, it is difficult to distinguish between prognostic effects of these genetic variants or predictive effects on treatment outcome of oxaliplatin. To differentiate between these prognostic effects and predictive effects, a control group consisting of a patient cohort treated with non-oxaliplatin based chemotherapy should be added. Most of the studies that were included had no control group. Nonetheless, the studies that did include a control group with non-oxaliplatin based-chemotherapy did find differences in the association between the genetic biomarker (*XPC* rs1043953, *XPD* rs13181, *MNAT* rs3783819/ rs973063/rs4151330, ATM protein expression, *HIC1* tandem repeat D17S5 and *PIN1* rs2233678) and treatment outcome of oxaliplatin-based chemotherapy and non-oxaliplatin based-chemotherapy, thereby suggesting these biomarkers to be more likely predictive than prognostic.

In addition, we included various types of biomarkers such as genetic polymorphism, mRNA expression and protein expression, these are quite different assays and normally we would not pile together these various types of biomarkers. However, our aim was to give a complete overview of all genetic biomarkers, in order to provide a selection of potential promising genetic biomarkers for further research.

As data scarcity and sparsity was encountered we decided to expand our search from intraperitoneal chemotherapy to systemic chemotherapy. No formal search in other databases than PubMed was conducted, since it was assumed that the majority of relevant literature was identified using this database. However, this might be considered a limitation of our study. Moreover, the addition of grey literature could have been of added value in terms of data scarcity and publication bias. Nonetheless, grey literature is mostly not peer-reviewed and not always traceable. In addition, the quality of data could potentially be improved by applying a standardized tool for the risk of bias assessment. However, as described in the methods section, a customized assessment of bias was performed which was mainly based on the Q-genie tool.

Lastly, not all studies corrected for additional covariates affecting treatment outcome such as clinical, molecular and pathological patient and tumor characteristics. This might have influenced the effect of the genetic biomarkers on treatment outcome. Therefore, additional prospective research including a multivariate analysis is needed, especially in patients with colorectal peritoneal metastases treated with CRS + HIPEC as literature is scarce in this population.

In this review, several genetic biomarkers in the DNA repair pathway were identified that showed promise for predicting outcome in colorectal cancer patients treated with oxaliplatin. These findings might be extrapolated to patients with colorectal peritoneal metastases treated with CRS + HIPEC and should be the subject of further investigation.

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SUPPLEMENTARY METHODS

Search string

((("Oxaliplatin"[majr] OR "oxaliplatin"[ti] OR oxaliplatin*[ti] OR "1,2-Diamminocyclohexane(trans-1)oxolatoplatinum(II)"[ti] OR "Oxaliplatine"[ti] OR "Eloxatine"[ti] OR "Eloxatin"[ti] OR "ACT 078"[ti] OR "ACT-078"[ti] OR "Mitomycin"[majr] OR "mitomycin C"[ti] OR Mitomycin*[ti] OR "ametycine"[ti] OR "mutamycin"[ti] OR "MMC"[ti] OR "hyperthermic intraperitoneal chemotherapy"[ti] OR "hyperthermic intraperitoneal"[ti] OR "hyperthermic intra peritoneal chemotherapy"[ti] OR "hyperthermic intra peritoneal"[ti] OR "HIPEC"[ti]) **AND** ("Genetic Markers"[Mesh] OR "Genetic Marker"[tw] OR "Genetic Markers"[tw] OR "Genetic Biomarker"[tw] OR "Genetic Biomarkers"[tw] OR "DNA Markers"[tw] OR "DNA Marker"[tw] OR "Chromosome Markers"[tw] OR "Chromosome Marker"[tw] OR "Pharmacogenetics"[mesh] OR "pharmacogenetics"[tw] OR pharmacogenetic*[tw] OR "pharmacogenomics"[tw] OR pharmacogenom*[tw] OR "Precision Medicine"[mesh] OR "precision medicine"[tw] OR "individualized"[tw] OR "personalized"[tw] OR "individualised"[tw] OR "personalised"[tw] OR "Polymorphism, Single Nucleotide"[mesh] OR "Single Nucleotide Polymorphism"[tw] OR "Single Nucleotide Polymorphisms"[tw] OR "SNPS"[tw] OR "SNP"[tw] OR "Polymorphism, Genetic"[mesh] OR Polymorphism*[tw] OR "Genetic Markers"[mesh] OR "Genetic Marker"[tw] OR "Genetic Markers"[tw] OR "Genetic Biomarker"[tw] OR "Genetic Biomarkers"[tw] OR "Genes"[mesh] OR "Gene"[tw] OR "Genes"[tw] OR "Mutation"[mesh] OR "Mutation"[tw] OR "Mutations"[tw] OR "DNA Damage"[mesh] OR "DNA Damage"[tw]) **AND** ("Treatment Outcome"[mesh] OR "outcome"[tw] OR "outcomes"[tw])) **OR** ((("Oxaliplatin"[majr] OR "oxaliplatin"[ti] OR oxaliplatin*[ti] OR "1,2-Diamminocyclohexane(trans-1)oxolatoplatinum(II)"[ti] OR "Oxaliplatine"[ti] OR "Eloxatine"[ti] OR "Eloxatin"[ti] OR "ACT 078"[ti] OR "ACT-078"[ti] OR "Mitomycin"[majr] OR "mitomycin C"[ti] OR Mitomycin*[ti] OR "ametycine"[ti] OR "mutamycin"[ti] OR "MMC"[ti] OR "hyperthermic intraperitoneal chemotherapy"[ti] OR "hyperthermic intraperitoneal"[ti] OR "hyperthermic intra peritoneal chemotherapy"[ti] OR "hyperthermic intra peritoneal"[ti] OR "HIPEC"[ti]) **AND** ("Genetic Markers"[majr] OR "Genetic Marker"[ti] OR "Genetic Markers"[ti] OR "Genetic Biomarker"[ti] OR "Genetic Biomarkers"[ti] OR "DNA Markers"[ti] OR "DNA Marker"[ti] OR "Chromosome Markers"[ti] OR "Chromosome Marker"[ti] OR "Pharmacogenetics"[majr] OR "pharmacogenetics"[ti] OR pharmacogenetic*[ti] OR "pharmacogenomics"[ti] OR pharmacogenom*[ti] OR "Precision Medicine"[majr] OR "precision medicine"[ti] OR "individualized"[ti] OR "personalized"[ti] OR "individualised"[ti] OR "personalised"[ti] OR "Polymorphism, Single Nucleotide"[majr] OR "Single Nucleotide Polymorphism"[ti] OR "Single

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Supplementary Table S5.1: Overview of studies on the association between ERCC1 biomarkers and treatment outcome of oxaliplatin-based chemotherapy in CRC patients

									Univariate analysis			Multivariate analysis		
Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	PFS	DFS	OS	PFS	DFS	OS
Rao et al., 2019 [26]	24	Stage II–III mCRC	CAPOX or FOLFOX	ERCC1-118	n.a.	Blood	Protein expression	**Underexpression +normal (11)** overexpression (13)	HR=2.35 (95% CI: 1.00–5.48) p=0.02					
Kassem et al., 2017 [44]	65	Stage III–IV	CAPOX or FOLFOX-4	*ERCC1*	n.a.	Tumor tissue	mRNA expression	**Low (50)** High (15)			HR=2.80 (95% CI: 1.27–6.21) p=0.01			
Basso et al., 2013 [45]	60	mCRC	FOLFOX-6	*ERCC1*	n.a.	Normal and tumor tissue	mRNA expression	**Overexpression (30)** Underexpression (30)	HR=1.09 (95% CI: 0.63–1.95) p=0.71					
Sfakianaki et al., 2019 [46]	246	Stage II–III	CAPOX or FOLFOX	*ERCC1*	n.a.	Tumor tissue	mRNA expression	**Low (118)** High (128)		HR=1.16 (95% CI: 0.61–1.57) p=0.93	HR=1.00 (95% CI: 0.56–1.80) p=0.99			
Li et al., 2014 [47]	112	Stage II–III	FOLFOX-4 or mFOLFOX or CAPOX	*ERCC1*	n.a.	Tumor tissue	mRNA expression	**Low (-)** High (-)		HR=1.05 (95% CI: 0.85–1.30) p=0.64				
Monzo et al., 2007 [43]	42	aCRC	CAPOX	ERCC1-Lys259Thr *c.776A>C*	rs735482	Blood	Polymorphism	A/A (33) A/C (4) + C/C (5)			14.4 mo vs 30.0 mo p=0.55			

Supplementary Table S5.1: ***Continued***

Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	Univariate analysis			Multivariate analysis		
									PFS	DFS	OS	PFS	DFS	OS
Huang et al., 2011 [25]	157	mCRC	FOLFOX-4	ERCC1-Asn118= *c.354T>C*	rs11615	Blood	Polymorphism	**T/T (19)** C/T (58 C/C (80)	C/C HR=0.06 (95% CI: 0.01–0.27) p<0.01 C/T HR=0.48 (95% CI: 0.13–1.74) p=0.26		C/C HR=0.07 (95% CI: 0.01–0.38) p<0.01 C/T HR=0.39 (95% CI: 0.08–1.89) p=0.25			
Rao et al., 2019 [26]	97	Stage II–III mCRC	CAPOX or FOLFOX	ERCC1-Asn118= *c.354T>C*	rs11615	Tumor tissue	Polymorphism	C/C (42) T/C (40) T/T (15)	211 days vs 196 days vs 590 days p=0.03					
Li et al., 2012 [27]	335	aCRC	FOLFOX-6	ERCC1-Asn118= *c.354T>C*	rs11615	Blood	Polymorphism	**T/T (166)** T/C (140) C/C (29)			T/C HR=0.87 (95% CI: 0.60–1.26) C/C HR= 0.22 (95% CI: 0.12–0.81)			T/C HR=0.81 (95% CI: 0.52–1.14) p=0.16 C/C HR=0.20 (95% CI: 0.10–0.79) p<0.05
Lamas et al., 2011 [28]	72	aCRC	mFOLFOX-6	ERCC1-Asn118= *c.354T>C*	rs11615	Blood	Polymorphism	C/C C/T T/T	9 mo vs 10 mo vs 10 mo p=1.0					

Supplementary Table S5.1 continues on next page.

Supplementary Table S5.1: ***Continued***

Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	Univariate analysis			Multivariate analysis		
									PFS	DFS	OS	PFS	DFS	OS
van Huis-Tanja et al., 2014 [29]	145	aCRC	CAPOX	ERCC1-Asn118= *c.354T>C*	rs11615	Blood	Polymorphism	**T/T (59)** C/T (72) C/C (14)	4.2 mo vs 4.2 mo vs 4.5 mo p=0.19		10.0 mo vs 12.1 mo vs 10.8 mo p=0.19			
Zaanan et al., 2014 [30]	202	Stage III	FOLFOX-4 or FOLFOX-6	ERCC1-Asn118= *c.354T>C*	rs11615	Tumor tissue	Polymorphism	**C/C (49)** C/T (88) + T/T (65)		HR=2.29 (95% CI: 0.97–5.41) p= 0.06				
Stoehlmacher et al., 2004 [31]	106	mCRC	FUOX	ERCC1-Asn118= *c.354T>C*	rs11615	Blood	Polymorphism	**C/C (30)** C/T (45) T/T (31)	C/T RR=1.24 (95% CI: 0.73– 2.11) T/T RR=1.36 (95% CI: 0.76–2.41) p=0.51		C/T RR=2.29 (95% CI: 1.19–4.41) T/T RR=1.86 (95% CI: 0.91–3.83) p=0.02			C/T + T/T RR=2.05 (95% CI: 1.00–4.20) p=0.04
Liang et al., 2010 [32]	113	mCRC	mFOLFOX-4 or CAPOX	ERCC1-Asn118= *c.354T>C*	rs11615	Blood	Polymorphism	**C/C (55)** C/T (43) T/T (15)						C/T HR=1.46 (95% CI: 0.94–2.27) p=0.10 T/T HR=1.66 (95% CI: 0.91–3.01) p=0.10

Supplementary Table S5.1: ***Continued***

Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	Univariate analysis			Multivariate analysis		
									PFS	DFS	OS	PFS	DFS	OS
Paré et al., 2008 [33]	106	mCRC	FOLFOX	ERCC1-Asn118= *c.354T>C*	rs11615	Leukocytes	Polymorphism	**T/T (42) + C/T** (52) C/C (24)	10 mo vs 6 mo p<0.001		30 mo vs 11 mo p<0.01			RR=1.8 (CI 95%: 1.1–3.0) p=0.02
Martinez-Balibrea et al., 2008 [34]	47	mCRC	XELOX	ERCC1-Asn118= *c.354T>C*	rs11615	Blood	Polymorphism	**T/T (18)** C/T + C/C (29)	HR=1.13 (95% CI: 0.57–2.24) p=0.74					
Martinez-Balibrea et al., 2008 [34]	49	mCRC	FUOX	ERCC1-Asn118= *c.354T>C*	rs11615	Blood	Polymorphism	**T/T (21)** T/C + C/C (28)	HR=1.96 (95% CI: 0.99–3.92) p=0.05			HR=2.12 (95% CI: 1.05–4.28) p=0.04		
Chang et al., 2009 [35]	168	mCRC	FOLFOX-4	ERCC1-Asn118= *c.354T>C*	rs11615	Blood	Polymorphism	**T/T (21) + C/T** (67) C/C (80)	7 vs 13 mo p<0.01		16 mo vs 25 mo p<0.01			
Chen et al., 2010 [36]	166	mCRC	FOLFOX-4	ERCC1-Asn118= *c.354T>C*	rs11615	Blood	Polymorphism	**C/C (78)** C /T + T/T (88)						HR=3.15 (95% CI: 1.89–5.23) p<0.01
Nishina et al., 2013 [38]	68	aCRC and/or recurrent CRC	mFOLFOX-6 + bevacizumab	ERCC1-Asn118= c.354T>C	rs11615	Blood	Polymorphism	**C/C (29)** C/T + T/T (39)	13.5 mo vs 12.6 mo HR=1.08 p=0.80					

Supplementary Table S5.1 continues on next page.

Supplementary Table S5.1: ***Continued***

Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	Univariate analysis			Multivariate analysis		
									PFS	DFS	OS	PFS	DFS	OS
Chua et al., 2009 [39]	115	mCRC	FOLFOX	ERCC1-Asn118= *c.354T>C*	rs11615	Tumor tissue	Polymorphism	**C/C (10)** C/T (64) T/T (41)	C/T HR=2.68 (95% CI: 1.15–6.23) p=0.02 T/T HR=2.54 (95% CI: 1.07–6.04) p=0.04 C/T + T/T HR=2.62 (95% CI: 1.14–6.02) p=0.02		C/T HR=1.88 (95% CI: 0.75–4.71) p=0.20 T/T HR=1.55 (95% CI: 0.60–4.00) p=0.40 C/T + T/T HR=1.74 (95% CI: 0.70–4.30) p=0.20	C/T + T/T HR=2.16 (95% CI: 0.94–4.97) p=0.07		
Ruzzo et al., 2007 [40]	166	mCRC	FOLFOX-4	ERCC1-Asn118= *c.354T>C*	rs11615	Blood	Polymorphism	**C/C (31)** C/T (85) T/T (50)	C/T HR=1.23 (95% CI: 0.78–1.94) p=0.27 T/T HR=0.53 (95% CI: 1.51–4.25) p<0.01			C/T HR=1.32 (95% CI: 0.78–2.24) p=0.29 T/T HR=2.34 (95% CI: 1.28–4.27) p<0.01		

Supplementary Table S5.1: ***Continued***

Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	Univariate analysis			Multivariate analysis		
									PFS	DFS	OS	PFS	DFS	OS
Sarasqueta et al., 2011 [41]	48	Stage III	CAPOX or FOLFOX	ERCC1-Asn118= *c.354T>C*	rs11615	Normal tissue	Polymorphism	**T/T -** T/C - C/C -					T/C HR=0.67 (95% CI: 0.23–1.89) p=0.45 C/C HR=0.94 (95% CI: 0.26–3.36) p=0.92	
Kumamoto et al., 2013 [42]	63	n.s.	mFOLFOX-6	ERCC1-Asn118= *c.354T>C*	rs11615	Blood	Polymorphism	**C/C (30)** C/T (23) T/T (10)	9.9 mo vs 8.1 mo vs 8.3 mo p=0.63		27.4 mo vs 22.5 mo vs 32.9 mo p=0.38			
Nishina et al., 2013 [38]	68	aCRC and/or recurrent CRC	mFOLFOX-6 + bevacizumab	ERCC1-Gln504Lys *c.1516C>A*	rs3212986	Blood	Polymorphism	**C/C (41)** C/A + A/A (27)	13.8 mo vs 12.6 mo HR=1.18 p=0.71					
Huang et al., 2011 [25]	157	mCRC	FOLFOX-4	ERCC1-Asn118= c.354T>C and XRCC1-Gln399Arg *c.1196A>G*	rs11615 and rs25487	Blood	Polymorphism	2 favorable genotypes (ERCC1 C/C and XRCC1 G/G) - vs ≤1 favorable genotype -			25 mo vs 16.5 mo p<0.01			

Supplementary Table S5.1 continues on next page.

Supplementary Table S5.1: ***Continued***

									Univariate analysis			Multivariate analysis		
Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	PFS	DFS	OS	PFS	DFS	OS
Liang et al., 2010 [32]	113	mCRC	Modified FOLFOX-4 or CAPOX	ERCC1-Asn118= *c.354T>C* and XRCC1-Gln399Arg *c.1196A>G*	rs11615 and rs25487	Blood	Polymorphism	**2 favorable genotypes (*XRCC1* A/A and *ERCC1* C/C) 38** vs 1 favorable genotype 40 vs 0 favorable genotype 35						1 HR=2.25 (95% CI: 1.38–3.67) p=0.01 0 HR=2.60 (95% CI: 1.56–4.31) p<0.01
Zaanan et al., 2014 [30]	210	Stage III	FOLFOX-4 or FOLFOX-6	ERCC1-Asn118= *c.354T>C* and XRCC1-Gln399Arg *c.1196A>G*	rs11615 and rs25487	Tumor tissue	Polymorphism	**≥1 favorable genotype (*ERCC1* C/C and/ or *XRCC1* G/G + G/A)** vs 0 favorable genotype		HR=2.42 (95% CI: 1.16–5.03) p=0.02			HR=2.03 (95% CI: 0.96–4.28) p=0.06	

Abbreviations: aCRC = advanced colorectal cancer, CAPOX = capecitabine and oxaliplatin, CI = confidence interval, *ERCC1 = excision repair cross-complementing group 1*, DFS = disease-free survival, FOLFOX = 5-fluorouracil, leucovorin and oxaliplatin, FUOX = 5-fluorouracil and oxaliplatin, HR = hazard ratio, mo = months, n.a. = not applicable, n.s. = not specified, OR = odds ratio, OS = overall survival, PFS = progression-free survival, RR = relative risk, *XRCC1 = X-ray repair cross-complementation group 1.* * Reference group in **bold**.

