Evidence Report/Technology Assessment

Can *UGT1A1* Genotyping Reduce Morbidity and Mortality in Patients with Metastatic Colorectal Cancer Treated with Irinotecan?

Prepared for:
Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group

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The purpose of this report is to provide insight into the ongoing development of EGAPP review methods and processes. This report is not a recommendation, and it is not intended as medical advice or as a substitute for clinical judgment. Findings presented in this report reflect evidence from studies available at the time the literature review was completed. The EGAPP Working Group therefore makes no warranties regarding the current accuracy or completeness of information contained in the report. A summary evidence-review, based upon the findings of this report, contains additional analyses and updates, and is available at: http://www.geneticsinmedicine.org/pt/re/gim/pdfhandler.00125817-200901000-00004.pdf.

Select figures, tables and text in this report also appear in the evidence-review mentioned above (Palomaki GE, Bradley LA, Douglas MP, Kolor K, Dotson WD. Can *UGT1A1* genotyping reduce morbidity and mortality in patients with metastatic colorectal cancer treated with irinotecan? An evidence-based review. *Genet Med* 2009:11(1):21–34; © 2009 American College of Medical Genetics) and these are reprinted with permission of Lippincott Williams & Wilkins.

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EXECUTIVE SUMMARY

Introduction

Colorectal cancer (CRC) is the third leading cause of new cancer in the US, with about 150,000 new cases per year. More than 55,000 deaths from CRC were expected in 2006. At least 15% of individuals with new CRC cancers (20,000 to 25,000) might be candidates for irinotecan therapy.[1-3] Surgery is the primary treatment for localized CRC. Between 70% and 80% of patients present with "apparently resectable localized disease;" optimal treatment in such cases is surgery followed by adjuvant therapy for high risk cases.[3-6] CRC patients with advanced disease at diagnosis may receive first-line systemic chemotherapy, or chemotherapy and radiation therapy may be combined with surgery, done before surgery, or used palliatively if surgery is not indicated.[5] Fluorouracil (5-FU) continues to be the first choice of chemotherapy, and may be used in combination with leucovorin.[4,6,7] However, other combination chemotherapy regimens using irinotecan with oxaliplatin and other drugs appear to improve median survival over fluorouracil and leucovorin, and are increasingly prescribed for first-line and sequential therapy for metastatic CRC.[5,8,9]

Irinotecan is a topoisomerase I inhibitor that interrupts DNA replication in cancer cells, resulting in cell death.[10-12] The irinotecan pro-drug is activated by the enzyme carboxylesterase to the active metabolite SN-38, which is 100-1,000 fold more cytotoxic than the parent drug.[10] SN-38 is further catalyzed into an inactive glucuronide derivative, SN-38G by several hepatic and extra-hepatic UGT enzymes. The major isozyme involved is UGT1A1, but others also have some role (UGT1A 6, 7, 9 10).[13] Decrease in the level of functional UGT1A1 enzyme, which may result from the presence of *UGT1A1*28*, reduces the ability to metabolize SN-38 to an inactive form, and has been associated with a higher risk of adverse reactions due to the higher levels and/or prolonged exposure to the active form of the drug.[6,14] Based on available tests and the proposed clinical scenario, the UGT1A1 enzyme was the focus of the evidence review.

Testing for *UGT1A1* variants

At least 63 *UGT1A1* variant alleles have been described, including single base pair changes, frame shift mutations, insertions, and deletions in the promoter region, 5 exons and 2 introns of the gene[15]. Most are associated with absent, reduced or inactive UGT1A1 enzyme; one is associated with increased enzyme, and some are unknown. This review focuses on the more

commonly tested mutations, the wild type $(TA)_6TAA$ promoter sequence designated *UGT1A1*1*, and the variant promoter $(TA)_7TAA$ sequence designated *UGT1A1*28*. The $(TA)_5TAA$ (*UGT1A1*36*) and $(TA)_8TAA$ (*UGT1A1*37*) promoter and exon 1 variants, 211G>A (*UGT1A1*6*) and 686C>A (*UGT1A1*27*), are also described but are not the focus of the review.

Clinical Scenario

The specific clinical scenario for use of *UGT1A1* genotyping addressed in the report is patients with metastatic colorectal cancer whose treatment regimen includes irinotecan. The proposed rationale is that, if drug selection or dosage of irinotecan in patients with metastatic CRC can be modified based on genotype, some proportion of serious side effects could be reduced.

Methods

The methods used to identify, review, evaluate, analyze, and summarize the existing evidence are detailed in Appendix C. Briefly, contracted investigators at RTI International and Evaluation of Genomic Applications in Practice and Prevention (EGAPP) consultants and staff conducted a systematic review on the analytic and clinical validity and clinical utility of *UGT1A1* testing in a specific clinical scenario. After development of an analytic framework (Figure 1) and key questions, reviewers conducted a search of published and grey literature through December, 2006, reviewed abstracts and selected articles, abstracted data into evidence tables, and assessed the quality of individual articles. See Appendix C for detailed information on search terms, inclusion/exclusion criteria, data abstraction, and rating quality of studies. EGAPP staff and consultants performed the final summarization and statistical analyses, integrated the component sections, and produced a draft evidence report for consideration by the EGAPP Working Group. With a focus on the application of study data to specific key questions, the reviewers assessed quality of evidence based on standard criteria, including study design and conduct, consistency and generalizability of data, and appropriate statistical analyses. Feedback was sought throughout the review from the Technical Expert Panel, other technical consultants, and nine external expert peer reviewers (Appendix A). The draft report was revised in response to comments from the reviewers, and submitted to the EGAPP Working Group along with a summary of comments and their disposition.