Table S5.2: Overview of studies on the association between XPA biomarkers and treatment outcome of oxaliplatin-based chemotherapy in CRC patients

									Univariate analysis			Multivariate analysis	
Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	PFS	DFS	OS	DFS	OS
Hu et al., 2019 [51]	580	aCRC	FOLFOX4 or CAPOX	XPA *g.100452435C>T*	rs2808668	Blood	Polymorphism	**C/C -** TT + C/T -				HR=1.19 (95% CI: 0.90–1.57) p=0.22	HR=1.17 (95% CI: 0.88–1.54) p=0.28
Hu et al., 2019 [51]	580	Stage III–IV	FOLFOX4 or CAPOX	XPA *g.100462409T>C*	rs10817938	Blood	Polymorphism	**T/T (306)** C/C + C/T (274)		46 mo vs 48 mo p=0.06	55 mo vs 62 mo p<0.01	HR=0.79 (95% CI: 0.63–1.00) p=0.05	HR=0.73 (95% CI: 0.58–0.92) p=0.01
Stoehlmacher et al., 2004 [31]	93	rCRC	FUOX	*XPA c.-4A>G*	rs1800975	Blood	Polymorphism	**G/G (24)** A/G (53) A/A (16)	A/G RR=1.24 (95% CI: 0.70–2.18) A/A RR=1.37 (95% CI: 0.66–2.84) p=0.61		A/G RR=0.88 (95% CI: 0.47–1.62) A/A RR=1.13 (95% CI: 0.49–2.45) p=0.76		
Monzo et al., 2007 [43]	42	aCRC	CAPOX	*XPA c.-4A>G*	rs1800975	Blood	Polymorphism	A/A (17) G/A (20) + A/A (5)			19.2 mo vs 18.1 mo p=0.29		
Monzo et al., 2007 [43]	42	aCRC	CAPOX	XPG-His46= *c.138T>C* and *XPA c.-4A>G*	rs1047768 + rs1800975	Blood	Polymorphism	**Favorable genotype (*XPG* (C/C) + *XPA* (G/A or G/G))** vs unfavorable genotype			49.6 mo vs 14.0 mo p<0.01		RR=34 (95% CI: 6.3–183) p<0.01

Abbreviations: aCRC = advanced colorectal cancer, CAPOX = capecitabine and oxaliplatin, CI = confidence interval, DFS = disease-free survival, FOLFOX = 5-fluorouracil, leucovorin and oxaliplatin, FUOX = 5-fluorouracil and oxaliplatin, HR = hazard ratio, mo = months, OS = overall survival, PFS = progression-free survival, rCRC = refractory colorectal cancer, RR = relative risk, *XPA = xeroderma pigmentosum complementation group A*, *XPG = xeroderma pigmentosum complementation group G*. * Reference group in **bold**.

Table S5.3: Overview of studies on the association between XPC biomarkers and treatment outcome of oxaliplatin-based chemotherapy in CRC patients

									Univariate analysis	Multivariate analysis	
Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/comparator (n)	OS	DFS	OS
Liu et al., 2012 [49]	432	n.s.	CAPOX or FOLFOX	XPC-Gln939Lys *c.2815C>A*	rs2228001	Blood	Polymorphism	**A/A -** A/C + C/C -	HR=0.97 (95% CI: 0.75–1.32) p=0.99		
Kap et al., 2015 [50]	201	Stage II–IV	Oxaliplatin-based chemotherapy	*XPC c.*463A>G*	rs1043953	Blood/ saliva	Polymorphism	**AA -** AG + GG -	HR=0.45 (95%CI: 0.29–0.70) p<0.01		
Hu et al., 2019 [51]	580	Stage III–IV	CAPOX or FOLFOX4	*XPC c.-27G>C*	rs2607775	Blood	Polymorphism	**C/C -** C/G + G/G		HR=0.91 (95% CI: 0.70–1.17) p=0.44	HR=0.91 (95% CI: 0.70–1.17) p=0.46

Abbreviations: CAPOX = capecitabine and oxaliplatin, CI = confidence interval, CRC = colorectal cancer, FOLFOX = 5-fluorouracil, leucovorin and oxaliplatin, HR = hazard ratio, n.s. = not specified, OS = overall survival, PFS = progression-free survival, **XPC = xeroderma pigmentosum complementation group C**. * Reference group in **bold**.

Table S5.4: Overview of studies on the association between XPD biomarkers and treatment outcome of oxaliplatin-based chemotherapy in CRC patients

									Univariate analysis		Multivariate analysis		
Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	PFS	OS	PFS	DFS	OS
Kassem et al., 2017 [44]	64	Stage III–IV	CAPOX or FOLFOX	XPD	n.a.	Tumor tissue	mRNA expression	**Low (48)** High (16)		HR=1.36 (95% CI: 0.59–3.14) p=0.47			
Kjersem et al., 2015 [54]	508	mCRC	FOLFOX or Nordic FLOX + cetuximab	XPD-Arg156= *c.468A>C*	rs238406	Blood	Polymorphism	C/C (173) + C/A (233) A/A (102)	7.8 mo vs 9.1 mo p<0.01	23.4 mo vs 20.3 mo p=0.33			
Stoehlmacher et al., 2004 [31]	103	rCRC	FUOX	XPD-Arg156= *c.468A>C*	rs238406	Blood	Polymorphism	**A/A (14)** C/A (59) C/C (30)	C/A RR=0.81 (95% CI: 0.44–1.49) C/C RR=0.73 (95% CI: 0.36–1.48) p=0.64	C/A RR=1.22 (95% CI: 0.55–2.75) C/C RR=1.18 (95% CI: 0.49–2.82) p=0.88			
Park et al., 2001 [55]	69	rCRC	FUOX	XPD-Arg156= *c.468A>C*	rs238406	Blood	Polymorphism	**C/C (22)** C/A (38) A/A (9)		11.7 mo vs 13.2 mo vs 8.5 mo C/A RR=0.94 A/A RR=1.60 p=0.50			

Supplementary Table S5.4 continues on next page.

Table S5.4: ***Continued***

Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	Univariate analysis		Multivariate analysis		
									PFS	OS	PFS	DFS	OS
Liu et al., 2019 [56]	106	Stage IV	mFOLFOX4 or CAPOX	XPD-Asp312Asn *c.934G>A*	rs1799793	Blood	Polymorphism	**G/G (49)** G/A (42) A/A (15)		G/A HR=1.26 (95% CI: 0.83–1.91) p=0.28 A/A HR=1.65 (95% CI: 0.92–2.97) p=0.09 G/A + A/A HR=1.34 (95% CI: 0.91–1.98) p=0.14			G/A HR=1.51 (95% CI: 0.98–2.34) p=0.06 A/A HR=2.43 (95% CI: 1.31–4.53) p<0.01
Park et al., 2001 [55]	59	rCRC	FUOX	XPD-Asp312Asn *c.934G>A*	rs1799793	Blood	Polymorphism	**A/A (7)** G/A (24) G/G (28)		Not reached 9.2 mo vs 26.5 mo G/A RR=2.17 G/G RR=1.29 p=0.27			

Table S5.4: ***Continued***

Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	Univariate analysis		Multivariate analysis		
									PFS	OS	PFS	DFS	OS
Ruzzo et al., 2007 [40]	165	mCRC	FOLFOX-4	XPD-Asp312Asn *c.934G>A*	rs1799793	Blood	Polymorphism	**G/G (57)** G/A (86) A/A (22)	G/A HR=1.02 (95% CI: 0.62–1.69) p=0.93 A/A HR=1.40 (95% CI: 0.83–2.37) p=0.21		G/A HR=1.13 (95% CI: 0.73–1.78) p=0.58 A/A HR=1.65 (95% CI: 0.73–3.21) p=0.12		
Sarasqueta et al., 2011 [41]	43	Stage III	CAPOX or FOLFOX	XPD-Lys751Gln *c.2251A>C*	rs13181	Normal tissue	Polymorphism	**A/A -** A/C - C/C -				AC HR=0.65 (95% CI: 0.24–1.8) p=0.41 CC HR=0.73 (95% CI: 0.13–4.11) p=0.72	
Lamas et al., 2011 [28]	72	aCRC	mFOLFOX-6	XPD-Lys751Gln *c.2251A>C*	rs13181	Blood	Polymorphism	A/A 28 A/C 33 C/C 11	8 mo vs 16 mo vs 10 mo p=0.02				

Supplementary Table S5.4 continues on next page.

Table S5.4: ***Continued***

Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	Univariate analysis		Multivariate analysis		
									PFS	OS	PFS	DFS	OS
Gan et al., 2012 [59]	289	aCRC	FOLFOX	XPD-Lys751Gln *c.2251A>C*	rs13181	Blood	Polymorphism	**A/A (138)** A/C (125) C/C (26)		A/C HR=0.91 (95% CI: 0.66–1.87) C/C HR=0.51 (95% CI: 0.33–0.94)			
Li et al., 2012 [27]	335	aCRC	FOLFOX-6	XPD-Lys751Gln *c.2251A>C*	rs13181	Blood	Polymorphism	**A/A (153)** A/C (150) C/C (32)		A/C HR=0.88 (95% CI: 0.61–1.28) C/C HR=0.52 (95% CI: 0.23–1.09)			A/C HR=0.86 (95% CI: 0.56–1.20) p=0.48 C/C HR=0.48 (95% CI: 0.19–0.97) p<0.05
Huang et al., 2011 [25]	157	mCRC	FOLFOX-4	XPD-Lys751Gln *c.2251A>C*	rs13181	Blood	Polymorphism	**C/C (1)** A/C (19) A/A (137)	A/C HR=0.76 (95% CI: 0.09–6.58) p=0.81 A/A HR=0.28 (95% CI: 0.03–2.38) p=0.24	A/C HR=0.86 (95% CI: 0.10–7.74) p=0.86 A/A HR=0.30 (95% CI: 0.03–2.82) p=0.30			

Table S5.4: ***Continued***

Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	Univariate analysis		Multivariate analysis		
									PFS	OS	PFS	DFS	OS
Le Morvan et al., 2007 [57]	59	mCRC	Oxaliplatin-based chemotherapy	XPD-Lys751Gln *c.2251A>C*	rs13181	Blood	Polymorphism	A/C (33) + C/C (6) A/A (20)		15.6 mo vs 26.3 mo p=0.02			
Stoehlmacher et al., 2004 [31]	106	rCRC	FUOX	XPD-Lys751Gln *c.2251A>C*	rs13181	Blood	Polymorphism	**A/A (40)** A/C (53) C/C (13)	A/C RR=1.13 (95% CI: 0.72–1.78) C/C RR=1.25 (95% CI: 0.59–2.67) p=0.76	A/C RR=1.87 (95% CI: 1.06–3.31) C/C RR=2.44 (95% CI: 1.09–5.44) p=0.05			A/C RR=1.50 (95% CI: 0.79–2.87) C/C RR=3.33 (95% CI: 1.39–7.99) p=0.04
Paré et al., 2008 [33]	121	mCRC	FOLFOX	XPD-Lys751Gln *c.2251A>C*	rs13181	Leukocytes	Polymorphism	**A/A (52)** A/C (45) + C/C (24)	12 mo vs 8 mo p<0.01	41 mo vs 17 mo p=0.02	RR=1.7 (95% CI: 1.1–2.8) p=0.02		RR=1.6 (95% CI: 1.1–2.5) p=0.03
Martinez-Balibrea et al., 2008 [34]	47	mCRC	CAPOX	XPD-Lys751Gln *c.2251A>C*	rs13181	Blood	Polymorphism	**A/A (20)** A/C (21) C/C (6)	A/C HR=1.05 (95% CI: 0.51–2.15) C/C HR=0.54 (95% CI: 0.19–1.52) p=0.39				

Supplementary Table S5.4 continues on next page.

Table S5.4: ***Continued***

Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	Univariate analysis		Multivariate analysis		
									PFS	OS	PFS	DFS	OS
Martinez-Balibrea et al., 2008 [34]	48	mCRC	FUOX	XPD-Lys751Gln *c.2251A>C*	rs13181	Blood	Polymorphism	**A/A (22)** A/C (19) C/C (8)	A/C HR=0.94 (95% CI: 0.45–1.97) C/C HR=1.50 (95% CI: 0.61–3.69) p=0.61				
Chen et al., 2010 [36]	166	mCRC	FOLFOX-4	XPD-Lys751Gln *c.2251A>C*	rs13181	Blood	Polymorphism	**A/A (139)** A/C (27)					HR=4.41 (95% CI: 2.51–7.75) p<0.01
Etienne-Grimaldi et al., 2010 [60]	115	aCRC	mFOLFOX7	XPD-Lys751Gln *c.2251A>C*	rs13181	Blood	Polymorphism	A/A (41) A/C (58) C/C (16)	6.4 mo vs 8.0 mo vs 6.4 mo p=0.33				
Lai et al., 2009 [58]	188	mCRC	FOLFOX-4	XPD-Lys751Gln *c.2251A>C*	rs13181	Blood	Polymorphism	A/A (158) A/C (30)	11 mo vs 7 mo p<0.01	22 mo vs 14 mo p<0.01			
Park et al., 2001 [55]	71	mCRC	FUOX	XPD-Lys751Gln *c.2251A>C*	rs13181	Blood	Polymorphism	**A/A (22)** A/C (39) C/C (10)		17.4 mo vs 12.8 mo vs 3.3 mo A/C RR=1.31 C/C RR=4.01 p<0.01			

Table S5.4: ***Continued***

Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	Univariate analysis		Multivariate analysis		
									PFS	OS	PFS	DFS	OS
Monzo et al., 2007 [43]	42	aCRC	CAPOX	XPD-Lys751Gln *c.2251A>C*	rs13181	Blood	Polymorphism	A/A (21) A/C (17) + C/C (4)	14.4 mo vs 19.2 mo p=0.83	8.3 mo vs 8.4 mo, p=0.54			
Ruzzo et al., 2007 [40]	165	mCRC	FOLFOX-4	XPD-Lys751Gln *c.2251A>C*	rs13181	Blood	Polymorphism	**A/A (43)** A/C (97) C/C (25)	A/C HR=1.67 (95% CI: 0.96–2.89) p=0.06 C/C HR=1.79 (95% CI: 1.13–3.09) p=0.03		A/C HR=1.81 (95% CI: 1.01–3.25) p=0.04 C/C HR=2.21 (95% CI: 1.17–4.17) p=0.01		
Kumamoto et al., 2013 [42]	63	n.s.	mFOLFOX-6	XPD-Lys751Gln *c.2251A>C*	rs13181	Blood	Polymorphism	**A/A (58)** A/C (5)	10.3 mo vs 6.1 mo p=0.05	25.5 mo vs 29.2mo p=0.26			

Abbreviations: aCRC = advanced colorectal cancer, CAPOX = capecitabine and oxaliplatin, CI = confidence interval, DFS = disease-free survival, FOLFOX = 5-fluorouracil, leucovorin and oxaliplatin, FLOX = 5-fluorouracil and folinic acid, FUOX = 5-fluorouracil and oxaliplatin, HR = hazard ratio, mCRC = metastatic colorectal cancer, mo = months, n.s. = not specified, OR = odds ratio, OS = overall survival, PFS = progression-free survival, rCRC = refractory colorectal cancer, RR = relative risk, *XPG = xeroderma pigmentosum complementation group G.* * Reference group in **bold**.

Table S5.5: Overview of studies on the association between XPG biomarkers and treatment outcome of oxaliplatin-based chemotherapy in CRC patients

									Univariate analysis		Multivariate analysis	
Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/comparator (n)	PFS	OS	PFS	OS
Monzo et al., 2007 [43]	42	aCRC	CAPOX	XPG-His46= *c.138T>C*	rs1047768	Blood	Polymorphism	C/C (19) C/T (19) + TT (4)		32.2 mo vs 12.0 mo p<0.01		
Kweekel et al., 2009 [65]	91	aCRC	CAPOX	XPG-His46= *c.138T>C*	rs1047768	Blood	Polymorphism	**T/T (28)** T/C (46) C/C (17)		No difference	T/C HR=1.71 (95% CI: 0.98–2.98) C/C HR=2.85 (95% CI: 1.42–5.71) p<0.01	
Chen et al., 2016 [64]	170	aCRC	FOLFOX	*XPG +25A>G*	n.s.	Blood	Polymorphism	**AA (32) + AG (83)** GG (55)	HR=1.59 (95% CI: 1.14–2.22) p<0.01	HR=1.58 (95% CI: 1.14–2.22) p<0.01	HR=1.50 (95% CI: 1.07–2.11) p=0.02	HR=1.68 (95% CI: 1.18–2.39) p<0.01
Liu et al., 2012 [49]	432	n.s.	CAPOX or FOLFOX	XPG-Asp1104His *c.3310G>C*	rs17655	Blood	Polymorphism	**G/G** G/C + C/C		HR=1.43 (95% CI: 1.01–2.00) p=0.04		HR 1.69 (95% CI: 1.20–2.38) p<0.01

Table S5.5: ***Continued***

									Univariate analysis		Multivariate analysis	
Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/comparator (n)	PFS	OS	PFS	OS
Chen et al., 2016 [64]	170	aCRC	FOLFOX	*XPG* -763A>G	n.s.	Blood	Polymorphism	**GG (35) + GA (78)** AA (57)	HR=1.75 (95% CI: 1.14–2.22) p<0.01	HR=1.73 (95%CI: 1.24–2.40) p<0.01	HR=1.72 (95% CI: 1.23–2.41) p<0.01	HR=1.88 (95% CI: 1.33–2.66) p<0.01
Monzo et al., 2007 [43]	42	aCRC	CAPOX	XPG-His46= *c.138T>C* and *XPA c.-4A>G*	rs1047768 and rs1800975	Blood	Polymorphism	**Favorable genotype (XPG (C/C) + XPA (G/A or G/G)** vs unfavorable genotype		49.6 mo vs 7.8 mo p<0.01		RR=34 (95% CI: 6.3–183) p<0.01

Abbreviations: aCRC = advanced colorectal cancer, CAPOX = capecitabine and oxaliplatin, CI = confidence interval, CRC = colorectal cancer, DFS = disease-free survival, FOLFOX = 5-fluorouracil; leucovorin and oxaliplatin, HR = hazard ratio, mo = months, n.s. = not specified, OS = overall survival, OR = odds ratio, PFS = progression-free survival, *XPA = xeroderma pigmentosum complementation group A*, *XPG = xeroderma pigmentosum complementation group G*. * Reference group in **bold**.

Table S5.6: Overview of studies on the association between MNAT1 biomarkers and treatment outcome of oxaliplatin-based chemotherapy in CRC patients

Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/comparator (n)	Univariate analysis OS
Kap et al., 2015 [50]	192	Stage II–IV	Oxaliplatin-based chemotherapy	*MNAT1 c.688-30168A>G*	rs3783819	Bblood/saliva	Polymorphism	**A/A -** vs G/A + G/G -	HR=0.51 (95% CI: 0.36–0.73) p<0.01
Kap et al., 2015 [50]	201	Stage II–IV	Oxaliplatin-based chemotherapy	*MNAT1 c.562-88A>G*	rs973063	Blood/saliva	Polymorphism	**A/A -** vs G/A + G/G -	HR=0.52 (95% CI: 0.37–0.72) p<0.01
Kap et al., 2015 [50]	201	Stage II–IV	Oxaliplatin-based chemotherapy	*MNAT1 c.809+24992A>G*	rs4151330	Blood/saliva	Polymorphism	**A/A -** vs G/A + G/G -	HR=0.53 (95% CI: 0.38–0.75) p<0.01

Abbreviations: CI = confidence interval, CRC = colorectal cancer, HR = hazard ratio, OS = overall survival. * Reference group in **bold**.

Table S5.7: Overview of studies on the association between XRCC1 biomarkers and treatment outcome of oxaliplatin-based chemotherapy in CRC patients

									Univariate analysis			Multivariate analysis	
Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	PFS	DFS	OS	DFS	OS
Huang et al., 2011 [25]	157	mCRC	FOLFOX-4	XRCC1-Gln399Arg *c.1196A>G*	rs25487	Blood	Polymorphism	**A/A (10)** G/A (57) G/G (90)	G/G HR=0.31 (95% CI: 0.10–0.91) p=0.03 G/A HR=1.25 (95% CI: 0.51–3.07) p=0.62		G/G HR=0.15 (95% CI: 0.04–0.57) p<0.01 G/A HR=0.63 (95% CI: 0.22–1.76) p=0.38		
Suh et al., 2006 [81]	51	aCRC	mFOLFOX-4	XRCC1-Gln399Arg *c.1196A>G*	rs25487	Tumor tissue	Polymorphism	G/G (31) G/A (16) A/A (4)			30.0 mo vs 16.5 mo vs 12.8 mo p=0.02		
Lamas et al., 2011 [28]	72	aCRC	FUOX	XRCC1-Gln399Arg *c.1196A>G*	rs25487	Blood	Polymorphism	A/A - G/A – G/G -	6 mo vs 10 mo vs 12 mo p=0.67				
Zaanan et al., 2014 [30]	207	Stage III	FOLFOX-4 or FOLFOX-6	XRCC1-Gln399Arg *c.1196A>G*	rs25487	Tumor tissue	Polymorphism	**G/G (94) + G/A (80)** A/A (33)		HR=1.61 (95% CI: 0.82-3.12) p=0.16			

Supplementary Table S5.7 continues on next page.

Table S5.7: ***Continued***

									Univariate analysis			Multivariate analysis	
Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	PFS	DFS	OS	DFS	OS
Stoehlmacher et al., 2004 [31]	105	rCRC	FUOX	XRCC1-Gln399Arg *c.1196A>G*	rs25487	Blood	Polymorphism	**G/G (44)** G/A (51) A/A (10)	G/A RR=0.95 (95% CI: 0.60–1.51) A/A RR=0.99 (95% CI: 0.47–2.09) p=0.97		G/A RR=1.07 (95% CI: 0.63–1.80) A/A RR=1.58 (95% CI: 0.71–3.55) p=0.50		
Liang et al., 2010 [32]	113	mCRC	mFOLFOX-4 or CAPOX	XRCC1-Gln399Arg *c.1196A>G*	rs25487	Blood	Polymorphism	**A/A (61)** A/G (39) G/G (13)					A/G HR=1.09 (95% CI: 0.57–2.08) p=0.80 G/G HR=1.31 (95% CI: 0.53–3.25) p=0.57
Gan et al., 2012 [59]	289	aCRC	FOLFOX	XRCC1-Gln399Arg *c.1196A>G*	rs25487	Blood	Polymorphism	**G/G (149)** G/A (88) A/A (51)			G/A HR=0.85 (95% CI: 0.51–1.23) A/A HR=0.66 (95% CI: 0.36–0.95)		

Table S5.7: ***Continued***

Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	Univariate analysis		Multivariate analysis	
									PFS	DFS	OS	DFS	OS
Chua et al., 2009 [39]	115	mCRC	FOLFOX	XRCC1-Gln399Arg *c.1196A>G*	rs25487	Tumor tissue	Polymorphism	**G/G (39)** A/G (61) A/A (15)	A/G HR=0.57 (95% CI: 0.28–1.19) p=0.10 A/A HR=1.01 (95% CI: 0.39–2.60) p=1.0 A/G + A/A HR=0.66 (95% CI: 0.34–1.28) p=0.20		A/G HR=0.92 (95% CI: 0.58–1.45) p=0.70 A/A HR=0.52 (95% CI: 0.24–1.14) p=0.10 A/G + A/A HR=0.83 (95% CI: 0.53–1.29) p=0.40		
Martinez-Balibrea et al., 2008 [34]	47	mCRC	CAPOX	XRCC1-Gln399Arg *c.1196A>G*	rs25487	Blood	Polymorphism	**G/G (19)** G/A (19) A/A (9)	G/A HR=0.82 (95% CI: 0.39–1.71) A/A HR=0.65 (95% CI: 0.25–1.66) p=0.64				
Martinez-Balibrea et al., 2008 [34]	48	mCRC	FUOX	XRCC1-Gln399Arg **c.1196A>G**	rs25487	Blood	Polymorphism	**G/G (21)** G/A (20) A/A (7)	G/A HR=0.85 (95% CI: 0.42–1.73) A/A HR=0.96 (95% CI: 0.31–3.00) p=0.90				

Supplementary Table S5.7 continues on next page.

Table S5.7: ***Continued***

									Univariate analysis			Multivariate analysis	
Author, year	n	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	PFS	DFS	OS	DFS	OS
Huang et al., 2011 [25]	157	mCRC	FOLFOX-4	ERCC1-Asn118= *c.354T>C* and XRCC1-Gln399Arg *c.1196A>G*	rs11615 and rs25487	Blood	Polymorphism	2 favorable genotypes (ERCC1 C/C and XRCC1 G/G) - vs ≤1 favorable genotype -			25 mo vs 16.5 mo p<0.01		
Liang et al., 2010 [32]	113	mCRC	Modified FOLFOX-4 or CAPOX	ERCC1-Asn118= *c.354T>C* and XRCC1-Gln399Arg *c.1196A>G*	rs11615 and rs25487	Blood	Polymorphism	**2 favorable genotypes (XRCC1 A/A and ERCC1 C/C) 38** vs 1 favorable genotype 40 vs 0 favorable genotype 35					1 favorable genotype HR=2.25 (95% CI: 1.38–3.67) p<0.01 0 favorable genotype HR=2.60 (95% CI: 1.56–4.31) p<0.01
Zaanan et al., 2014 [30]	210	Stage III	FOLFOX-4 or FOLFOX-6	ERCC1-Asn118= *c.354T>C* and XRCC1-Gln399Arg *c.1196A>G*	rs11615 and rs25487	Tumor tissue	Polymorphism	**≥ 1 favorable genotype (ERCC1 C/C and/ or XRCC1 G/G + G/A) 179** vs 0 favorable genotype 21		HR=2.42 (95% CI: 1.16–5.03) p=0.02		HR=2.03 (95% CI: 0.96–4.28) p=0.06	

Abbreviations: aCRC = advanced colorectal cancer, CAPOX = capecitabine and oxaliplatin, CRC = colorectal cancer, CI = confidence interval, FOLFOX = 5-fluorouracil, leucovorin and oxaliplatin, FUOX = 5-fluorouracil and oxaliplatin, HR = hazard ratio, mo = months, mCRC = metastatic colorectal cancer, OS = overall survival, PFS = progression-free survival, rCRC = refractory colorectal cancer. * Reference group in **bold**.

Table S5.8: Overview of studies on the association between biomarkers in the HR pathway and treatment outcome of oxaliplatin-based chemotherapy in CRC patients

									Univariate analysis	Multivariate analysis
Author, year	N	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/comparator (n)	PFS	PFS
Martinez-Balibrea et al., 2008 [34]	47	mCRC	CAPOX	XRCC3-Thr241Met *c.722C>T*	rs861539	Blood	Polymorphism	**C/C (23)** C/T (18) T/T (6)	C/T HR=0.91 (95% CI: 0.43–1.9) T/T HR=1.8 (95% CI: 0.66–4.95) p=0.48	
Martinez-Balibrea et al., 2008 [34]	48	mCRC	FUOX	XRCC3-Thr241Met *c.722C>T*	rs861539	Blood	Polymorphism	**C/C (18)** C/T (20) T/T (10)	C/T HR=1.22 (95% CI: 0.58–2.6) T/T HR=1.33 (95% CI: 0.54–3.29) p=0.80	
Ruzzo et al., 2007 [40]	165	mCRC	FOLFOX-4	XRCC3-Thr241Met *c.722C>T*	rs861539	Blood	Polymorphism	**T/T (31)** C/T (71) C/C (63)	C/T HR=1.41 (95% CI: 0.89–2.24) p=0.12 C/C HR=0.87 (95% CI: 0.54–1.38) p=0.54	C/T HR=1.67 (95% CI: 0.96–2.89) p=0.07 C/C HR=0.99 (95% CI: 0.57–1.71) p=0.96

Supplementary Table S5.8 continues on next page.

Table S5.8: ***Continued***

									Univariate analysis	Multivariate analysis
Author, year	N	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/comparator (n)	PFS	PFS
Ihara et al., 2016 [82]	78	mCRC or rCRC	mFOLFOX or CAPOX ±bevacizumab	MRE11	n.a.	Tumor tissue	Protein expression	Positive expression (48) Negative expression (30)	11.3 mo vs 11.8 mo p=0.50	
Ihara et al., 2016 [82]	78	mCRC or rCRC	mFOLFOX or CAPOX ±bevacizumab	RAD51	n.a.	Tumor tissue	Protein expression	**Positive expression (40)** Negative expression (38)	9.7 mo vs 13.5 mo p=0.04	HR 0.80 (95% CI: 0.35–1.83) p=0.60
Ihara et al., 2016 [82]	78	mCRC or rCRC	mFOLFOX or CAPOX ±bevacizumab	MRE11 + RAD51	n.a.	Tumor tissue	Protein expression	MREE1 and/or RAD51: **Positive expression 47** Negative expression 31	10.1 mo vs 13.2 mo p=0.02	HR 1.39 (95%CI: 0.58–3.34) p=0.50
Moutinho et al., 2014 [79] discovery cohort	131	Stage IV	FUOX–based chemotherapy	*SRBC*	n.a.	Tumor tissue	DNA methylation status	**Unmethylated (92)** Methylated (39)	HR=1.83 (95% CI: 1.15–2.92) p= 0.01	
Moutinho et al., 2014 [79] validation cohort	58	Stage IV	FUOX–based chemotherapy	*SRBC*	n.a.	Tumor tissue	DNA methylation status	**Unmethylated (44)** Methylated (14)	HR=1.90 (95% CI: 1.01–3.60) p= 0.05	

Abbreviations: CAPOX = capecitabine and oxaliplatin, CRC = colorectal cancer, CI = confidence interval, DFS = disease-free survival, FOLFOX = 5-fluorouracil, leucovorin and oxaliplatin, FUOX = 5-fluorouracil and oxaliplatin, HR = hazard ratio, mo = months, mCRC = metastatic colorectal cancer, OS = overall survival, PFS = progression-free survival, rCRC = refractory colorectal cancer. * Reference group in **bold**.

Table S5.9: Overview of studies on the association between biomarkers in the MMR pathway and treatment outcome of oxaliplatin-based chemotherapy in CRC patients

								Univariate analysis			Multivariate analysis	
Author, year	n	CRC type	Treatment	Biomarker	Type of sample	Type of assay	Comparator*/reference (n)	PFS	DFS	OS	DFS	OS
Kim et al., 2010 [70]	115	Stage II–IV	FOLFOX	MMR status	Tumor tissue	Protein expression	**pMMR (104)** dMMR (11)		HR=0.69 (0.24–1.97) p=0.49	HR=1.31 (0.17–9.98) p=0.79		
Sfakianaki et al., 2019 [46]	235	Stage II–III	FOLFOX or CAPOX	MMR status	Tumor tissue	Polymorphism	**dMMR (35)** pMMR (200)		HR=1.72 (95% CI: 1.29–3.51) p=0.03	HR=1.38 (95% CI: 1.04–2.71) p=0.04	HR=1.78 (95% CI: 1.34–3.01) p<0.01	HR=1.58 (95% CI: 1.24–3.00) p=0.02
Gallois et al., 2018 [69]	1867	Stage III	FOLFOX4 ± cetuximab	MMR status	Tumor tissue	Polymorphism	**dMMR (172)** pMMR (1560)					HR=1.80 (95% CI: 1.16–2.81) p<0.01

Abbreviations: aCRC = advanced colorectal cancer, CAPOX = capecitabine and oxaliplatin, CI = confidence interval, CRC = colorectal cancer, DFS = disease-free survival, dMMR = deficient mismatch repair, FOLFOX = 5-fluorouracil, leucovorin and oxaliplatin, FUOX = 5-fluorouracil and oxaliplatin, HR = hazard ratio, MMR = mismatch repair, mo = months, n.s. = not specified, OS = overall survival, PFS = progression-free survival, pMMR = proficient mismatch repair. * Reference group in **bold**.

Table S5.10: Overview of studies on the association between biomarkers in DNA damage response and DNA synthesis and treatment outcome of oxaliplatin-based chemotherapy in CRC patients

									Univariate analysis		Multivariate analysis	
Author, year	N	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	PFS	OS	PFS	OS
Sundar et al., 2018. [74]	121	mCRC	CAPOX± bevacizumab or FOLFOX± bevacizumab/ cetuximab	ATM	n.a.	Ttumor tissue	Protein expression	**Loss (9)** Proficient (113)		HR=2.52 (95% CI: 1.00–6.37) p=0.05		
Kweekel et al., 2009 [65]	91	aCRC	CAPOX	ATM-Asp1853Asn *c.5557G>A*	rs1801516	Blood	Polymorphism	**G/G (63)** G/A (24) A/A (4)		No difference	G/A HR=0.72 (95% CI: 0.43–1.21) A/A HR=4.25 (95% CI: 1.45–12.44) p<0.01	
Okazaki et al., 2017 [76]	218	mCRC	Oxaliplatin-based chemotherapy	*HIC1* *Tandem repeat D17S5 loci*	n.a.	Blood	Polymorphism	**S/S (≤4TRs in both alleles) 179 + S/L (≤4TRs in one allele) 19** L/L(≥5TRs in both alleles) 20	HR=1.93 (95% CI: 1.11–3.35) p=0.01	HR=1.25 (95% CI: 0.74–2.10) p=0.40	HR=2.00 (95% CI: 1.13–3.54) p=0.02	HR=1.20 (95% CI: 0.71–2.04) p=0.50

Table S5.10: ***Continued***

Author, year	N	CRC type	Treatment	Biomarker	rs number	Type of sample	Type of assay	Reference*/ comparator (n)	Univariate analysis		Multivariate analysis	
									PFS	OS	PFS	OS
Suenaga et al., 2018 [78]	143	mCRC	FOLFOX ± bevacizumab	*PIN1* *NC_000019.9:g.9945179G>C*	rs2233678	Blood	Polymorphism	**G/G (129)** G/C (13) + C/C (1)	HR=3.24 (95% CI: 1.60–6.54) p<0.01	HR=2.38 (95% CI: 1.32–4.30) p<0.01	HR= 2.67 (95% CI: 1.28–5.57) p<0.01	HR=1.91 (95%CI: 1.02–3.59) p=0.04
Suenaga et al., 2018 [78]	70	mCRC	FOLFOX or CAPOX + bevacizumab	*PIN1* *NC_000019.9:g.9945179G>C*	rs2233678	Blood	Polymorphism	**G/G (64)** G/C (6)	HR=1.11 (95% CI: 0.43–2.82) p=0.83	HR=2.43 (95% CI: 0.83–7.15) p=0.09	HR=1.15 (95% CI: 0.44–2.98) p=0.78	HR=3.01 (95% CI: 0.98–9.20) p=0.05
Park et al., 2010 [80]	88	mCRC	CAPOX or mFOLFOX4	*MGMT* *-535G>T*	rs1625649	Tumor tissue	Polymorphism	**G/G (39) + G/T (39)** T/T (10)	HR=2.65 (95% CI: 1.10–6.39) p=0.03	HR=2.09 (95% CI: 0.59–7.47) p=0.26	HR=3.14 (95% CI: 1.42–6.91) p<0.01	HR=2.06 (95% CI: 0.74–5.75) p=0.17

Abbreviations: *ATM = ataxia-telangiectasia mutated*, CAPOX = capecitabine and oxaliplatin, CRC = colorectal cancer, CI = confidence interval, FOLFOX = 5-fluorouracil, leucovorin and oxaliplatin, FUOX = 5-fluorouracil and oxaliplatin, HIC1 = hypermethylated in cancer 1, HR = hazard ratio, *MGMT = Human O6-alkylguanine-DNA alkyltransferase*, mo = months, mCRC = metastatic colorectal cancer, OS = overall survival, PFS = progression-free survival, *PIN1 = peptidyl-prolyl cis/trans isomerase NIMA-interacting 1*, TR = tandem repeat. * Reference group in **bold**.

CHAPTER 6

Genome-wide association study for predictors of survival after cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in patients with colorectal peritoneal metastases

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ABSTRACT

Aim

Patients with peritoneal metastases of colorectal origin are often treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (CRS + HIPEC). Careful patient selection for CRS + HIPEC is essential in order to reduce the number of patients unsuccessfully undergoing this invasive procedure. A promising approach for more adequate patient selection might be the search for pharmacogenetic biomarkers. Therefore, in this study we performed a genome wide association study (GWAS) to identify new genetic biomarkers that are associated with CRS + HIPEC clinical outcome.

Methods

This was a retrospective study in patients with CPM that were consecutively treated with CRS + HIPEC with MMC or oxaliplatin, between January 2007 and January 2020. We conducted a GWAS using germline DNA on disease-free survival (DFS) as primary, and overall survival (OS) as secondary endpoint. Each genetic marker was analyzed using cox-proportional hazards analysis including clinically relevant covariates. Corresponding genes were selected using LD proxy and HaploReg v4.1.