Results for Key Questions 1-4

KQ1. Does testing for *UGT1A1* mutations in patients with metastatic CRC treated with irinotecan lead to improvement in outcomes? (Overarching question)

This evidence-based review addresses the question of whether testing for *UGT1A1* mutations in patients with metastatic colorectal cancer treated with irinotecan leads to improvement in outcomes (*e.g.*, irinotecan toxicity, response to treatment, morbidity and mortality), as compared to no testing. No studies have been identified that addressed this question directly. No prospective observational or controlled trials have been done in which irinotecan dose was based on *UGT1A1* genotype to determine benefits and harms.

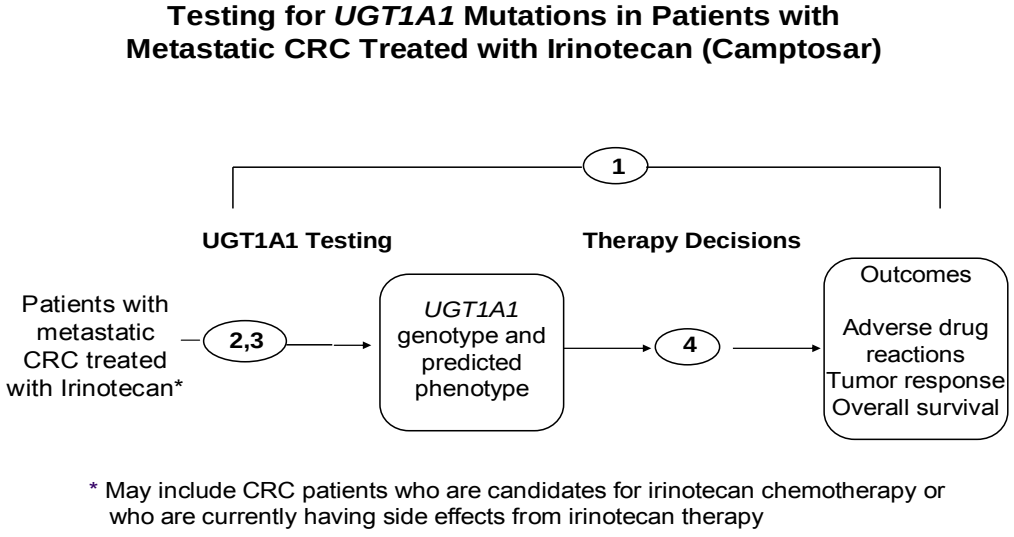


Figure 1. Analytic Framework

KQ2. What is the analytic validity of the test(s) that identify key *UGT1A1* mutations?

The quality of evidence for the analytic validity of current *UGT1A1* genetic testing methods is fair, but available data indicate that both analytic sensitivity and specificity for the common genotypes are high.

KQ3. What is the clinical validity of *UGT1A1* testing?

a. How well does *UGT1A1* testing predict phenotypic markers (*e.g.*, increased plasma SN-38 levels or decreased enzyme activity) and associated adverse drug reactions (*e.g.*, diarrhea or neutropenia)?

Comparison of exposure to the inactive metabolite (SN-38G) to the active form (SN-38) based on time and dose shows a clear association between *UGT1A1* genotype and the ratio of the area under the curve (AUC) for SN-38G (inactive form) to the AUC for SN38 (active form). Homozygous **28/*28* individuals have about twice the exposure to the active form due to slower metabolism of the drug. However, there is significant overlap in the ratios between the three main genotypes.

b. How well does *UGT1A1* testing in patients with metastatic CRC predict morbidity (diarrhea, neutropenia) and mortality (survival)?

The strength of evidence is fair for the studies reporting *UGT1A1* common genotypes versus concentration of the active form of irinotecan (SN-38), presence of severe diarrhea, and presence of severe neutropenia. The strongest association for a clinical endpoint is for severe neutropenia, where the risk ratio for individuals homozygous for the **28* allele compared to wild type individuals is 3.51 (95% CI 2.03 to 6.07).

Two studies provided estimates of tumor responsiveness, and together found risk ratios of 1.09 (95% CI 0.83 to 1.43) and 1.70 (95% CI 1.24 to 2.33) for **1/*28* heterozygotes and **28/*28* homozygotes, respectively. These two, and one additional study, also found median survival, 1 and 2 year survivals, and hazard ratios in the direction of benefit among **28/*28* homozygotes (although most were too small to be statistically significant).

c. Do other factors (*e.g.*, race/ethnicity, other medications) affect clinical validity?

The *UGT1A1* polymorphisms most common in Caucasians are also the most common in other racial/ethnic groups studied (*i.e.*, Asians, Africans/African Americans). However, the allele frequencies differ. For example, the **28* allele frequency in Caucasians is 0.334 (95% CI 0.309 to 0.361), but it is 0.139 (95% CI 0.112 to 0.171) in Asians and 0.40 (95% CI 0.34 to 0.45) in Africans/African Americans. Asians also have relatively high frequencies of some alleles infrequently found in Caucasians (*e.g.*, **6* allele frequency is < 0.01 in Caucasians but 0.11 in Asians).

KQ4. What are the benefits and harms related to UGT1A1 testing for patients with metastatic CRC treated with irinotecan?

a. Based on *UGT1A1* test results, what are the management options for patients?

At least three treatment options exist: modification of the irinotecan regime (*e.g.*, reduce initial dose), use of other drugs, and/or pre-treatment with colony-stimulating factors. The most commonly suggested patient option is to reduce the irinotecan initial and/or subsequent doses in **28* homozygotes or other genotypes known to result in poor metabolism of the drug. However, it has been suggested that the data may also indicate that **1/*1* patients may be under-dosed.[16] The FDA approved relabeling of the drug Camptosar (irinotecan), indicating that genetic testing for *UGT1A1* genotypes may be useful in dosing. While we did not find any published studies, there are ongoing structured trials studying the effects of modifying irinotecan dosage based on *UGT1A1* genotyping. [16-19]

b. Do these options improve patient outcomes or management decisions by patients or providers?

No prospective trials have examined whether a reduced dose of irinotecan results in a reduced rate of adverse drug events, a major gap in knowledge. Clinical utility of *UGT1A1* genotyping would be derived from a reduction in drug-related adverse reactions (benefits) while at the same time avoiding declines in tumor response rate and increases in morbidity/mortality (harms). Although the strength of evidence is marginal, two of three reviewed studies (and one recent publication) found that individuals homozygous for the **28* allele had improved survival. Three reviewed studies found statistically significant higher tumor response rates among individuals homozygous for the **28* allele. Little or no direct evidence exists to compare these benefits and harms, but preliminary modeling indicates that the effectiveness of reducing the dose would need to be high to be cost effective and to have benefits that outweigh harms. An alternative is to increase irinotecan dose among wild-type individuals to improve tumor response with minimal increases in adverse drug events. Given the large number of individuals diagnosed with colorectal cancer cases each year who are candidates for irinotecan, a randomized controlled trial is feasible and could clarify the tradeoffs between possible reductions in severe neutropenia and improved tumor response and/or survival in patients with various *UGT1A1* genotypes.

Strength of Evidence

Figure 2 below provides a graphic display of the strength of evidence for the key components of the current evidence review, analytic validity, clinical validity and clinical utility, with the strength of evidence represented by the text within the boxes. The actual findings (e.g., strength of association) are contained in the body of the text.

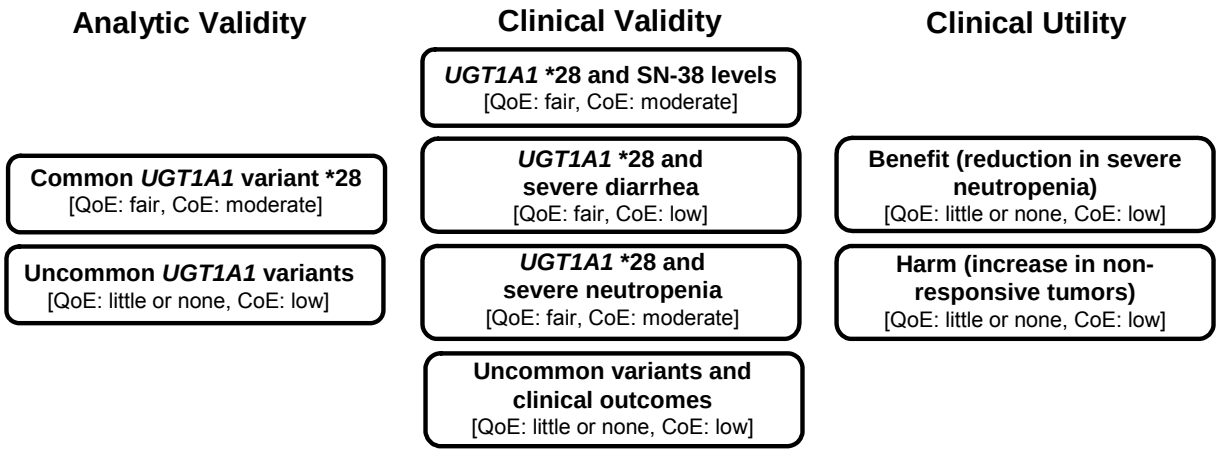


Figure 2. Graphic display of the quality of evidence (QoE) and certainty of evidence (CoE) for selected components of the current evidence review. For analytic validity, clinical validity and clinical utility, each of the main components of the evidence review is represented by the text within a box. The QoE and CoE for each component is indicated by the bracketed text.

Major Gaps in Knowledge

1) Although there appears to be a clear relationship between *UGT1A1* genotype and severe neutropenia (and some evidence of a relationship with severe diarrhea) there is no evidence to support or refute the hypothesis that a modified initial and/or subsequent dose of irinotecan will change the rate of these severe adverse drug reations.
2) Even if adverse drug events were reduced, this may come at the expense of a reduction in tumor responsiveness leading to an overall net harm.
3) If the test were recommended for use in clinical practice, additional studies would be needed to understand the potential effects of alleles that are rare in Caucasians but more common in other racial/ethnic groups (*e.g., *6* in Asians).