Results

In total, 258 patients underwent CRS + HIPEC and were eligible for genotyping. After quality control 206 patients (79.8%) remained. Twelve markers were significantly associated with reduced DFS, i.e. chr21:42667563:I, rs79200189, rs188864712, rs76946962, rs116774218, rs62018875, rs182474777, rs72766414, rs3902655, chr3:136477662:I, rs114242526, and rs186272589. These markers influence the expression of the following genes *FAM3B*, *STAG1*, *SCL35G2*, *TMEM114*, *METTL22*, and *LINC00351*. For four out of six of these identified genes (*FAM3B*, *STAG1*, *SCL35G2*, and *METTL22*) one or more biological mechanisms were identified that are in support of the observed associations of the genetic biomarkers with reduced DFS.

Conclusion

Several new potentially prognostic or predictive genetic biomarkers for clinical outcome of CRS + HIPEC patients were identified. Further research and validation of our findings are warranted.

INTRODUCTION

Peritoneal metastases of a colorectal origin affect approximately 10% of colorectal cancer patients and are associated with a dismal prognosis [1]. Colorectal peritoneal metastases (CPM) were regarded as terminal illness, until a randomized controlled trial from 2003 proved the efficacy of radical surgery (cytoreductive surgery [CRS]) followed by hyperthermic intraperitoneal chemotherapy (HIPEC) [2]. Despite the curative potential of CRS + HIPEC treatment, 70% of patients still experience disease recurrence [3]. Furthermore, morbidity (20–40% of patients) and mortality (3% of patients) are often observed after CRS + HIPEC [4–6]. Careful selection of patients who might benefit from CRS + HIPEC with acceptable risk of morbidity and mortality is thus essential, but remains challenging [7].

Patient selection can be improved through the use of prognostic and predictive models. Multiple such models have been constructed, covering a wide variety of parameters [7–11]. In addition, it has been demonstrated that BRAF and KRAS mutations negatively influence prognosis of CPM patients undergoing the CRS + HIPEC procedure [12, 13]. Although undeniably contributing to a more optimal CRS + HIPEC patient selection, these clinical and pathological models, and parameters still do not fully predict CRS + HIPEC clinical outcome. Moreover, most of these biomarkers can only be identified in patients in an intraoperative-, or postoperative setting. Therefore, different approaches for identification of prognostic and/or predictive factors for CRS + HIPEC treatment outcome are warranted, in order to further optimise patient selection for this treatment.

A promising approach for more adequate patient selection might be the exploration of the prognostic and/or predictive value of pharmacogenetic biomarkers. The effect of the HIPEC agents oxaliplatin and MMC is influenced by several pharmacological mechanisms such as tumour tissue absorption, drug activation and pharmacodynamic mechanisms [14–16]. A well-known pharmacodynamic mechanism is the DNA repair pathway. Both oxaliplatin and MMC interfere with DNA by forming inter- and intrastrand lesions, DNA adducts and -crosslinks, resulting in termination of DNA replication and transcription, cell-cycle arrest and apoptosis [17–19]. However, the DNA repair pathway detects and repairs DNA lesions caused by adduct-forming chemotherapeutic agents such as oxaliplatin and MMC, decreasing their therapeutic effect [20–22].

While many studies have searched for pharmacogenetic biomarkers that are predictive for treatment outcome in patients treated with systemic chemotherapy, almost no studies are available on patients treated with intraperitoneal chemotherapy. Especially in the CRS +

HIPEC population, there is a lack of association studies that identify possible pharmacogenetic biomarkers [15, 16, 23, 24].

Therefore, in this study we performed a genome wide association study (GWAS) to identify new genetic biomarkers that are associated with CRS + HIPEC clinical outcome and thus may aid in patient selection.

MATERIALS AND METHODS

Study design

This was a retrospective study in patients with CPM that were consecutively treated with CRS + HIPEC with MMC or oxaliplatin. We conducted a GWAS on disease-free survival (DFS) and overall survival (OS). The study was approved by the local medical ethical review board and was not subject to the medical research involving human subjects act. Data was pseudonymised before data analysis.

Patients

All patients with histologically proven isolated CPM that were consecutively treated with CRS + HIPEC with MMC or oxaliplatin in a large tertiary HIPEC centre in the Netherlands between January 2007 and January 2020 were included in this study. MMC was administered in a dosage of 35 mg/m^2 at 40°C–42°C and was circulated for 90 minutes. Oxaliplatin was administered in a dosage of 460 mg/m^2 at 39°C–41°C for 30 minutes. Patients with appendiceal or rectal cancers, or with synchronous liver metastases were excluded, as well as any patient that did not undergo complete cytoreduction. Completeness of cytoreduction was determined by the completeness of cytoreduction score (CCR), and as such patients with a CCR score >1 were excluded [25]. Furthermore, patients who died within 30 days after CRS + HIPEC treatment were excluded.

Data collection

Clinical data regarding patient-, disease-, treatment- and outcome characteristics were collected from the electronic patient charts. Selection and description of characteristics, and the follow-up procedures that were performed are described previously [15]. Shortly, standard follow-up was performed after successful CRS and HIPEC, according to the colorectal cancer guidelines and consisted of laboratory testing of carcinoembryonic antigen (CEA) every three

months, a liver ultrasound every six months, and an abdominal CT-scan every 12 months. In case recurrent disease was suspected based on clinical parameters, CEA elevation, or liver ultrasound outcomes, an additional abdominal CT-scan was performed. In this study, data about two additional treatment characteristics was collected: the type of chemotherapeutic agent used in HIPEC treatment (oxaliplatin or MMC), and whether patients were treated with neoadjuvant therapy within 3 months before the CRS + HIPEC procedure. The latest data on vital status was collected from the Municipal Administrative Database in June 2021 in which deaths of the Dutch population are recorded.

DNA isolation and genotyping

Formalin-fixed, paraffin embedded (FFPE) material from normal tissue was used for DNA isolation. Normal tissue was selected based on pathology reports. Briefly, DNA was isolated from FFPE tissue cores using the automated tissue preparation system (Siemens Healthcare Diagnostics, Tarrytown, NY), as described previously [26]. DNA samples were genotyped with the Infinium Global Screening Array-24 v3 (Illumina, San Diego, CA, USA) according to the manufacturer's instructions at the Human Genotyping Facility (HuGe-F) at Erasmus Medical Centre (EMC), The Netherlands.

Endpoints

The primary endpoint of this study was DFS, defined as the period between CRS + HIPEC procedure and disease recurrence or death of any cause. Patients not experiencing disease recurrence or death of any cause were censored at the last moment of follow-up. The secondary endpoint was OS, defined as the period between CRS + HIPEC treatment and death of any cause. Patients not experiencing death were censored at the last moment of follow-up.

Statistical analysis

Quality control

Genetic markers were excluded based on a minor allele frequency (MAF) threshold of 0.5% (excluding 145,105 markers; 20.0%) and missingness threshold of 25% (excluding 202,688 markers; 27.9%). The missingness threshold was chosen leniently due to the presence of FFPE embedded tumor derived samples with low DNA quality. Hardy-Weinberg equilibrium (HWE) was evaluated per genetic biomarker, using a χ^2 goodness-of-fit statistic with a cut-off p-value of $\leq 1x10^{-7}$ (excluding 21,384 markers; 2.9%). In total, 392,608 genetic markers remained in the analysis.

Individuals were excluded based on missingness >30% (52 excluded individuals; 20.2%), again choosing a lenient exclusion threshold. Multidimensional scaling (MDS) was used to investigate possible population stratification and outliers. No individuals were identified as outliers. Association analysis was performed with and without the first four MDS coordinates as covariates. Ranking and p-values of the top 30 SNPs were almost identical and only analyses without MDS coordinates are shown.

Association model

For each genetic marker, the association with DFS and OS was assessed using a Cox-proportional hazards model using R (http://www.r-project.org/). For DFS, presentation of CPM (synchronous/metachronous), peritoneal cancer index (PCI), N stage and the administration of (neo)adjuvant chemotherapy (yes/no) were included as covariates. For OS, age (years), signet ring cell differentation (yes/no), PCI and N stage were included as covariates. An initial set of covariates was based on the Colorectal Peritoneal Metastases Prognostic Surgical Score (COMPASS) as described by Simkens et al. [7]. The covariates identified for inclusion into the models were based on a cut-off p-value of <0.1 for the marginal association of the variable with the respective outcome.

Markers were evaluated using an additive genetic model. To assess possible anti-conservative behaviour of the statistical tests, the inflation factor was computed for each models, transforming P-values to the χ^2-scale and comparing the median, observed test statistic to the theoretical expectation. Post association QC was performed by visual inspection of p-values in the Quantile-Quantile (QQ) plots. Formal significance for a marker was assumed for a two-sided p-value $<5x10^{-8}$ (Bonferroni correction). Associations between $p=5x10^{-8}$ and $p=1x10^{-7}$ were deemed suggestive and included in the evaluation of possible biological significance.

Selection of corresponding genes

The genes that corresponded to the significant and suggestive markers were selected with LD proxy (https://ldlink.nci.nih.gov/?tab=ldproxy), with a base pair window of 500,000, an R^2 of 0.8 and the EURO population. When LD proxy returned no results, as an alternative HaploReg 4.1 was used to select the corresponding genes. In addition, HaploReg v4.1 was used to evaluate whether each marker that was identified potentially influences the outcome through regulatory motifs and/or expression of the corresponding gene (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) through variants showing high linkage disequilibrium.

RESULTS

Patient characteristics

In total, 258 patients underwent CRS + HIPEC and were eligible for genotyping. After quality control 206 patients (79.8%) remained. Baseline patient characteristics are described in **Table 6.1**. The median age of the patients was 71 (IQR=62–76). Six percent of the patients had signet ring cell differentiation and 42 percent of the patients had a nodal of stage of 2. More than half of the patients presented with synchronous peritoneal metastases and the median PCI was 8 (IQR=4–13). Almost 60% of the patients were treated with a form of (neo)-adjuvant chemotherapy. Median DFS and OS for all patients were 13 and 39 months, respectively.

Genetic association analysis

For the primary genetic association analysis, twelve markers were significantly associated with DFS, and two markers were assumed suggestive (**Table 6.2** and **Figure 6.1**). All markers were unfavourable for clinical outcome. Of the significant markers rs79200189, rs188864712, rs76946962, rs116774218, chr3:136477662:I, rs114242526, and rs186272589 are located on chromosome 3, and correspond with the following genes: *STAG1*, *SCL35G2*, *NCK1-DT* (other name *NCK1-AS1*), and *NCK1*. The remaining significant markers are located on chromosome 13, 16, 17 and 21 and are positioned close to or correspond with the following genes: *FAM3B*, *TMEM114*, *METLL22*, *LINC02003* and *LINC00351*. Lastly, HaploReg affirmed that the above mentioned markers influence regulatory motifs and/or expression of the genes *FAM3B*, *STAG1*, *SCL35G2*, *TMEM114*, *METLL22* and *LINC00351*.

For OS, 23 markers reached the significance level and seven markers were considered suggestive (**Table 6.3** and **Figure 6.2**). All significant markers were unfavourable for clinical outcome, only one suggestive marker was favourable for clinical outcome (rs2543317). Nine out of twenty-three of the significant markers are located in proximity to each other on chromosome 2 and correspond with the following four genes: *SRD5A2*, *MEMO1*, *DPY30* and *SLC30A6*. Another six significant markers are located on chromosome 11, are highly in linkage and correspond with two genes: *OR5AK2* and *OR5AK4P*. The remaining significant markers are located on chromosome 1, 4, 7, 10, 11 and 17 and are positioned closed to or correspond with the following genes: *PCGF5*, *LOC339620*, *EPO*, *LINC02548*, *KLHL2*, *MSMO1*, *CR1*, *CR1L*, *CR2*, *MIR29B2CHG*, *CD34*, *PLXNA2* and *AC116655.1*. HaploReg affirmed that the above markers influenced regulatory motifs and/or expression of the genes *SRD5A2*, *DPY30*, *SLC30A6*, *OR5AK2*, *PCGF5*, *EPO*, *KLHL2*, *MSMO1*, *CR1*, and *AC116655.1*.

The QQ plots for DFS and OS are presented in **Figure S6.1** and **Figure S6.2**, with inflation factors of 1.02 and 1.09, respectively.

Table 6.1: Patient characteristics

	n=206	(%)
Sex		
Male	102	(49.5)
Female	104	(50.5)
Age (median + IQR in years)	71	62–76
Primary tumour location		
Right colon	81	(39.3)
Sigmoid	76	(36.9)
Other (appendix, transverse, left colon)	35	(17.0)
Missing	14	(6.8)
Signet ring cell differentiation		
Yes	12	(5.8)
No	194	(94.2)
Tumour stage		
T1–2	5	(2.4)
T3	81	(39.3)
T4	115	(55.9)
Missing	5	(2.4)
Nodal stage		
N0	61	(29.6)
N1	59	(28.6)
N2	85	(41.3)
Missing	1	(0.5)
Presentation of peritoneal metastases		
Synchronous	111	(53.9)
Metachronous	95	(46.1)
ASA score		
1	15	(7.3)
2	165	(80.1)
3	21	(10.2)
Missing	5	(2.4)
Peritoneal cancer index (median + IQR)	8	4–13
Type of HIPEC agent		
Mitomycin C	190	(92.2)
Oxaliplatin	16	(7.8)
(Neo)-adjuvant chemotherapy		
Yes	117	(56.8)
No	89	(43.2)

Patient cohort consists out of patients with peritoneal metastasis from colorectal cancer treated with CRS + HIPEC with mitomycin C or oxaliplatin. CRS = cytoreductive surgery, HIPEC = Hyperthermic Intraperitoneal Chemotherapy, SD = standard deviation, ASA = American Society of Anaesthesiology.

Table 6.2: Overview of most promising genetic biomarkers for disease-free survival in patients with colorectal peritoneal metastasis treated with cytoreductive surgery-hyperthermic intraperitoneal chemotherapy

SNP	Chromosome	Position	MAF (%)	Allele	Imputation quality score	Gene(s)	p-value	Hazard ratio	95% CI
Significant associations									
chr21:42667563:I	21	42667563	2.5	A/AG	0.92	Position close to *FAM3B*	$1.1x10^{-9}$	11.0	5.1–23.7
rs79200189	3	136514862	2.6	G/T	0.93	*STAG1, SLC35G2*	$7.3x10^{-9}$	8.2	4.0–16.6
rs188864712	3	136134155	2.0	A/T	0.70	*STAG1, SLC35G2, NCK1-DT, NCK1*	$7.5x10^{-9}$	17.7	6.7–46.7
rs76946962	3	136382163	3.6	C/A	0.79	*STAG1, SLC35G2*	$1.0x10^{-8}$	7.1	3.6–13.9
rs116774218	3	136516111	2.6	A/G	0.95	*STAG1, SLC35G2, NCK1-DT, NCK1*	$1.2x10^{-8}$	8.0	3.9–16.4
rs62018875	16	8692738	11.2	G/C	0.89	*TMEM114, METLL22*	$1.9x10^{-8}$	2.6	1.8–3.6
rs182474777	17	70307338	2.1	A/C	0.56	Position close to *LINC02003*	$3.2x10^{-8}$	25.6	8.1–80.9
rs72766414	16	8690404	11.4	G/A	0.90	*TMEM114, METLL22*	$3.5x10^{-8}$	2.5	1.8–3.5
rs3902655	13	85864047	2.1	C/T	1.00	Position close to *LINC00351*	$3.6x10^{-8}$	5.5	3.0–10.2
chr3:136477662:I	3	136477662	2.4	T/TGCGATCTCA	1.00	*STAG1, SLC35G2, NCK1-DT, NCK1*	$3.6x10^{-8}$	7.7	3.7–15.9
rs114242526	3	136363965	2.4	C/G	1.00	*STAG1, SLC35G2, NCK1-DT, NCK1*	$3.7x10^{-8}$	7.7	3.7–15.9
rs186272589	3	136563367	2.4	G/T	0.99	*STAG1, SLC35G2, NCK1-DT, NCK1*	$4.2x10^{-8}$	7.6	3.7–15.8
Suggestive associations									
chr3:195399925:D	3	195399925	4.3	CA/C	0.54	*SDHAP2*	$6.1x10^{-8}$	6.6	3.3–13.0
rs75852846	1	185386211	3.6	G/T	1.00	Position close to *GS1-279B7.1*	$9.6x10^{-8}$	4.8	2.7–8.4

Table 6.3: Overview of most promising genetic biomarkers for overall survival in patients with colorectal peritoneal metastasis treated with cytoreductive surgery-hyperthermic intraperitoneal chemotherapy

SNP	Chromosome	Position	MAF (%)	Allele	Imputation quality score	Gene(s)	p-value	Hazard ratio	95% CI
Significant associations									
rs71446010	2	31859714	6.2	T/C	0.80	Position close to *SRD5A2*	$9.0x10^{-11}$	5.7	3.4–9.6
chr10:92923875:D	10	92923875	2.9	ACG/A	0.93	*PCGF5*	$1.5x10^{-9}$	7.3	3.8–13.9
rs35741321	2	31802395	2.3	C/G	0.86	*SRD5A2*	$2.5x10^{-9}$	9.1	4.4–18.8
rs79379878	2	32152861	4.5	A/C	0.63	*MEMO1, DPY30, SLC30A6*	$3.1x10^{-9}$	7.6	3.9–14.8
rs12218128	10	92914103	2.9	T/C	0.89	*PCGF5*	$3.7x10^{-9}$	7.2	3.7–13.8
rs117574388	17	20863966	3.2	G/A	0.67	*LOC339260*	$4.2x10^{-9}$	7.9	4.0–15.7
rs12995113	2	32221763	4.2	T/C	0.59	*MEMO1, DPY30, SLC30A6*	$1.1x10^{-8}$	8.5	4.1–17.7
rs34093135	2	31843946	3.4	T/C	0.94	Position close to *SRD5A2*	$1.3x10^{-8}$	6.1	3.3–11.4
rs189469899	7	100336598	6.1	C/T	0.42	Position close to *EPO*	$1.5x10^{-8}$	7.9	3.9–16.1
rs72870052	11	13859182	2.8	T/C	0.78	*LINC02548*	$2.8x10^{-8}$	8.6	4.0–18.3
rs71446025	2	32084129	4.7	C/T	0.64	*MEMO1, DPY30, SLC30A6*	$3.0x10^{-8}$	6.4	3.3–12.4
rs11228914	11	56871958	6.8	C/T	0.96	*OR5AK2, OR5AK4P*	$3.1x10^{-8}$	3.1	2.1–4.6
rs114467817	2	32083640	4.7	T/C	0.64	*MEMO1, DPY30, SLC30A6*	$3.2x10^{-8}$	6.4	3.3–12.4
rs114619308	2	32400896	2.0	G/T	0.56	*SLC30A6*	$3.2x10^{-8}$	19.5	6.8–55.8
rs74374586	11	56843250	6.7	G/A	0.97	*OR5AK2, OR5AK4P*	$3.3x10^{-8}$	3.1	2.1–4.6
rs79286680	11	56890335	6.9	C/T	0.97	*OR5AK2, OR5AK4P*	$4.2x10^{-8}$	3.0	2.0–4.5
rs75575884	4	166243491	2.3	T/C	0.61	*KLHL2, MSMO1*	$4.4x10^{-8}$	12.2	5.0–30.0