Given these major gaps, prospective studies (including randomized controlled trials) are warranted to determine whether or not *UGT1A1* genotyping to determine drug dose results in net benefit to the patient.

Introduction

Purpose of the Review

In a process similar to that used by the U.S. Preventive Services Task Force, the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group will utilize the information presented in this report to inform the development of evidence-based recommendations based on current knowledge of the validity and utility of *UGT1A1* testing for predicting response to irinotecan in patients with metastatic colorectal cancer (CRC). Because the literature on this topic is relatively limited, this report serves as a model for developing more flexible approaches to evidence review that maintain a high level of quality.

Scope and Structure of the Review: Key Questions and Analytic Framework

The analytic framework is shown in Figure 1 (Executive Summary) with the numbers indicating the key questions. Table 1 lists the overarching question and other key questions addressed in the evidence review. The analytic framework and key questions were developed by the EGAPP Working Group, and further refined in discussions with the technical contractors and the Technical Expert Panel (Appendix A). Key question (KQ) 1 is the overarching question that is being asked about clinical utility: *Does testing for UGT1A1 mutations in patients with metastatic CRC treated with irinotecan lead to improvement in outcomes (e.g., irinotecan toxicity, response to treatment, morbidity and mortality) compared to no testing?* In the absence of direct evidence to answer KQ 1, KQ 2-4 are intended to elicit intermediate information to addresse the overarching question through a 'chain of evidence'. In reviewing the available evidence, questions from the ACCE review framework (Appendix B) were often used to identify and organize the specific information needed to address the key questions.[20]

As shown in the analytic framework (Figure 1) and key questions (Table 1), the focus of this report is on patients with metastatic colorectal cancer (CRC) treated with irinotecan. However, the scope of the literature search was expanded to include irinotecan treatment for all cancers because there is limited evidence available on CRC alone, and because some management issues related to irinotecan use are similar regardless of the type of cancer. Available studies mainly provide data on testing for the *UGT1A1*28* allele in Caucasian populations. This is due both to the strength of the association between the **28* allele and irinotecan toxicity, and to the prevalence of this allele in the majority Caucasian population of North America. If this test were to be recommended for routine use in this clinical scenario, additional studies would be needed

Table 1. Key Questions

1. Does testing for *UGT1A1* mutations in patients with metastatic CRC treated with irinotecan lead to improvement in outcomes (*e.g.*, irinotecan toxicity, response to treatment, morbidity and mortality) compared to no testing? (Overarching question)

2. What is the analytic validity of the test(s) that identify key *UGT1A1* mutations?

3. What is the clinical validity of *UGT1A1* testing?
    a. How well does *UGT1A1* testing predict phenotypic markers (*e.g.,* increased plasma SN-38 levels or decreased enzyme activity) and associated adverse drug reactions (*e.g.*, diarrhea or neutropenia)?
    b. How well does *UGT1A1* testing in patients with metastatic CRC predict morbidity and mortality?
    c. Do other factors (*e.g.*, race/ethnicity, other medications) independently affect clinical validity?

4. What are the benefits and harms (clinical utility) related to UGT1A1 testing for patients with metastatic CRC treated with irinotecan?
    a. Based on *UGT1A1* test results, what are the management options for patients?
    b. Do these options improve patient outcomes or management decisions by patients or providers?

to obtain better estimates of frequencies of other alleles/genotypes in different populations, assess analytic and clinical validity of testing for the less common markers, and investigate their utility in testing of other racial/ethnic groups, particularly Asians/Asian Americans and Africans/African Americans.

Methods

The methods used to identify, review, evaluate, analyze, and summarize the existing evidence are detailed in Appendix C. Investigators at RTI International were contracted by the National Office of Public Health Genomics (NOPHG) at the Centers for Disease Control and Prevention (CDC) to conduct the initial stage of the review, focusing on clinical validity and utility. RTI's

work included a literature search (through May, 2006), review of abstracts and selected articles, abstraction of data into evidence tables, assessment of the quality of individual articles, and preparation of a preliminary report (see Appendix C, pp. 1-5 for detailed information on search terms, inclusion/exclusion criteria, data abstraction, and rating quality of studies). The approach developed by RTI for assessing quality of individual studies involves numerical scoring based on: 1) study design; 2) study population; 3) comparability of studies; 4) statistical analyses; and 5) measures of effect and precision (Appendix C). Ratings were summed across the five categories to assign an overall rating of *Good*, *Fair*, or *Poor*. RTI provided a preliminary report to CDC on "*The Evidence for the Benefits of UGT1A1 Promoter Region Polymorphism Testing in Cancer Patients Treated with Irinotecan*" (RTI evidence tables in Appendix E).

When the RTI report was submitted, EGAPP's CDC staff and consultants performed searches and review of articles on analytic validity and allele/genotype frequencies, and updated the clinical validity and utility searches through December, 2006. They also performed additional summarization and statistical analyses, integrated the component sections, and produced a draft evidence report for consideration by the EGAPP Working Group and selected expert reviewers. With a focus on the application of study data to specific key questions, EGAPP reviewers assessed quality of evidence based on standard criteria, including study design and conduct, consistency and generalizability of data, and appropriate statistical analyses. Short summaries were developed for all individual studies included for analytic and clinical validity and clinical utility, and included EGAPP and RTI quality ratings for comparison (Appendix C). Overall quality assessments of *Good*, *Fair,* or *Marginal*[a] were provided for analytic and clinical validity and clinical utility along with the rationale. Briefly, *good* quality indicated existence of reliable data to support conclusions that are not likely to change based on further studies. *Fair* quality indicated that, although some reasonable quality data were available, they were insufficient to allow firm conclusions to be drawn. *Marginal* quality indicated insufficient data or flaws in study design or conduct that would not allow any conclusions to be drawn. Feedback was sought throughout the review from the Technical Expert Panel, technical consultants, and the EGAPP Working Group. In addition, a draft of this evidence report was sent to nine expert peer reviewers (Appendix A) in August, 2007, and the report was revised in response to comments from the reviewers. Targeted reviews of the literature in August and December, 2007, were conducted to ensure that newer findings were included or acknowledged. The report was

[a] The designation *Marginal* acknowledges that some studies used may not have been "poor" in overall design or conduct, but were among the lowest quality for addressing a key question in this review.

then resubmitted to the Working Group along with a summary of comments and their disposition.

Defining the Disorder, Test, and Clinical Scenario

Disorder

The specific clinical disorder is colorectal cancer (CRC), the third leading cause of new cancer cases in the United States (US). A total of 148,610 new CRC cases were estimated to occur in 2006.[1,2] Although improved screening and treatment have reduced the death rate slightly during the last 15 years, more than 55,000 deaths from CRC were expected in 2006. In patients with newly diagnosed CRC, staging of tumors is performed to determine the extent and location of the tumor, in order to inform appropriate treatment strategies and provide information on prognosis.[21] Methods for staging colorectal cancer include the American Joint Committee on Cancer (AJCC), Dukes, and Astler-Coller systems.[21,22] The staging for rectal cancer closely approximates the staging for colon cancer, although survival rates for these cancers at a similar stage may differ. These systems describe the spread of the cancer based on its identification in tissue layers in the colon or rectum, organs nearby, or organs more distant. Pathologic staging on the removed tissue describes the extent of the primary tumor, whether there is metastasis to nearby lymph nodes, and whether there are distant metastases.

Surgery is the primary treatment for most patients with localized CRC. A recent review on management of CRC estimates that as many as 70-80% of patients present with "apparently resectable localized disease"; optimal treatment in such cases is surgery followed by adjuvant therapy for high risk cases.[4-6] It is also reported that about half of CRC patients eventually develop advanced, or disseminated, cancer. CRC patients who present with advanced disease at diagnosis may receive first-line systemic chemotherapy, or chemotherapy and radiation therapy may be combined with surgery, done before surgery, or used as palliative therapy if surgery is not indicated.[5] Flourouracil (5-FU) continues to be the first choice of chemotherapy, and may be used in combination with leucovorin.[4,6] However, the incorporation of two cytotoxic agents, irinotecan and oxaliplatin, into first-line chemotherapy regimens appears to improve median survival over fluorouracil and leucovorin, and these drugs are increasingly prescribed for first-line treatment for metastatic CRC.[5,8,9] In addition, multiple treatment options (some including irinotecan) are now available in the second-line setting for patients with metastatic CRC who have progressed despite prior chemotherapy.

Table 2. Stages of Colorectal Cancer According to Three Staging Systems [21,22]

AJCC Staging System	Corresponding Staging Systems		General Description
	Dukes	Astler-Coller	
I	A	A, B1	The cancer is limited to the lining or muscular wall of the colon and has not spread
IIa	B	B2	The cancer has spread through the colon wall or into adjacent organs but has not entered the lymph nodes
IIb		B3	
IIIa	C	C1	The cancer has spread beyond the colon to nearby lymph nodes
IIIb		C2/C3	
IIIc		C1/C2/C3	
IV	NA	D	The cancer has spread to distant organs (*e.g.*, liver, lungs, or bones)

No published data were identified that provide a direct estimate of potential irinotecan use. In order to estimate the number of CRC patients that might receive irinotecan therapy, incident data from 1998 to 2001 were utilized from the National Program of Cancer Registries (NPCR) and the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) registries, containing staging in 476,871 CRC cases.[3] In patients greater than 50 years of age, 15% were classified as having "distant" cancer and would likely be candidates. An additional 6% were classified as "in situ", 34% as "localized", 37% as "regional", and 8% as "unknown". Some patients with a "regional" classification might also be candidates, but the proportion is not clear. The reviewers for this report chose to use the conservative estimate of 15%, but acknowledge that it could be 50% or more. In addition, it seems likely that the number of patients who ultimately receive irinotecan will continue to increase with the approval of new biologic agents (*e.g.*, signaling inhibitors bevacizumab, cetuximab) that may be used with irinotecan in second-line therapy of CRC, and with the increase in the number of patients surviving to progress from first-line to second-line treatment.[5,23]