Table 6.3: ***Continued***

SNP	Chromosome	Position	MAF (%)	Allele	Imputation quality score	Gene(s)	p-value	Hazard ratio	95% CI
rs713550	11	56894225	6.9	G/T	0.97	*OR5AK2, OR5AK4P*	$4.4x10^{-8}$	3.0	2.0–4.5
rs75241437	11	56898883	7.0	C/T	0.97	*OR5AK2, OR5AK4P*	$4.4x10^{-8}$	3.0	2.0–4.5
rs76535254	11	56898941	7.0	C/A	0.97	*OR5AK2, OR5AK4P*	$4.4x10^{-8}$	3.0	2.0–4.5
rs11118216	1	207808529	2.2	G/A	0.50	*CR1, CR1L, CR2, MIR29B2CHG, CD34, PLXNA2*	$4.5x10^{-8}$	14.8	5.6–38.9
rs144862583	4	9476655	4.1	G/A	0.51	Position close to *AC116655.1*	$4.5x10^{-8}$	8.1	3.8–17.2
rs71446057	2	32414391	3.8	C/T	0.64	*MEMO1, DPY30, SLC30A6*	$4.7x10^{-8}$	7.5	3.6–15.4
Suggestive associations									
rs2543317	12	9725060	93.7	T/C	0.43	Position close t0 *KLRB1*	$5.0x10^{-8}$	0.1	0.1–0.3
rs189134266	18	24555632	3.4	T/A	0.42	*AQP4, AQP4-AS1, CHST9*	$5.3x10^{-8}$	13.5	5.3–34.4
rs117280856	13	52533230	2.2	C/G	0.78	*ATP7B, NEK3, THSD1, VPS36*	$5.3x10^{-8}$	9.1	4.1–20.2
rs115158822	4	166246866	2.4	T/C	0.61	*KLHL2, MSMO1*	$6.2x10^{-8}$	11.9	4.8–29.1
rs10792074	11	56785625	7.8	G/T	0.94	*OR5AK2, OR5AK4P*	$7.5x10^{-8}$	2.8	1.9–4.0
rs77984426	11	56798620	7.8	A/T	0.95	*OR5AK2, OR5AK4P*	$8.2x10^{-8}$	2.7	1.9–4.0
rs11228855	11	56711467	8.1	C/T	0.92	*OR5AK2, OR5AK4P*	$9.1x10^{-8}$	2.8	1.9–4.0

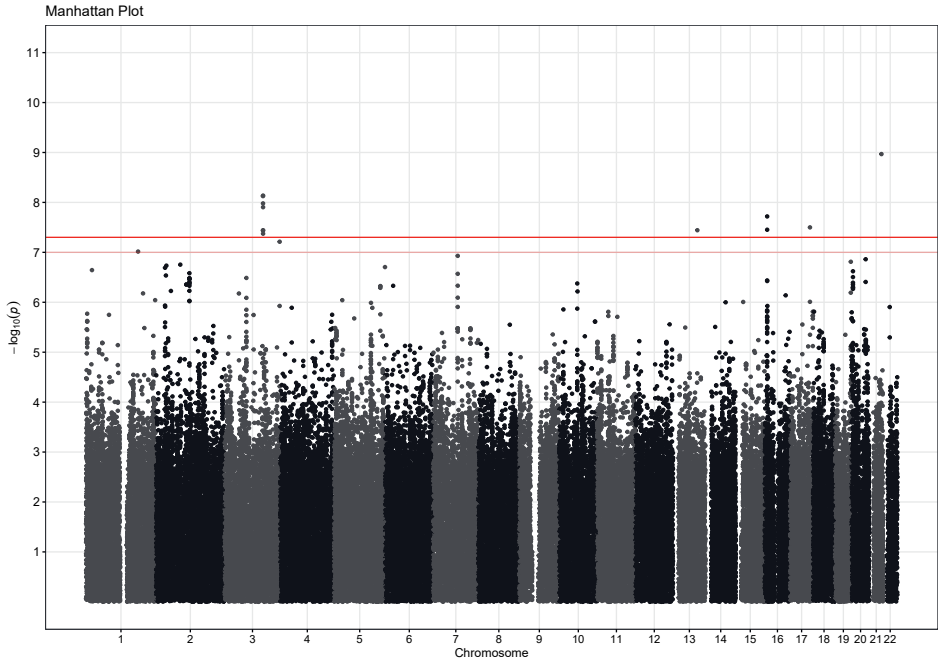

Figure 6.1: Genome-wide association analysis for predictors of disease-free survival on cytoreductive surgery-hyperthermic intraperitoneal chemotherapy in patients with colorectal peritoneal metastases.

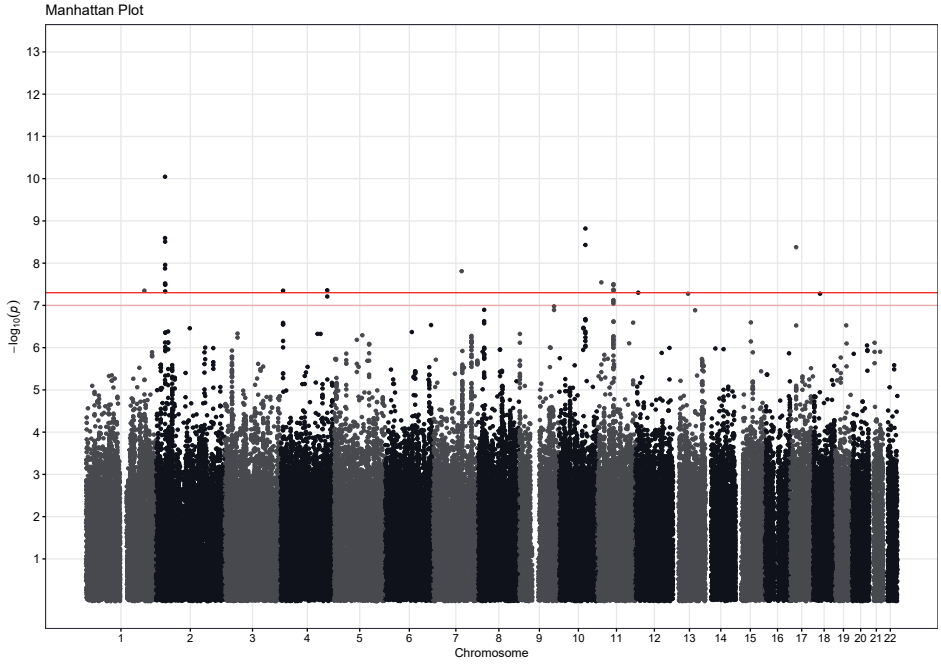

Figure 6.2: Genome-wide association analysis for predictors of overall survival on cytoreductive surgery-hyperthermic intraperitoneal chemotherapy in patients with colorectal peritoneal metastases.

DISCUSSION

We conducted a genome wide association analysis in patients with peritoneal metastases of colorectal origin treated with CRS + HIPEC. To the best of our knowledge, this is the first genome wide association analysis conducted in this type of patient population. With this study, we aimed to identify new genetic biomarkers that are associated with clinical outcome of CRS + HIPEC.

We identified twelve genetic biomarkers that were significantly associated with a worse DFS. These genetic biomarkers influence the regulatory motifs and/or expression of the following six corresponding genes: *FAM3B*, *STAG1*, *SCL35G2*, *TMEM114*, *METLL22*, and *LINC00351*. For OS, a total of 23 genetic biomarkers were significantly associated with shorter survival, corresponding to the following ten genes whose expression is influenced by these markers: *SRD5A2*, *DPY30*, *SLC30A6*, *OR5AK2*, *PCGF5*, *EPO*, *KLHL2*, *MSMO1*, *CR1*, and *AC116655.1*.

For 11 out of 16 identified genes (*FAM3B*, *STAG1*, *SCL35G2*, *METLL22*, *SRD5A2*, *DPY30*, *SLC30A6*, *PCGF5*, *EPO*, *KLHL2*, and *MSMO1*) one or more biological mechanisms were found that are in support with our finding that these genes are associated with DFS and OS. The genes that seemed the most promising are further discussed here. *FAM3B* encodes for the FAM3B protein that induces apoptosis of alpha and beta cells and is present in insulin secretory cells. However, FAM3B expression is also high in most colon cancer cell lines and it is suggested that it promotes colon cancer cell invasion and metastasis and could therefore potentially serve as a prognostic marker [27]. In addition, upregulation of *FAM3B* has been shown to trigger cisplatin resistance in gastric cancer cells which indicates that this could possibly be a predictive marker because in CRS + HIPEC treatment oxaliplatin similarly forms platinum-DNA adducts as cisplatin [28]. With regard to *STAG1*, a gene encoding for a key subunit of cohesin; this protein mediates sister chromatid cohesion, and is also involved in the DNA repair pathway. Cohesin is required for repair of DNA double-strand breaks, and thereby may biologically explain our finding that a genetic variant of *STAG1* could potentially be prognostic and/or predictive for clinical outcome of CPM patients treated with CRS + HIPEC [29]. *METTL22* encodes for a protein which belongs to the methyltransferase-like family, a diverse group of methyltransferases that methylate nucleotides, proteins, and small molecules. Several of these transferases, such as METTL3 and METTL14, have an established role in cancer progression [30]. METTL22 interacts with its substrate, Kin17, which is involved in DNA repair and replication and mRNA processing. As has been mentioned before defects in the DNA repair pathway could potentially be prognostic and/or predictive for clinical outcome

of CPM patients treated with CRS + HIPEC. *DPY30* encodes for DPY30 which is a core subunit of the SET1A methyltransferase complex. This complex seems to be involved in the methylation of YAP and histone H3 in colorectal cancer cells which in turn leads to cancer stemness. Cancer stemness is seen as a source of development and progression of colorectal cancer and therefore *DPY30* might be of prognostic value in colorectal cancer patients [31]. Finally, *MSMO1* encodes for a protein that is involved in cholesterol biosynthesis. In cancer, it has been mentioned as a prognostic factor for the progression of cervival squamous cell carcinoma and pancreatic cancer. MSMO1 seems to act as a tumour suppressor, positive MSMO1 expression indicated a significantly better prognosis and an independent favourable prognostic factor [32, 33].

The results of this genome wide association analysis show that there lies potential in the search for new genetic biomarkers in the colorectal CRS + HIPEC population. This is one of the few pharmacogenetics studies in CPM patients, and is the first GWAS in CPM patients. Patients were derived from a consecutively treated patient cohort which reflects current clinical practice and can be considered free of selection bias.

A limitation of our cohort is the low number of patients and the patient heterogeneity; patients were treated with HIPEC with mitomycin C and oxaliplatin and patients were included over a long time span. Moreover, because of this long time span we were not able to report on several biomarkers such as BRAF, KRAS mutations or CMS classification. However, the most important biomarkers were included as covariates in our analysis based on the COMPASS. Another limitation is the DNA quality used for genotyping resulting in low call rates per patient. We have no indication that there is differential genotyping error as a result, but improving the protocols about sample acquisition and DNA extraction might help follow-up studies. The inflation factor of 1.09 for our OS results is slightly elevated potentially leading to increased rate of false positives. We investigated population stratification by including two MDS components into the regression models which did not substantially change the inflation factor. This suggests that our results need further replication.

Future research should further elaborate on whether the association between the markers that were identified and (disease-free) survival is prognostic or predictive. We were not able to make a distinction between prognostic and/or predictive genetic biomarkers for treatment outcome because there was no untreated patient cohort available. All patients that we included were treated with CRS + HIPEC. In addition, this study was conducted in germline DNA, it would be of great interest to also study these genetic biomarkers in tumour DNA.

In conclusion, several new genetic biomarkers were identified that were associated with clinical outcome in patients with peritoneal metastases treated with CRS + HIPEC, and for which potential biological mechanism could be found. Further research and validation of our findings are warranted.

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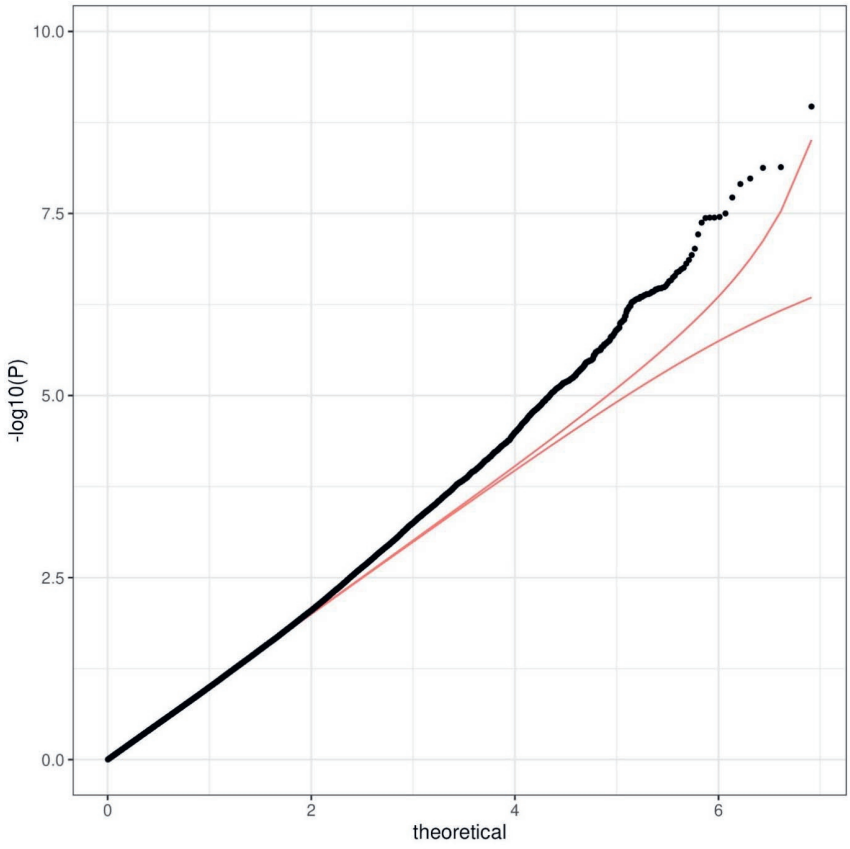
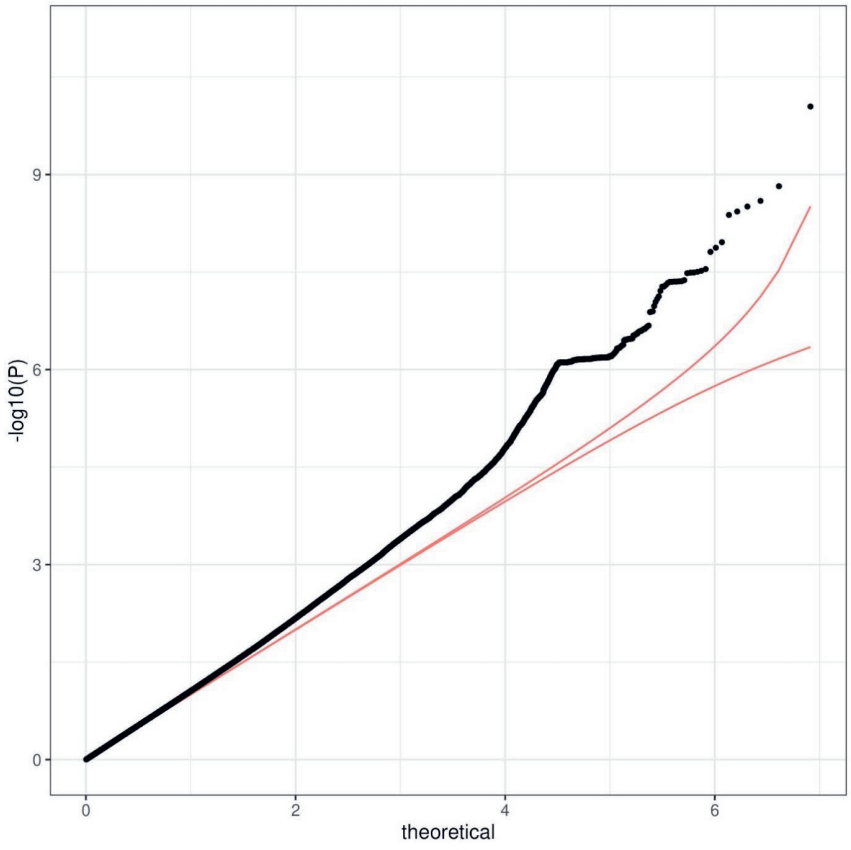
SUPPLEMENTARY MATERIAL

Supplementary figures



Supplementary Figure S6.1: Quantile-Quantile (QQ) plot for disease-free survival.



Supplementary Figure S6.2: Quantile-Quantile (QQ) plot for overall survival.

CHAPTER 7

Identification of pharmacogenetic biomarkers for efficacy of cytoreductive surgery plus hyperthermic intraperitoneal mitomycin C in patients with colorectal peritoneal metastases

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ABSTRACT

Introduction

Mitomycin C (MMC) is commonly used in patients with colorectal peritoneal metastases (CPM) treated with cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy (CRS + HIPEC). MMC requires metabolic activation prior to exert its cytotoxic effect of which the main activating enzymes are NQO1 and POR. However, not all patients are able to activate MMC for example due to polymorphisms in the genes encoding these enzymes. The aim of this study was to investigate the association of *NQO1*2*, *NQO1*3*, and *POR*28* with the efficacy of CRS + HIPEC with MMC in patients with CPM.

Method

A retrospective follow-up design was used to study genetic association in patients with histologically proven CPM treated with CRS + HIPEC with MMC with respect to peritoneal recurrence rate after 3 months (primary endpoint), after 6 months, disease-free survival and overall survival. Genetic polymorphisms *NQO1*2*, *NQO1*3*, and *POR*28* were tested for association.

Results

A total of 253 patients were included. In *NQO1*3* carriers the peritoneal recurrence rate 3 and 6 months after HIPEC was significantly higher than in wild type patients, respectively 30.0% vs 3.8% (p=0.009) and 40.0% vs 12.1% (p=0.031). In line with these results, *NQO1*3* was associated with a shorter disease-free survival (HR 2.04, 95% CI [1.03–4.03]). There was no significant association with overall survival (HR 1.42, 95% CI [0.66–3.07]).

Conclusion

Carriership of the *NQO1*3* allele is associated with worse peritoneal recurrence rate and disease-free survival. These results suggest that individualization of patients treated with CRS + HIPEC based upon pharmacogenetics may be beneficial.