UGT Enzymes and Metabolism of Irinotecan

The drug irinotecan, also referred to as CPT-11 (7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin) is a topoisomerase I inhibitor that interrupts DNA replication in cancer cells, resulting in cell death.[10-12,12] The irinotecan pro-drug is activated by the enzyme carboxylesterase to the active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin), which is 100-1,000 fold more cytotoxic than the parent drug.[10] SN-38 is further catalyzed into an inactive glucuronide derivative, SN-38G (7-ethyl-10-[3,4,5-trihydroxy-pyran-2-carboxylic acid]-camptothecin) by several hepatic and extra-hepatic UGT enzymes. The major isozyme involved is UGT1A1, but others also have some role (UGT1A 6, 7, 9 10).[13] Glucuronidation (*i.e.,* conjugation with glucuronic acid) is a mechanism for clearance of a range of drugs in humans.[11] Reduction in levels of functional UGT1A1 enzyme reduces the ability to metabolize SN-38 to an inactive form, and has been associated with a higher risk of adverse reactions. This is suggested to be the result of the higher SN-38 levels and/or prolonged exposure to of the active form of the drug.[11,14] Based on available tests and the proposed clinical scenario, the UGT1A1 enzyme is the focus of this review.

Heritable Disorders Related to the UGT1A1 Enzyme

The main role of the enzyme UGT1A1 is glucuronidation of bilirubin. As a consequence, three heritable unconjugated hyperbilirubinemias have been described that result from altered levels and activity of this enzyme: Gilbert syndrome, and Crigler-Najjar syndrome types I and II.[11] Reports emerged of observed higher metabolic ratios of SN-38 to SN-38G in patients with Gilbert syndrome or Crigler-Najjar type II who have reduced levels of UGT1A1 activity, and in Crigler-Najjar type I patients lacking any UGT1A1 activity.[24-26] There were reports that patients with these disorders were at increased risk for toxicity related to treatment with irinotecan.[27,28]

Irinotecan Treatment of CRC

Irinotecan was introduced for treatment of ovarian and colorectal cancers, and was approved by the U.S. Food and Drug Administration (FDA) in 1996 as Camptosar® (Millenium Pharmaceuticals) for first-line therapy in metastatic CRC. Use of Camptosar is approved as "a component of first-line therapy in combination with 5-fluorouracil and leucovorin for patients with metastatic carcinoma of the cancer or rectum", or "for patients with metastatic carcinoma of the colon or rectum whose disease has recurred or progressed following initial fluorouracil-based therapy".[29] Combined treatments of irinotecan and oxaliplatin in combination with 5-fluorouracil have been shown to significantly increase median survival and time to tumor progression.[5] As

with other chemotherapy drugs, irinotecan dosing is based on body surface area (mg/m^2). Treatment with irinotecan is associated with significant adverse side effects, the most clinically important of which are neutropenia and diarrhea. Trials showed that patients at particular risk for adverse reactions included the elderly with co-morbid conditions, those with a baseline performance status of 2[(see footnote b)], and patients who had previously received pelvic/abdominal radiation.[29]

Neutropenia is a decrease in the number of circulating *neutrophils* (a type of white blood cell that usually accounts for 50-70% of circulating white blood cells), due in this case to bone marrow suppression by the anti-neoplastic chemotherapy drugs. Because neutrophils are important in defense against bacterial infections, chronic neutropenia can be life-threatening. Neutropenia is defined as Grade 1, 2, 3, or 4 based on the absolute neutrophil count (ANC: Grade 1-1500-1999; Grade 2-1000-1499; Grade 3- 500-999; Grade 4-<500 cells/mm^3).[30]

Late diarrhea (generally occurring more than 24 hours after drug administration) may be prolonged and lead to dehydration, electrolyte imbalance, or sepsis. The severity of diarrhea is graded on a more subjective scale from 1 (mild) to 4 (severe or life-threatening).[31] The inactive SN-38G metabolite is eliminated in the bile and deconjugation of SN-38G to the active SN-38 can be catalyzed by an intestinal enzyme (β-glucuronidase), resulting in levels of SN-38 in the intestine that are believed to cause diarrhea.[13]

Clinical trials involving irinotecan have reported rates of grade 3-4 neutropenia ranging from 16% (10 of 63 patients undergoing second-line treatment with irinotecan alone)[14] to as high as 54% (121 of 225 patients undergoing first-line treatment with fluorouracil, irinotecan, and leucovorin).[32] Rates of grade 3-4 diarrhea range from 5 to 36%.[14,23,32-34] Variability in these reported rates is likely to be associated with the specific population studied (*e.g.*, patients undergoing first-line or second-line treatment) and the treatment regimen (*e.g.*, number of drugs, dosages, schedule and route of administration). In some reported trials, toxicity-related deaths have occurred, mainly related to grade 4 or febrile neutropenia and sepsis.[14,32,34,35] A trial including first-line treatment with irinotecan alone (125 mg/m^2) in 2000 reported that 5.8% of

[b] The ECOG (or WHO/Zubrod) score is used to assess how a patient's disease is progressing, assess how the disease affects the daily living abilities of the patient, and determine appropriate treatment and prognosis. The ECOG score runs 0 to 5, with 0 denoting perfect health and 5 death; a score of 2 indicates the patient is symptomatic and unable to carry out work activities, but ambulatory and up more than 50% of waking hours.