INTRODUCTION

Peritoneal metastases occur frequently in patients with colorectal cancer (CRC), affecting about 10% of patients and is characterized by a poor outcome [1, 2]. Before the introduction of cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC), prognosis of these patients was characterized by a poor quality of life and very limited survival despite treatment with palliative chemotherapy [3, 4]. The implementation of CRS + HIPEC has led to a major increase in overall survival or even cure in some patients and is standard of care for patients with limited peritoneal metastases [1, 5]. Unfortunately, many people experience recurrent disease. Over the past decades, multiple factors have been identified to increase the risk of recurrence, such as the extent of peritoneal disease, the completeness of cytoreduction, and signet ring cell differentiation [6]. This knowledge allows selection of patients with more favourable characteristics for treatment with CRS + HIPEC.

Another factor that could contribute to early recurrence of disease are the pharmacokinetic and pharmacodynamic properties of the chemotherapeutic agent used during HIPEC. Mitomycin C (MMC) is one of the most commonly used drugs in this setting, which is an anti-tumour antibiotic isolated from Streptomyces Caespitosus [7]. MMC is a bio-reductive drug, requiring metabolic activation prior to be able to exert its cytotoxic effects, i.e. the generation of DNA crosslinks [8–11]. However, in some patients this activation does not occur, as demonstrated by Van der Speeten et al.: no traces of the active metabolites of MMC were found in peritoneal fluid, plasma and urine in 6 (4%) out of 145 patients that received CRS + HIPEC with MMC [12]. Clearly, this could reduce the efficacy of CRS + HIPEC with MMC.

The main enzymes involved in metabolic activation of MMC are NADPH:quinone oxidoreductase 1 (NQO1) and NAD(P)H:cytochrome P450 (oxido) reductase (POR) [11] (**Figure 7.1**) [9]. NQO1 is a cytosolic flavoprotein that catalyses the two-electron reduction of MMC and is highly expressed in tumour tissues [13–15]. POR is a microsomal flavoprotein which acts through a one-electron reductive mechanism. Polymorphisms that are known to result in diminished or even absent activity of NQO1 and POR are *NQO1*2* (rs1800566), *NQO1*3* (rs1131341), and *POR*28* (rs1057868), respectively [16–22]. However, results on the association between these polymorphisms and response to MMC are conflicting and scarce [16, 18, 20, 21], and has thus far not properly been investigated in a large cohort of patients.

We hypothesized that patients with reduced activation capacity by NQO1 or POR due to a genetic polymorphism have a decreased response to MMC chemotherapy in the CRS + HIPEC setting. Therefore, the aim of this study was to investigate the association of *NQO1*2*,

*NQO1*3*, and *POR*28* with the efficacy of CRS + HIPEC treatment with MMC in patients with colorectal peritoneal metastases (CPM).

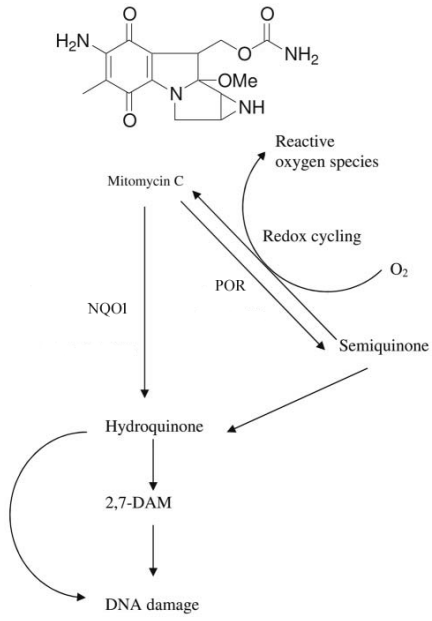

Figure 7.1: Schematic overview of MMC metabolic activation [9].
POR = NAD(P)H:cytochrome P450 (oxido) reductase, NQO1 = NADPH:quinone oxidoreductase 1, other reductases might also play a role in metabolic activation of MMC [9].

MATERIALS AND METHODS

Study design

This was a retrospective follow-up study in patients with CPM that were treated with CRS + HIPEC with MMC. We studied the effect of *NQO1*2*, *NQO1*3*, and *POR*28* genotype on the peritoneal recurrence rate (PRR) after 3 months as primary endpoint. Secondary endpoints were PRR after 6 months, disease-free survival (DFS) and overall survival (OS). The study was approved by the local medical ethical review board and was not subject to the medical research involving human subjects act. Data was anonymised before data analysis.

Patients

All patients with histologically proven CPM that were treated with CRS + HIPEC with MMC in a large tertiary HIPEC centre in the Netherlands between January 2007 and December 2017 were included in this study. During this period there were no changes in patient selection

and treatment regimen. MMC was administered in a dosage of 35 mg/m^2 at 40°C–42°C and was circulated for 90 minutes. Patients with appendiceal cancers or neoplasms were excluded, as well as any patient that did not receive a complete cytoreduction since these patients had residual disease after CRS. Completeness of cytoreduction was determined by the completeness of cytoreduction score (CCR), patients with a CCR score >1 were excluded [6] As peritoneal recurrence was the primary endpoint, systemic metastases were not considered a contra-indication in the current analysis.

Data collection

Relevant patient, tumour and treatment characteristics were collected retrospectively. The edition of the Tumour-Nodal-Metastases (TNM) classification that was registered at the time of diagnosis was used for classification of the primary tumour. The sixth TNM edition was used from 2007 to 2009; the seventh TNM edition was used from 2010 to 2016; the eight TNM edition was used from 2017 onwards.

CPM were considered synchronous metastases when diagnosed before or within three months after primary tumour resection, and were considered metachronous metastases when diagnosed more than three months after primary tumour resection. The extent of CPM was determined according to the peritoneal cancer index (PCI) as described by Jacquet and Sugarbaker [23]. The CCR was used to measure the amount of visible remaining tumour after CRS [6].

After successful CRS + HIPEC, standard follow-up was performed according to the colorectal cancer guidelines and consisted of laboratory testing of carcinoembryonic antigen (CEA) every three months, a liver ultrasound every six months, and an abdominal CT-scan every 12 months. In case recurrent disease was suspected based on clinical parameters, CEA elevation, or liver ultrasound outcomes, an additional abdominal CT-scan was performed.

Patients considered fit for chemotherapy were offered adjuvant chemotherapy. However, a systematic review from 2017 questioning the effect of adjuvant chemotherapy after CRS + HIPEC resulted in a decrease in the administration of adjuvant chemotherapy [24]. Patients treated with adjuvant chemotherapy received their first cycle of chemotherapy within 3 months after primary tumour resection.

Latest data on vital status was collected from the Municipal Administrative Database in December 2018 in which all births, deaths and emigrations of the Dutch population are recorded.

DNA isolation and genotyping

Formalin-fixed, paraffin embedded (FFPE) material from normal tissue was used for DNA isolation. Normal tissue was selected based on pathology reports. Normal tissue was used since *NQO1*2* (rs1800566), *NQO1*3* (rs1131341), and *POR*28* (rs1057868) are germline variants and previous studies presented no discrepancies in *NQO1*2* genotype status between DNA isolated from normal tissue compared to tumour tissue [17,20]. *NQO1*2*, *NQO1*3*, and *POR*28* genotype status was determined using TaqMan SNP genotyping assays. Briefly, DNA was isolated from FFPE tissue cores using the automated tissue preparation system (Siemens Healthcare Diagnostics, Tarrytown, NY), as described previously [25]. Genotyping reactions were performed using the ViiA 7 real-time PCR system and QuantStudio 12K Flex Real-Time PCR system. Commercial primers and probes kits were used and originated from Thermo Fisher Scientific.

Endpoints

PRR was defined as peritoneal recurrence or peritoneal plus systemic recurrence and was calculated as the proportion of patients who had a peritoneal recurrence at t=3 or 6 months after HIPEC. These time points were chosen due to our hypothesis that patients not able to activate MMC are expected to have an early local recurrence.

DFS and OS were calculated from the date of HIPEC until first recurrence or death, or until death, respectively. All recurrences, both peritoneal as well as extra-peritoneal recurrence, were included.

Statistical analysis

Patient characteristics were analysed using descriptive statistics. Genotype frequencies were tested for Hardy-Weinberg equilibrium (HWE), a p-value of $p>0.05$ was considered consistent with HWE.

The association between each polymorphism (*NQO1*2*, *NQO1*3*, and *POR*28*) and baseline patient characteristics and PRR within 3 and 6 months after HIPEC was analysed through the Chi-squared test or Fisher's Exact Test, depending on the number of events. A dominant model was used. These analyses were not corrected for covariates as the low number of events does not allow to fit logistic models reliably when including covariates. The secondary endpoints are used to confirm these analyses.

In order to correct for other known predictive or prognostic markers for treatment outcome such as PCI score or signet ring cell carcinoma, the association between each allele (*NQO1*2*,

*NQO1*3*, and *POR*28*) and DFS and OS was tested using multivariate cox-regression analysis. Univariate analysis was conducted using log-rank test. The following pre-specified covariates, known to affect clinical outcome, were used: age (years), signet ring cell differentiation (yes/no), N stage (N0-N1 versus N2), presentation of CPM (synchronous/metachronous), PCI (PCI<10 versus PCI≥10), and adjuvant chemotherapy (yes/no). The covariates were chosen based on the Colorectal Peritoneal Metastases Prognostic Surgical Score (COMPASS) as described by Simkens et al. [26]. All tables report nominal p-values and a significance-level of 0.05 was applied. Multiple testing correction was used for the primary endpoint. All other analyses are considered exploratory and no correction for multiple testing was performed. The statistical analysis was performed with SPSS for Windows (version 25.0; IBM Corp., Armonk, NY, USA).

RESULTS

Patient characteristics

In total, 253 patients underwent CRS + HIPEC with MMC and were eligible for genotyping of *NQO1*2*, *NQO1*3*, and *POR*28*. The mean age was 62±10.4 years. Seven percent of the patients had signet ring cell differentiation. A total of 44% of the patients had a nodal stage of 2 and half of the patients presented with synchronous CPM. The PCI during CRS + HIPEC was ≥10 in 42% of the patients and 46% of the patients received adjuvant chemotherapy within 3 months after surgery (**Table 7.1**). *NQO1*2*, *NQO1*3*, and *POR*28* were not associated with any of the baseline patient characteristics. The median follow-up of patients was 22 months, ranging from 11 days to 9.6 years. In addition, the median DFS and OS of patients were 10 months and 29 months, respectively (**Figure 7.2**). The observed allele frequencies of *NQO1*2*, *NQO1*3*, and *POR*28* were 21.0%, 2.0% and 31.0%, respectively and were all consistent with HWE (**Table 7.2**).

Peritoneal recurrence rate

PRR at 3 and 6 months could be obtained in 92% (233/253) and 90% (228/253) of the patients, respectively. PRR status in the remaining patients could not be obtained because follow-up was too short or recurrence location was unknown. The PRR 3 months after CRS + HIPEC was significantly higher in the *NQO1*3* HE/HO group than in the WT group (30.0% vs 3.8%, OR=10.9, p=0.009). After Bonferroni correction, the association remained significant (p=0.027, corrected for three tests performed). Also at six months after HIPEC the differ-

ence in PRR remained (40.0% vs. 12.1%, OR=4.9, p=0.031). No association was observed between *NQO1*2* or *POR*28* genotype status and PRR 3 and 6 months after CRS + HIPEC (**Table 7.3**).

Table 7.1: Patient characteristics

	n=253	(%)
Sex		
Male	115	(45.5)
Female	138	(54.5)
Age (mean ±SD in years)	62	±10.4
Primary tumour location		
Right colon	91	(36.0)
Sigmoid	89	(35.2)
Rectum	38	(15.0)
Other (transverse, left colon)	33	(13.0)
Missing	2	(0.8)
Signet ring cell differentiation		
Yes	17	(6.7)
No	236	(93.3)
Tumour stage		
T1-2	9	(3.6)
T3	110	(43.5)
T4	132	(52.2)
Missing	2	(0.8)
Nodal stage		
N0-N1	139	(54.9)
N2	112	(44.3)
Missing	2	(0.8)
Presentation of peritoneal metastases		
Synchronous	128	(50.6)
Metachronous	125	(49.4)
ASA score		
1	23	(9.1)
2	207	(81.8)
3	23	(9.1)
Peritoneal cancer index		
<10	145	(57.3)
≥10	105	(41.5)
Missing	3	(1.2)
Adjuvant chemotherapy		
Yes	116	(45.8)
No	137	(54.2)

Patient cohort consists out of patients with peritoneal metastasis from colorectal cancer treated with CRS + HIPEC with mitomycin C. CRS = cytoreductive surgery, HIPEC = Hyperthermic Intraperitoneal Chemotherapy, SD = standard deviation, ASA = American Society of Anaesthesiology.

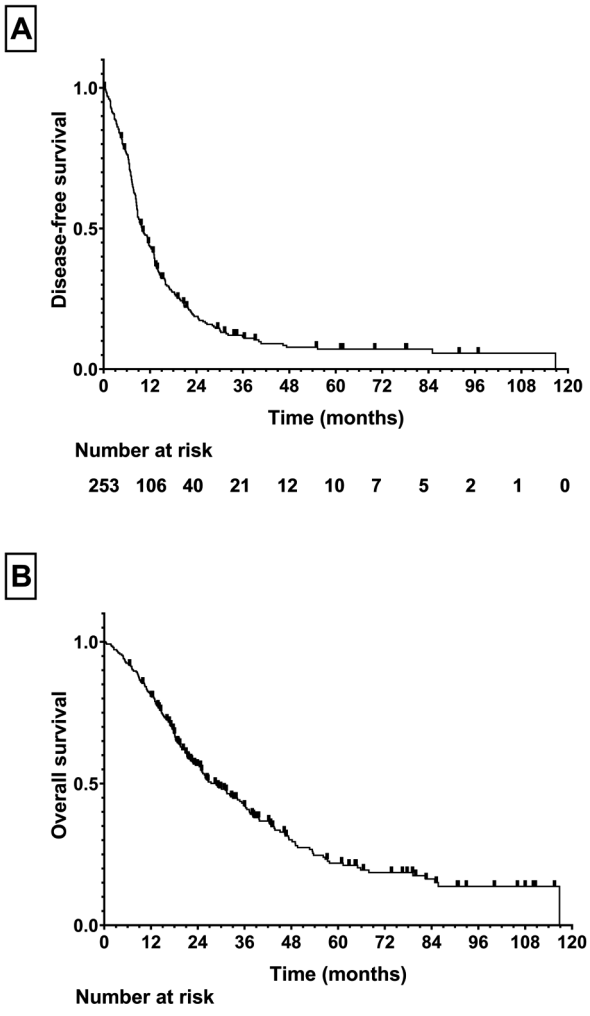

Figure 7.2: Disease-free survival (A) and overall survival (B) of the total patient cohort consisting of patients with peritoneal metastasis from colorectal cancer treated with CRS + HIPEC with mitomycin C.

Survival

In univariate analysis none of the polymorphisms were associated with DFS or OS. Results of the multivariate cox-regression analyses of the effect of genotype on DFS and OS including all other clinically relevant covariates are provided in **Table 7.4**. Carriers of the *NQO1*3* variant allele had a decreased DFS (HR 2.04, 95% CI [1.03–4.03], p=0.040) compared to wild type patients. This was not observed for carriers of the *NQO1*2* and *POR*28* polymorphisms. No association was observed between any of the three polymorphisms and overall survival.

Table 7.2: Genotype frequencies of total patient cohort

Genotype status	n=253	(%)	HWE p-value	MAF
*NQO1*2*				
GG	155	(61.3)	0.70	0.21
GA	85	(33.6)		
AA	10	(4.0)		
Unknown	3	(1.2)		
*NQO1*3*				
GG	232	(91.7)	0.74	0.02
GA	10	(4.0)		
AA	0	(0.0)		
Unknown	11	(4.3)		
*POR*28*				
CC	122	(48.2)	0.057	0.31
CT	90	(35.6)		
TT	29	(11.5)		
Unknown	12	(4.7)		

Patient cohort consists out of patients with peritoneal metastasis from colorectal cancer treated with CRS + HIPEC with mitomycin C. CRS = cytoreductive surgery, HIPEC = Hyperthermic Intraperitoneal Chemotherapy, HWE = Hardy Weinberg Equilibrium, MAF= minor allele frequency, GG = wild type, GA = heterozygous, AA = homozygous, CC = wild type, CT = heterozygous, TT = homozygous.

Table 7.3: Association of *NQO1*2*, *NQO1*3*, and *POR*28* with peritoneal recurrence rate 3 and 6 months after CRS + HIPEC

Genotype status	PRR within 3 months			PRR within 6 months		
	PRR (%)	OR [95% CI]	p-value	PRR (%)	OR [95% CI]	p-value
*NQO1*2*						
GG	8/144 (5.6)	1.0 [0.33–3.32]	1.00	16/140 (11.4)	1.9 [0.92–4.08]	0.08
GA/AA	5/86 (5.8)			17/85 (20.0)		
*NQO1*3*						
GG	8/212 (3.8)	10.9 [2.38–50.3]	0.009	25/207 (12.1)	4.9 [1.28–18.4]	0.031
GA/AA	3/10 (30.0)			4/10 (40.0)		
*POR*28*						
CC	7/111 (6.3)	0.7 [0.22–2.30]	0.56	16/108 (14.8)	0.9 [0.40–1.86]	0.69
CT/TT	5/110 (4.5)			14/108 (13.0)		

PRR = peritoneal recurrence rate, OR = Odds ratio, 95% CI = 95% confidence interval, GG = wild type, GA = heterozygous, AA = homozygous, CC = wild type, CT = heterozygous, TT = homozygous.