patients developed grade 3-4 neutropenia and fever, about 2% progressed to serious infection, and 0.9% (about 1 in 110) to drug-related death (2 of 223; 95% CI 0.1-3.2).[32] In patients in that same study treated under a different regimen (fluorouracil, irinotecan 125 mg/m^2, and leucovorin), the corresponding rates were 7.1%, 1.8%, and 0.9% (2 of 225; 95% CI 0.1-3.2), respectively.[32] A 2003 trial of second-line treatment with irinotecan alone administered at two different intervals (125 mg/m^2 weekly; 350 mg/m^2, or 300 mg/m^2 if >70 years of age, every 3 weeks) reported that 2% and 3% of patients in these regimens developed neutropenia with fever or infection, respectively; drug-related death occurred in 5.3% (5 of 94; 95% CI 1.7-12) in the weekly regimen and 1.6% (3 of 190; 95% CI 0.3-4.5) of those receiving treatment every 3 weeks.[34]

Testing for *UGT1A1* Variants

The *UGT1A* gene family includes nine protein coding genes and four pseudogenes, and encodes 13 different isoforms of the UGT1A enzyme (*UGT1A1* through *UGT1A13p*). The isoforms result from alternative splicing of promoters and regions encoding substrate binding domains (multiple exon 1 sites) to common exons 2-5 (Figure 3).[12,36-40] At least 63 *UGT1A1* variant alleles have been described, including single base pair changes, frame shift mutations, insertions, and deletions in the promoter region, 5 exons and 2 introns of the gene.[15] Most are associated with absent, reduced or inactive enzyme; one is associated with increased enzyme, and the effects of some are unknown. This review focuses on the more commonly tested mutations (Table 3).

The first is a 2-base pair insertion (TA) in the TATA box in the promoter region of the gene. The result is that the $(TA)_6TAA$ sequence, found in the promoter of the wild-type *UGT1A1*1* allele, becomes $(TA)_7TAA$; this variant has been designated *UGT1A1*28*. The $(TA)_5TAA$ (*UGT1A1*36*) and $(TA)_8TA$ (*UGT1A1*37*) variants are also described (Table 3), but are less common and less routinely tested. Others included are single base pair changes in exon 1, and cause non-synonymous amino acid changes. *UGT1A1*6*, a guanine to adenine substitution (c.211G>A), changes amino acid 71 from glycine to arginine (G71R); *UGT1A1*27*, a cytosine to adenine substitution (c.686C>A), changes amino acid 229 from proline to glycine (P229Q)(Table 3). Information on additional functional polymorphisms in *UGT1A1* promoter (*e.g.,* -3279T>G; *UGT1A1*60)* and coding regions (*e.g.,* 1456T>G; *UGT1A1*7*) were even more limited at the time of the initial review, and they were not included[41-44]. These, and more recent

studies[45,46], have shown that some polymorphisms are relatively common in specific racial/ethnic groups (e.g., Asians) and may influence metabolism of irinotecan.

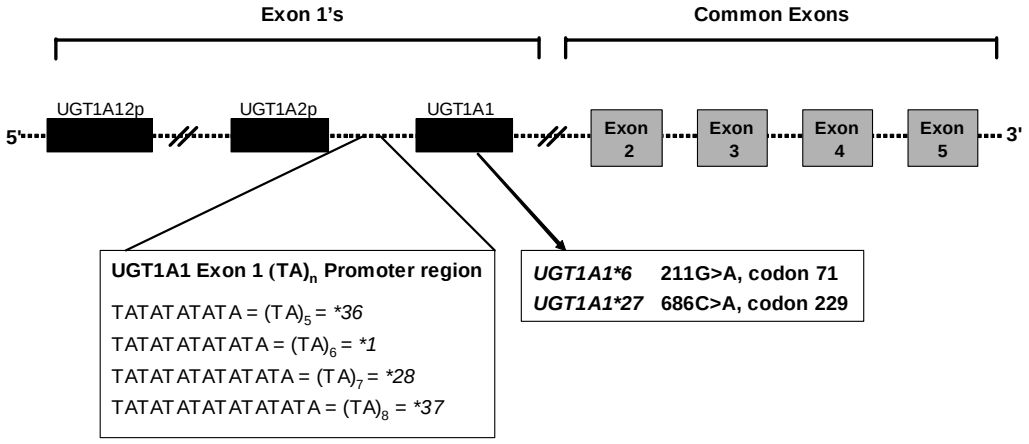


Figure 3. Schematic of the partial *UGT1A1* gene showing locations of the polymorphisms of interest for this review in the Exon 1 promoter region and in Exon 1. First exons are alternatively spliced to common exons to produce UGT isoforms. Figure adapted from Innocenti & Ratain, Oncology (Williston Park). 2003;17(Suppl 5):52-5., and Innocenti & Ratain, Clin Pharmacol Ther. 2004;75(6):495-500.