Table 7.4: Association of *NQO1*2*, *NQO1*3*, and *POR*28* with disease-free survival and overall survival

	DFS		OS		
Polymorphism/covariate	HR [95% CI]	p-value	HR [95% CI]	p-value	n*
*NQO1*2*	0.88 [0.696–1.117]	0.30	0.86 [0.654–1.127]	0.27	245
Age	1.00 [0.982–1.008]	0.46	1.02 [1.000–1.031]	0.049	
Signet ring cell differentiation	1.09 [0.593–2.000]	0.78	1.98 [1.034–3.788]	0.039	
N stage	1.20 [0.901–1.609]	0.21	1.23 [0.882–1.707]	0.23	
PC synchronous/metachronous	1.00 [0.739–1.366]	0.98	0.75 [0.527–1.056]	0.10	
PCI	2.08 [1.572–2.763]	<0.001	2.59 [1.876–3.582]	<0.001	
Adjuvant chemotherapy	0.51 [0.376–0.685]	<0.001	0.66 [0.469–0.925]	0.016	
*NQO1*3*	2.04 [1.032–4.025]	0.040	1.42 [0.656–3.086]	0.37	237
Age	0.99 [0.982–1.007]	0.39	1.02 [1.001–1.032]	0.035	
Signet ring cell differentiation	1.15 [0.637–2.080]	0.64	2.03 [1.084–3.813]	0.027	
N stage	1.13 [0.842–1.523]	0.41	1.18 [0.838–1.651]	0.35	
PC synchronous/metachronous	1.07 [0.779–1.466]	0.68	0.78 [0.548–1.119]	0.18	
PCI	2.15 [1.611–2.861]	<0.001	2.62 [1.883–3.645]	<0.001	
Adjuvant chemotherapy	0.52 [0.381–0.702]	<0.001	0.68 [0.478–0.956]	0.027	
*POR*28*	0.92 [0.752–1.129]	0.43	1.09 [0.865–1.366]	0.47	236
Age	0.99 [0.980–1.006]	0.27	1.01 [0.999–1.030]	0.067	
Signet ring cell differentiation	1.06 [0.576–1.954]	0.85	1.93 [1.003–3.697]	0.049	
N stage	1.29 [0.952–1.754]	0.10	1.23 [0.872–1.730]	0.24	
PC synchronous/metachronous	1.08 [0.786–1.496]	0.62	0.80 [0.562–1.146]	0.23	
PCI	2.09 [1.573–2.788]	<0.001	2.59 [1.868–3.598]	<0.001	
Adjuvant chemotherapy	0.54 [0.396–0.731]	<0.001	0.70 [0.499–0.991]	0.044	

* Due to missing covariates, some patients were excluded from this analysis. DFS = disease-free survival, OS = overall survival, HR = hazard ratio, 95% CI = 95% confidence interval, PC = peritoneal cancer, PCI = peritoneal cancer index.

DISCUSSION

The aim of this study was to investigate the association of *NQO1*2*, *NQO1*3*, and *POR*28* with the efficacy of CRS + HIPEC treatment with MMC in patients with CPM. We hypothesized that patients with reduced activation capacity by NQO1 or POR due to a genetic polymorphism have a decreased response to MMC chemotherapy in the CRS + HIPEC setting. It is of utmost importance to identify these patients that might not benefit from HIPEC with MMC since this is a toxic and high cost treatment. This study was conducted as a proof of principle for our hypothesis that *NQO1* or *POR* polymorphisms could lead to treatment failure with HIPEC MMC.

A clear association was observed between the *NQO1*3* polymorphism and both the peritoneal recurrence rate as well as disease-free survival in patients treated with CRS + HIPEC with mitomycin C. No association was found for the other two polymorphisms, i.e. *NQO1*2* and *POR*28*. Thereby, the study results are in line with the hypothesis and suggest a decreased efficacy of MMC in *NQO1*3* carriers.

This is the first study to report on the association between *NQO1*3* variant allele carriers and the efficacy in patients treated with CRS + HIPEC with MMC. Our findings are supported by preclinical findings in human colon cancer cells in which the *NQO1*3* polymorphism proved to be associated with resistance to treatment with MMC [16]. Also in human tumour xenograft models, knockout mice and human bladder tumours decreased NQO1 activity was associated with reduced activity of MMC, in line with the observations in our study [27–29].

Whereas for *NQO1*3* significant associations were observed, for *NQO1*2* there was no association. This is unlike the results of another clinical study as reported by Fleming et al. [20]. Possible explanations for this conflicting result with Fleming et al. could be the different number of patients included in the two studies and the different way in which HIPEC was administered.

The exact effect needs to be further investigated; of note, a study in xenograft [18] and a clinical study in bladder cancer patients [21] did not show an association, in line with our findings. Although an association with PRR and DFS could be demonstrated, none of the tested polymorphisms were associated with OS in our patient population. However, this is perhaps not entirely surprising; a possible explanation why an effect on OS could not be observed is the fact that HIPEC treatment with MMC is a local treatment given locally in the peritoneum, and it is administered only once. Therefore, a single administration may potentially not be expected to have large effects on a long term endpoint such as OS. For HIPEC with oxaliplatin this was presented in a randomized multicentre trial (n=265), the addition of HIPEC with oxaliplatin

to CRS did not change OS in patients with CPM [30]. For HIPEC with MMC the effect on OS has not yet been established. In addition, we did not correct for additional lines of treatment more than 3 months after HIPEC which also largely affect the overall survival rate. If patients have a recurrence after HIPEC, these patients will often receive other treatments such as palliative systemic chemotherapy.

Limitations of the present study may be, firstly, patients were included over a long time period of 10 years which may have led to heterogeneity in our population and the follow-up. However, the treatment protocol during this period did not change. Secondly, our follow-up method may not have been fully ideally for determination of the endpoint peritoneal recurrence at 3 months since there was no standardized CT at 3 months. Notwithstanding, follow-up for all patients was conducted following standardized procedures: in case recurrent disease was suspected based on clinical parameters, CEA elevation, or liver ultrasound outcomes, an abdominal CT-scan was performed. Thirdly, our primary analysis with PRR did not correct for covariates. Although these results are consistent with covariate corrected secondary analysis on DFS plus our hypothesis about the pharmacological mechanism, we cannot completely exclude spurious confounding as an explanation of our findings. Nonetheless, supporting evidence is available, as preclinical studies have demonstrated that NQO1 levels and NQO1 activity are critical determinants of sensitivity to MMC [16, 31–33]. Fourthly, the group of patients with the *NQO1*3* polymorphism was rather small compared to the wild type group. However, this is a direct consequence from the fact that only a limited number of patients may have the genetic disorder to not be able to fully activate mitomycin into its active form. An estimated 4% of the population has decreased enzyme activity for activation of mitomycin, based on the study by van der Speeten et al. [12]. Our findings are in line with this hypothesis and the event rate was thereby also not expected to be any larger. Lastly, not all important predictive or prognostic biomarkers such as BRAF which could affect early recurrence have been included in our analysis [34]. Notwithstanding, this was a proof of principle study, in order to identify novel pharmacogenetic biomarkers, thereby allowing improved patient selection besides selection on the already known molecular, clinical and pathological biomarkers. Moreover, it is also important to note that patients with an R2 status were excluded, which decreases the likelihood of early recurrence due to failure of cytoreduction within our patient group.

In our study we assessed the effect of genetic variation in only two enzymes within the MMC activation pathway, while other factors in this pathway and DNA repair pathways could also play a role [9]. Further prospective research is needed in an independent cohort of patients to assess whether our findings can be replicated.

In conclusion, the *NQO1*3* polymorphism led to a higher peritoneal recurrence rate and a decreased disease-free survival in patients with peritoneal metastasis from colorectal cancer treated with CRS + HIPEC with MMC. These results suggest that individualization of patients treated with CRS + HIPEC based upon pharmacogenetics may be beneficial and should be subject of further investigation.

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CHAPTER 8

General discussion

Colorectal cancer (CRC) is a widespread disease for which one of the main treatment modalities is chemotherapy. Chemotherapeutic treatment comes with challenges, such as severe adverse events leading to loss of quality of life, treatment discontinuation and sometimes even toxic death. In addition, chances for curation in the metastatic setting are low. Therefore, there is a window of opportunity to improve safety and efficacy of chemotherapeutic treatment of CRC for the individual patient.

The studies described in this thesis aimed to improve the safety and efficacy of chemotherapeutic drugs in patients with colorectal cancer by individualising drug dosing and choice of drug based on germline genetic biomarkers. Within this last chapter, the findings are discussed in a broader perspective and potential clinical implications and future perspectives are provided.

PART I

In **Part I** of this thesis we aimed to implement *UGT1A1* genotype-guided dosing of irinotecan in clinical practice. We showed that the combined conclusion of multiple dose-finding studies indicate that the current standard way of dosing of irinotecan is not safe for UGT1A1 poor metaboliser patients (UGT1A1 PM patients) [1]. Therefore, the DPWG provided a dose recommendation for UGT1A1 PM patients, an initial dose reduction of irinotecan of 30% [1]. This 30% dose reduction for UGT1A1 PM patients was implemented in clinical practice and hereby we proved that this is feasible and a safe starting dose with adequate systemic exposure of the active metabolite SN-38 [2]. The incidence of febrile neutropenia and chemotherapy-related hospital admissions in UGT1A1 PM patients was significantly reduced. *UGT1A1* genotype-guided dosing of irinotecan proved to be feasible in daily practice as there was no delay in start of treatment. Moreover, it proved to be cost-saving compared to non-screening, with a total saving of €183 per patient.

All taken together, *UGT1A1* genotype-guided dosing was successfully implemented in four hospitals in the Netherlands. This thesis has led to acceptance of *UGT1A1* genotype-guided dosing of irinotecan as a new standard of care in the field of oncology in the Netherlands, approximately 70% of the medical oncologists would like to implement *UGT1A1* genotype-guided dosing of irinotecan. This number is based on a survey that was held at a post-ASCO congress in 2021 by the Dutch Society of Medical Oncology.

Wider implementation of *UGT1A1* genotype-guided dosing of irinotecan provides an opportunity for assessment of further efficacy outcomes and this might lead to further incorporation of *UGT1A1* genotype-guided dosing of irinotecan in clinical treatment guidelines, one of the main challenges in the near future. In addition, it is important to point out four other challenges to further personalise CRC treatment with irinotecan.

First, in our study we adapted the dose of irinotecan in UGT1A1 PM patients [3]. No dose adaptations were made in UGT1A1 intermediate metaboliser (IM) and extensive metaboliser (EM) patients. However, literature data suggest that safety-wise it seems possible to escalate the irinotecan dose in UGT1A1 EM patients which might lead to a higher efficacy in these patients [1]. Also, in our study subgroup analysis we noted that the incidence of severe irinotecan-related toxicity in EM patients was lower compared to IM patients which provides an additional argument for dose escalation studies in EM patients [3]. Therefore, further research on clinical efficacy outcomes of irinotecan dose escalation in EM patients is warranted.

Secondly, in our study we did not report on the *UGT1A1*6* variant allele and therefore we excluded patients of Asian origin but the *6 variant allele is also important in this population [4, 5]. The effect of the *6 variant allele on UGT1A1 functionality is comparable to the effect of *28 on UGT1A1 functionality [6]. Therefore, for the Asian population, it is of importance to not only incorporate the *UGT1A1*28* variant in clinical practice, but also the *6 variant. This test should not only be available to patients of Asian origin living in Asian countries, but also to patients of Asian origin in the Netherlands.

Thirdly, although *UGT1A1* genotype-guided dosing showed to reduce severe toxicity, patients may still encounter severe adverse advents such as late onset diarrhoea. Therefore, it is of importance to identify other biomarkers that are predictive for irinotecan-induced severe diarrhoea and to incorporate these in clinical practice. There are two interesting hypotheses that need further research. First, a genetic biomarker that seems a promising predictor of irinotecan-induced severe diarrhoea is *ABCB1* rs1128503. The *ABCB1* gene encodes for P-glycoprotein (P-gp), an ATP-mediated transporter that participates in the biliary excretion of irinotecan and SN-38. It is hypothesized that enhanced P-gp expression increases biliary secretion of SN-38 and thereby increases the risk of severe diarrhoea [7]. Second, it is hypothesized that high activity of the gut microbiota-derived enzyme β-glucuronidase (GUS) will be associated with increased late-onset gastrointestinal activation of SN38-G back to SN38 and thereby leading to irinotecan related-diarrhoea [8, 9]. At the same time, interpatient variability of these gut microbiota-derived GUS enzymes is high, in a sample of 139

individuals a high variability in the number of different GUS enzymes was observed ranging from a minimum of 4 to a maximum of 38 per individual [10]. It is hypothesized that possible targeted interventions such as the use of prebiotic compounds, fecal transplant therapy or antibiotics in selected patient with high GUS activity could reduce the risk of irinotecan-related late onset diarrhoea [8, 9].

Fourthly, with regard to clinical and biological characteristics, CRC is a very diverse illness, resulting in a high variability in disease development and treatment response and therefore calls for a personalised treatment. This has led to the development of the consensus molecular subtypes (CMS) of CRC, a well-studied and robust stratification strategy. CRC can be categorised into four subtypes (CMS1-4) based on differences in gene expression in tumour tissue, which includes both cancer cells and the microenvironment. This tumour molecular profiling by means of CMS classification can potentially predict the efficacy of irinotecan-based systemic therapy. Several studies have reported that irinotecan based-regimens rather than oxaliplatin based-regimens seem to be more effective in metastatic CRC CMS subtypes 1 and 4. Therefore it is of great importance to further investigate this possible biomarker for treatment selection because the first-choice regimen in the first-line setting often is oxaliplatin based [11].

PART II

In **Part II** we aimed to discover genetic biomarkers that are predictive for treatment outcome of colorectal peritoneal metastases patients treated with CRS + HIPEC. It appeared that only two studies investigated the association of biomarkers related to DNA repair and treatment outcome of CRS + HIPEC with mitomycin C or oxaliplatin [12]. However, in patients with colorectal cancer that were treated with intravenously administered oxaliplatin, a clear association between genetic biomarkers in the DNA repair pathway and treatment outcome was reported in literature. Therefore, we conducted a genome-wide association analysis and several genetic biomarkers were identified that were significantly associated with disease-free survival and/or survival in CPM patients that were treated with CRS + HIPEC [13]. In addition, a proof of principle was provided [14]. We hypothesized that patients with reduced activation capacity by NQO1 or POR due to a genetic polymorphism have a decreased response to MMC chemotherapy in the CRS + HIPEC setting. The aim of this candidate gene study was to investigate the association of *NQO1*2*, *NQO1*3*, and *POR*28* with the efficacy of CRS + HIPEC treatment with MMC in patients with CPM. In line with the hypothesis, a

clear association was observed between the *NQO1*3* polymorphism treatment outcome in patients treated with CRS+ HIPEC with mitomycin C. This study shows that pharmacogenetic biomarkers may potentially be useful for treatment selection in this population. However, our exploratory data first need to be confirmed. This was a candidate gene driven approach, potentially also other pharmacogenetic biomarkers within the PK/PD pathway may further explain interpatient variability in treatment response.

Thereby, **Part II** points us further towards the prognostic and/or predictive value of genetic biomarkers for CRS plus HIPEC treatment outcome. However, the amount of evidence is still scarce. Therefore, there is a need for replication studies to validate the genetic biomarkers that were identified. In addition, it would be of great interest to further distinct between the prognostic or predictive effect of these biomarkers. Our patient cohort only consisted out of CPM patients that were treated with CRS + HIPEC, we had no untreated cohort available which is required to conclude whether a genetic biomarker is predictive for treatment outcome [15].

In addition, the genetic biomarkers that were identified all have a low minor allele frequency and therefore a limited clinical relevance in the total patient population. This power to estimate and individual's phenotype based on genotype data can potentially be improved by the introduction of a polygenic risk score. A polygenic risk score can be defined as: "a single value estimate of an individual's common genetic liability to a phenotype, calculated as a sum of their genome-wide genotypes, weighted by corresponding genotype effect size estimates derived from summary statistic GWAS data" [16]. The development of this score seems a worthwhile step from GWA studies towards precision medicine in clinical practice.

In conclusion, the described studies brought us a few steps closer to safe and effective use of chemotherapeutic drugs in the individual colorectal cancer patient. Irinotecan should no longer be administered without a *UGT1A1* genotype test and a start has been made towards personalised medicine for colorectal cancer patients with peritoneal metastases.

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SUMMARY

Colorectal cancer (CRC) is often treated with chemotherapy. However, it is well known that treatment with chemotherapy comes with challenges, such as (severe) adverse events leading to loss of quality of life, treatment discontinuation and sometimes even death. Moreover, chances for curation in the metastatic setting are low. Therefore, a large window of opportunity to improve both safety as well as efficacy of chemotherapeutic treatment for the individual patient exists. A possible approach to improve chemotherapeutic treatment for CRC patients could be the discovery, validation and implementation of new genetic biomarkers. The use of genetic biomarkers allows to identify patients that are at higher risk for severe adverse drug events and to select patients which will benefit the most from chemotherapy. The aim of this thesis was therefore to improve the safety and efficacy of chemotherapeutic drugs in patients with colorectal cancer by individualising drug dosing and choice of drug based on germline genetic biomarkers.

In Part I we focused on the optimisation of systemic treatment with irinotecan. We aimed to evaluate the added value and clinical utility of *UGT1A1* genotype-guided dosing of irinotecan by a systematic review of the literature and the development of a guideline on the drug-gene interaction between irinotecan and *UGT1A1*. For the ultimate evaluation we conducted a prospective implementation study on *UGT1A1* genotype-guided dosing of irinotecan.

In **Chapter 2** we assessed the available evidence and the potential value of *UGT1A1* genotype-guided dosing of irinotecan in order to reduce the risk of severe toxicity based on five pre-specified criteria, including: 1] the level of evidence for associations between *UGT1A1* polymorphisms and irinotecan-induced severe toxicity, 2] the clinical validity and utility of pre-therapeutic genotyping of *UGT1A1*, 3] the safety and tolerability of irinotecan in carriers of *UGT1A1* polymorphisms, 4] the availability of specific dose recommendations for irinotecan in carriers of *UGT1A1* polymorphisms, and 5] the evidence of cost benefits of pre-therapeutic genotyping of *UGT1A1*. On all five criteria, study results were favourable for pre-therapeutic genotyping of *UGT1A1*, namely: the highest level of evidence (level I) was found for a higher incidence of irinotecan-induced severe toxicity in homozygous carriers of *UGT1A1*28* or *UGT1A1*6* (UGT1A1 PMs); the clinical validity and utility of testing for *UGT1A1*28* or *UGT1A1*6* proved to be acceptable; dose-finding studies showed a lower maximum tolerated dose in homozygous variant allele carriers; and most of the drug labels and guidelines recommend a dose reduction of 25 to 30% in these patients. Also, pre-therapeutic genotyping of UGT1A1 is likely cost-saving.

In **Chapter 3** we aimed to facilitate implementation of UGT1A1 genotype guided dosing of irinotecan by writing a guideline – on behalf of the Dutch Pharmacogenetics Working Group (DPWG)

– for physicians and pharmacists, based on a systematic review of the literature on *UGT1A1* genotype-guided dosing of irinotecan. As also has been reported in **Chapter 2**, for UGT1A1 PMs there is ample evidence for an increased risk of serious adverse events such as neutropenia or diarrhoea at normal doses (also when compared to all other genotypes/phenotypes), while convincing evidence for an increased efficacy has not been demonstrated. All nine meta-analyses investigating adverse events, and 16 of the 23 included studies reported this increased risk of toxicity. With regard to efficacy, four of the five meta-analyses and eight of the ten studies did not show the *28 and/or *6 variants to be associated with increased effectiveness of treatment. Based on pharmacokinetic studies, this DPWG guideline recommends a 70% starting dose in UGT1A1 PM patients that start treatment with irinotecan. In UGT1A1 intermediate metaboliser patients, an a priori dose reduction is not recommended. Based on the DPWG clinical implication score, *UGT1A1* genotyping is considered "essential", therefore directing towards pre-therapeutic *UGT1A1* testing in patients intended for treatment with irinotecan.

Chapter 4, describes the first prospective implementation study on *UGT1A1* genotype-guided dosing of irinotecan. In this chapter, *UGT1A1* genotype-guided dosing of irinotecan was implemented in clinical practice. We demonstrated that genotype-guided dosing significantly reduced the incidence of febrile neutropenia and chemotherapy-related hospital admissions in UGT1A1 PMs. Of the 350 evaluable patients, 31 (8.9%) patients were UGT1A1 PM and received a median 30% dose reduction. The incidence of febrile neutropenia in this group was 6.5% compared to 24% in historical UGT1A1 PMs (p=0.04) and was comparable to the incidence in UGT1A1 non-PMs treated with full dose therapy. Systemic exposure of SN-38 of reduced dosing in UGT1A1 PMs was still slightly higher compared to a standard-dosed irinotecan patient cohort (difference: +32%). Cost analysis showed that genotype-guided dosing was cost-saving with a cost reduction of €183 per patient. This chapter showed that *UGT1A1* genotype-guided dosing of irinotecan significantly improves patient safety without a risk of underdosing. As a result, *UGT1A1* genotype-guided dosing was successfully implemented in four hospitals in the Netherlands and we highly recommend to implement this in clinical practice.

In **Part II**, we focused on the optimisation of intraperitoneal chemotherapy in patients with colorectal peritoneal metastasis. We aimed to identify genetic biomarkers predictive for treatment outcome of cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy (CRS + HIPEC).

To do so, in **Chapter 5** we first conducted a systematic review of the literature on the association of genetic biomarkers in the DNA repair pathway and treatment outcome of patients

treated with CRS + HIPEC. As HIPEC agents we included both oxaliplatin or mitomycin C. Since literature on intraperitoneal chemotherapy and genetic biomarkers was scarce, we expanded our search strategy to systemic chemotherapy. In total, 43 papers were included in this review. No study reported potential pharmacogenomic biomarkers in patients with colorectal cancer undergoing MMC-based systemic chemotherapy. For oxaliplatin-based systemic chemotherapy, a total of 26 genetic biomarkers within 14 genes were identified that were significantly associated with treatment outcome. The most promising genetic biomarkers were *ERCC1* rs11615, *XPC* rs1043953, *XPD* rs13181, *XPG* rs17655, *MNAT* rs3783819/rs973063/rs4151330, MMR status, ATM protein expression, *HIC1* tandem repeat D17S5 and *PIN1* rs2233678. By extrapolation, these genetic biomarkers may also be predictive for the efficacy of intraperitoneal oxaliplatin. This should be the subject of further investigation.

In **Chapter 6** we conducted a retrospective genome-wide association study in a CRC patient cohort of 258 patients consecutively treated with CRS + HIPEC in order to identify new genetic biomarkers potentially associated with treatment outcome. The study revealed twelve markers that were significantly associated with reduced disease-free survival (DFS), which influence the expression of six genes. For four out of these six identified genes (*FAM3B*, *STAG1*, *SCL35G2*, and *METTL22*) one or more biological mechanisms could be identified that are in support of the observed associations of the genetic biomarkers with reduced DFS. Several new potentially prognostic or predictive genetic biomarkers for clinical outcome of CRS-HIPEC patients were identified. This is the first GWAS study in this type of patient population, and before clinical application of these findings the data require further validation in an independent patient cohort.

In **Chapter 7** we aimed to provide a proof of principle. We conducted a retrospective, hypothesis-driven study, in which *NQO1*2*, *NQO1*3*, and *POR*28* as possible genetic biomarkers for hyperthermic intraperitoneal mitomycin C were clinically validated. As a prodrug, mitomycin C requires metabolic activation prior to exert its cytotoxic effect, of which the main activating enzymes are NQO1 and POR. However, not all patients are able to activate mitomycin C, for example due to polymorphisms in the genes encoding these enzymes. The aim of this study was to investigate the association of *NQO1*2*, *NQO1*3*, and *POR*28* with the efficacy of CRS plus HIPEC with mitomycin C in patients with colorectal peritoneal metastasis. In this retrospective study, a total of 253 patients were included. Carriership of *NQO1*3* was associated with worse peritoneal recurrence rate, the peritoneal recurrence rate 3 and 6 months after HIPEC was significantly higher than in wild type patients, respectively 30.0% versus 3.8% (p=0.009) and 40.0% versus 12.1% (p=0.031). In line with these results, *NQO1*3* carriership

was associated with a shorter DFS (HR 2.04, 95% CI [1.03–4.03]). There was no significant association with overall survival (HR 1.42, 95% CI [0.66–3.07]). These results suggest that individualisation of patients treated with CRS plus HIPEC based upon pharmacogenetics may be beneficial and should be subject of further investigation.

In **conclusion**, the described studies in this thesis brought us a few steps closer to safe and effective use of chemotherapeutic drugs in the individual colorectal cancer patient. Irinotecan should no longer be administered without a UGT1A1 genotype test and a start has been made towards personalised medicine for colorectal cancer patients with peritoneal metastases.

SAMENVATTING

De behandeling van patiënten met colorectale kanker bestaat vaak uit chemotherapie. De behandeling met deze chemotherapie is echter niet zonder bijwerkingen. Deze bijwerkingen kunnen leiden tot een verminderde kwaliteit van leven, het vroegtijdig stopzetten van een behandeling en soms zelfs tot overlijden. Bovendien is de kans op genezing bij patiënten met uitgezaaide kanker klein. Er valt dus nog veel te verbeteren aan de behandeling van colorectale kanker, zowel op het gebied van veiligheid als werkzaamheid. Een oplossingsrichting om deze behandeling te verbeteren voor de individuele patiënt, zou het gebruik van nieuwe genetische biomarkers kunnen zijn. Het gebruik van deze biomarkers maakt het mogelijk om patiënten te identificeren die een hoger risico hebben op ernstige geneesmiddelgerelateerde bijwerkingen, en om patiënten te selecteren die het meest zullen profiteren van chemotherapie. Het doel van dit proefschrift was derhalve de verbetering van de veiligheid en werkzaamheid van chemotherapie bij patiënten met colorectale kanker door middel van de identificatie en validatie van genetische biomarkers die gepersonaliseerde dosering mogelijk maken en mogelijk voorspellend zijn voor behandeluitkomsten.

In **Deel I** van dit proefschrift hebben we ons gericht op het optimaliseren van de systemische behandeling met irinotecan. Ons doel was om de toegevoegde waarde en de klinische bruikbaarheid van het gepersonaliseerd doseren van irinotecan op basis van *UGT1A1* genotype te onderzoeken. Dit hebben we gedaan door middel van een systematische literatuurreview en de ontwikkeling van een richtlijn over de geneesmiddel-gen interactie tussen irinotecan en *UGT1A1*. Voor het ultieme bewijs hebben we een prospectieve implementatiestudie uitgevoerd waarbij we het gepersonaliseerd doseren van irinotecan op basis van *UGT1A1* genotype in de klinische setting hebben getoetst.

In **Hoofdstuk 2** hebben we de potentiële waarde van het gepersonaliseerd doseren van irinotecan op basis van *UGT1A1* genotype getoetst aan de hand van de beschikbare literatuur. Er is hierbij geëvalueerd of het gepersonaliseerd doseren van irinotecan het risico op ernstige toxiciteit vermindert. Dit is uitgevoerd op basis van vijf vooraf gespecificeerde criteria: 1] het niveau van bewijs voor de associatie tussen *UGT1A1* polymorfismen en door irinotecan-geïnduceerde ernstige toxiciteit, 2] de klinische validiteit en bruikbaarheid van *UGT1A1* genotypering voorafgaand aan start irinotecan, 3] de veiligheid en tolerantie van irinotecan bij dragers van *UGT1A1* polymorfismen, 4] de beschikbaarheid van doseringsaanbevelingen voor irinotecan bij dragers van *UGT1A1* polymorfismen, en 5] de financiële voordelen van gepersonaliseerd doseren van irinotecan. Alle vijf criteria gaven aan dat gepersonaliseerd doseren van irinotecan van waarde is. Het hoogste niveau van bewijs (niveau I) werd gevonden voor een hogere incidentie van irinotecan-geïnduceerde ernstige toxiciteit bij homozygote

dragers van *UGT1A1*28* of *UGT1A1*6* (UGT1A1 PMs); de klinische validiteit en bruikbaarheid van de *UGT1A1* genotypering bleken acceptabel te zijn; in dose-finding studies werd een lagere maximaal getolereerde dosis gezien bij UGT1A1 PMs; en de meeste drug labels en richtlijnen bevelen een dosisvermindering van 25 tot 30% aan UGT1A1 PMs. Bovendien is het gepersonaliseerd doseren van irinotecan waarschijnlijk kosteneffectief.

Hoofdstuk 3 had als doel om een richtlijn voor artsen en apothekers te ontwikkelen over het gepersonaliseerd doseren van irinotecan op basis van *UGT1A1* genotype. Deze richtlijn is geschreven namens de Nederlandse Farmacogenetica Werkgroep (DPWG) en met deze richtlijn wordt beoogd de implementatie van deze genotypering in de klinische praktijk te vergemakkelijken. De richtlijn is gebaseerd op een systematische literatuurreview. Zoals ook gerapporteerd in **Hoofdstuk 2** is er voor UGT1A1 PMs voldoende bewijs dat er sprake is van een verhoogd risico op ernstige bijwerkingen zoals neutropenie of diarree bij normale doses (ook in vergelijking met alle andere genotypen/fenotypen), terwijl overtuigend bewijs voor verhoogde werkzaamheid niet is aangetoond. Dit verhoogde risico op toxiciteit werd gerapporteerd in alle negen geïncludeerde meta-analyses en in 16 van de 23 geïncludeerde studies. Met betrekking tot de werkzaamheid bleek uit vier van de vijf meta-analyses en acht van de tien studies dat er geen verband bestond tussen de *28 en/of *6 varianten en een verhoogde effectiviteit van de behandeling. Op basis van farmacokinetische studies beveelt deze DPWG-richtlijn een startdosis van 70% aan bij UGT1A1 PM patiënten die starten met irinotecan. Bij UGT1A1 intermediaire metaboliser patiënten wordt geen dosisverlaging bij start irinotecan aanbevolen. Op basis van de DPWG clinical implication score wordt *UGT1A1* genotypering als "essentieel" beschouwd, hiermee geeft de DPWG aan dat *UGT1A1* genotypering standaard uitgevoerd zou moeten worden voorafgaand aan behandeling met irinotecan.

In **Hoofdstuk 4** wordt een prospectieve implementatiestudie beschreven naar het gepersonaliseerd doseren van irinotecan op basis van *UGT1A1* genotype. Dit is naar ons weten de eerste prospectieve implementatiestudie waarbij irinotecan wordt gedoseerd op basis van *UGT1A1*. Uit deze studie blijkt dat het gepersonaliseerd doseren leidt tot een significante verlaging van de incidentie van febriele neutropenie en het aantal door chemotherapie-geïnduceerde ziekenhuisopnames bij UGT1A1 PMs. Van de 350 geïncludeerde patiënten waren 31 (8,9%) patiënten UGT1A1 PM. Deze groep patiënten is behandeld met een mediane dosisintensiteit irinotecan van 70%. De incidentie van febriele neutropenie in deze groep was 6,5% in vergelijking met 24% bij historische UGT1A1 PMs die met een 100% dosering behandeld werden (p=0,04). De incidentie van 6,5% in UGT1A1 PMs was vergelijkbaar met de incidentie bij UGT1A1 non-PMs behandeld met de volledige dosis irinotecan. De systemische blootstelling

van SN-38 bij UGT1A1 PMs, die behandeld werden met een 70% dosisintensiteit, was nog steeds licht hoger in vergelijking met een patiëntencohort behandeld met een standaard 100% dosisintensiteit van irinotecan (verschil: +32%). De kostenanalyse toonde aan dat gepersonaliseerd doseren van irinotecan kostenbesparend was, de kosten werden verlaagd met €183 per patiënt. Met dit hoofdstuk is aangetoond dat het gepersonaliseerd doseren van irinotecan op basis van *UGT1A1* genotype de patiëntveiligheid significant verbetert zonder risico op onderdosering. Hiermee is het gepersonaliseerd doseren van irinotecan succesvol geïmplementeerd in vier Nederlandse ziekenhuizen en we bevelen dan ook landelijke implementatie aan.

In **Deel II** lag de focus op de optimalisatie van intraperitoneale chemotherapie bij patiënten met colorectale peritoneale metastasen. Het doel was om genetische biomarkers te identificeren die voorspellend zijn voor de behandeluitkomsten van cytoreductieve chirurgie plus hypertherme intraperitoneale chemotherapie (CRS + HIPEC).

Om dit te bereiken hebben we in **Hoofdstuk 5** eerst een systematische literatuurreview uitgevoerd naar de associatie tussen genetische biomarkers in de DNA repair pathway en behandeluitkomsten van patiënten die behandeld werden met CRS + HIPEC. Hierbij is gekeken naar patiënten die behandeld werden met mitomycine of oxaliplatine. Aangezien de literatuur over intraperitoneale chemotherapie en genetische biomarkers schaars was, is de zoekstrategie uitgebreid naar systemische chemotherapie. In totaal werden 43 artikelen opgenomen in deze review. Geen enkele studie meldde potentiële farmacogenetische biomarkers bij patiënten met colorectale kanker die mitomycine-gebaseerde systemische chemotherapie ondergingen. Voor oxaliplatine-gebaseerde systemische chemotherapie werden in totaal 26 genetische biomarkers binnen 14 genen geïdentificeerd die significant geassocieerd waren met de behandeluitkomsten. De meest veelbelovende genetische biomarkers waren *ERCC1* rs11615, *XPC* rs1043953, *XPD* rs13181, *XPG* rs17655, *MNAT* rs3783819/rs973063/rs4151330, MMR-status, ATM-eiwitexpressie, *HIC1* tandem repeat D17S5 en *PIN1* rs2233678. Deze resultaten voor de behandeluitkomsten van systemisch oxaliplatine kunnen mogelijk geëxtrapoleerd worden naar intraperitoneale oxaliplatine. Dit systematische review biedt de basis voor dit verdere onderzoek.

In **Hoofdstuk 6** hebben we een retrospectieve genoomwijde associatiestudie (GWAS) uitgevoerd in een colorectale kankerpatiëntencohort van 258 patiënten. Deze patiënten werden behandeld met CRS + HIPEC met oxaliplatine en mitomycine. Met deze GWAS wilden we nieuwe genetische biomarkers identificeren die mogelijk geassocieerd zijn met de behandeluitkomsten van CRS + HIPEC. De studie toonde twaalf markers aan die significant geassoci-

eerd waren met ziektevrije overleving en die de expressie van zes genen beïnvloeden. Voor vier van deze zes geïdentificeerde genen (*FAM3B*, *STAG1*, *SCL35G2* en *METTL22*) konden één of meer biologische mechanismen worden geïdentificeerd die de waargenomen associaties tussen de genetische biomarkers met verminderde ziektevrije overleving ondersteunen. Verschillende nieuwe potentiële prognostische of predictieve genetische biomarkers voor de klinische uitkomst van CRS + HIPEC patiënten werden geïdentificeerd. Dit is de eerste GWAS-studie in deze type patiëntenpopulatie, en voordat deze bevindingen klinisch kunnen worden toegepast, is verdere validatie van de gegevens in een onafhankelijk patiëntencohort nodig.

Hoofdstuk 7 omvat een retrospectieve studie waarbij *NQO1*2*, *NQO1*3* en *POR*28* als mogelijke genetische biomarkers voor HIPEC met mitomycine klinisch werden gevalideerd. Mitomycine is een prodrug die wordt geactiveerd door de enzymen NQO1 en POR. Echter, niet alle patiënten zijn in staat om mitomycine te activeren, bijvoorbeeld als gevolg van polymorfismen in de genen die voor deze enzymen coderen. Het doel van deze studie was om de associatie tussen *NQO1*2*, *NQO1*3* en *POR*28* en de behandeluitkomsten van CRS + HIPEC met mitomycine te onderzoeken. Dit werd uitgevoerd bij patiënten die peritoneale metastasen hadden van colorectale kanker. In deze retrospectieve studie werden in totaal 253 patiënten geïncludeerd. Hierbij werd een significante associatie gevonden tussen dragers van *NQO1*3* en een hogere peritoneal recurrence rate. De peritoneal recurrence rate 3 en 6 maanden na HIPEC was significant hoger dan bij wildtype patiënten, respectievelijk 30,0% versus 3,8% (p=0,009) en 40,0% versus 12,1% (p=0,031). In lijn met deze resultaten werd dragerschap van *NQO1*3* geassocieerd met een kortere ziektevrije overleving (HR 2,04, 95% BI [1,03–4,03]). Er was geen significante associatie met overall survival (HR 1,42, 95% BI [0,66–3,07]). Deze resultaten suggereren dat de individualisering van patiënten die behandeld worden met CRS + HIPEC op basis van farmacogenetica gunstig kan zijn en dat dit verder onderzocht zou moeten worden.

Dit proefschrift heeft ons een paar stappen dichterbij veilig en effectief gebruik van chemotherapie voor de individuele colorectale kankerpatiënt gebracht. Irinotecan zou niet meer moeten worden gestart zonder een *UGT1A1* genotypering vooraf en een eerste stap is gemaakt richting het gepersonaliseerd doseren van intraperitoneale chemotherapie bij colorectale kankerpatiënten met peritoneale metastasen.

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CURRICULUM VITAE

Emma Claire Hulshof was born on April 24th 1989 in Tubbergen, the Netherlands. After finishing pre-university education (VWO) in 2007 at Twents Carmel College de Thij in Oldenzaal, she studied Pharmacy at Utrecht University. As part of her Master's of Science programme she did a research internship at the World Health Organisation in Copenhagen, Denmark. In 2013 she graduated and started working as a pharmacist at Sint Jans Gasthuis in Weert. In 2015 she started a residency in hospital pharmacy at the Catharina Hospital in Eindhoven and Maxima Medical Center in Veldhoven, where she worked until 2019. During this residency she started with the IRI28 trial, the onset of this thesis. After registration as a hospital pharmacist in 2019, she started working as a hospital pharmacist at the Catharina Hospital Eindhoven, which was combined with her PhD research at Leiden University Medical Center. The results of this research are presented in this thesis. In 2022 she won the Catharina Science Award. Since August 2022 she is employed at Hospital Group Twente as a hospital pharmacist.