Table 3. *UGT1A1* allele naming conventions, locations, and associated phenotypes[a]

UGT1A1 Alleles	Variant or SNP	Location	Enzyme Activity	Associated Phenotype
*UGT1A1*1*	$(TA)_6TAA$	Promoter	Normal	Wild type
*UGT1A1*28*	$(TA)_7TAA$	Promoter	Reduced[b]	Gilbert syndrome
*UGT1A1*36*	$(TA)_5TAA$	Promoter	Increased[c]	
*UGT1A1*37*	$(TA)_8TAA$	Promoter	Reduced[b]	Crigler-Najjar syndrome, type II
*UGT1A1*6*	c.211G>A; G71R	Exon 1	Reduced	Gilbert syndrome
*UGT1A1*27*	g.686C>A; P229Q	Exon 1	Reduced	Gilbert syndrome

[a] This review does not address other functional polymorphisms in the promoter (*e.g.*, *UGT1A1*60*; g.-3279T>G) and coding regions (*e.g., UGT1A1*7*; c.1456T>G).
[b] Reduction in *UGT1A1* expression and UGT1A1 activity
[c] Normal to increased transcriptional activity

As described previously, the UGT enzyme is produced by the *UGT1A1* gene, and metabolizes SN-38 to the inactive form SN-38G. When the *UGT1A1* promotor has the wild type sequence $(TA)_6TAA$, average levels of the UGT1A1 enzyme will metabolize, inactivate and eliminate SN-38. The insertion of an extra dinucleotide repeat in the promoter region of the gene - $(TA)_7TAA$ (*UGT1A1*28* variant) - results in reduced gene expression. Resulting enzyme levels appear to vary substantially, but are reduced about 25% compared to normal in *UGT1A1*28* heterozygotes, and 50-70% compared to normal in *UGT1A1*28* homozygotes.[11,25] The homozygous *UGT1A1*28* genotype is found in about 10% of the North American population, and is almost always the causative genotype for Gilbert syndrome in Caucasians; *UGT1A1*6* is associated with Gilbert syndrome in a small number of cases.[15,40] Criglar-Nijjar syndrome, types I and II, are associated with other variants, most quite rare.[15]

Clinical Scenario for *UGT1A1* Testing

The *UGT1A1*28* allele is associated with reduced levels of enzyme. Therefore, individuals with the wild type sequence (**1/*1*) who have average levels of the enzyme will metabolize SN-38 more quickly than individuals who are either heterozygous (**1/*28*) or homozygous (**28/*28*). Higher or more prolonged exposure to the active form of the drug, which may result from the presence of *UGT1A1*28*, is thought to explain many adverse drug events observed, including severe neutropenia and severe diarrhea. If irinotecan dosage can be modified based on *UGT1A1* genotype, some proportion of the adverse events might be avoided. However, a reduction in dosage might also be associated with reduced tumor response and/or increased morbidity.

In late 2004, a change to the Camptosar (irinotecan) Injection Package Insert prescribing information[29,35] was announced through an email alert (NDA 20-571/S-024/S-027/S-028), stating that:

> "…a reduction in the starting dose by at least one level 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."

> "Individuals who are homozygous for the UGT1A1*28 allele are at increased risk for neutropenia following initiation of Camptosar treatment. A reduced initial dose should be considered….. Heterozygous patients… may be at increased risk for neutropenia; however, clinical results have been variable and such patients have been shown to tolerate normal starting doses."

Subsequently, in August 2005, the Invader® UGT1A1 Molecular Assay (Third Wave Technologies, Inc., Madison, Wisconsis) that tests for *UGT1A1*28* and *UGT1A1*1* was cleared by the US Food and Drug Administration (FDA) under 510(k) rules for Drug Metabolizing Enzyme Genotyping Systems.[47,48] The Invader test and other laboratory developed *UGT1A1* tests are currently available from multiple laboratories in the United States (see Table KQ2-1)[49], and are being marketed to oncologists and pathologists as an aid to clinical decision making.[50,51] In its package insert, Third Wave, Inc. describes the product as :

> "…..an *in vitro* diagnostic test for the detection and genotyping of the **1* (TA6) and **28* (TA7) alleles of the UDP glucuronosyltransferase 1A1 (UGT1A1) gene in genomic DNA from whole peripheral blood as an aid in the identification of patients with greater risk for decreased UDP-glucuronosyltransferase activity".

Results

Key Question 1 (Overarching Question): Does testing for *UGT1A1* mutations in patients with metastatic colorectal cancer treated with irinotecan lead to improvement in outcomes?

No controlled trials of *UGT1A1* genotyping versus clinical outcomes have been reported, and, thus, the overarching question cannot be answered directly. Most available studies genotyped all patients, but either did not use the information in clinical decision-making, or genotyped after the trial had ended. More studies provided information on toxicity rather than on clinical outcomes (*e.g.*, survival, response to treatment).

Key Question 2: What is the analytic validity of the test(s) that identify key *UGT1A1* mutations?

<u>Testing Methods</u>

A variety of methods have been described to detect the *UGT1A1*28* variant, including polymerase chain reaction (PCR) with capillary electrophoresis or high resolution polyacrylamide gel electrophoresis (PAGE)[52,53], direct sequencing[54], pyrosequencing[41,55], denaturing high pressure liquid chromatography (dHPLC)[56,57], real-time fluorescence PCR[58], and fluorescence resonance energy transfer (FRET) with melting curve analysis.[59,60] Table KQ2-1 shows examples of different laboratories in the U.S. that are offering clinical testing for *UGT1A1* variants using the Invader assay or validated laboratory developed tests. This list was compiled through a search of Google and the *GeneTests* website[61] and is meant to illustrate a range of offerings rather than a comprehensive listing. Testing costs are about $250[19] and testing is being offered for both blood and buccal samples.

Table KQ2-1. Examples of US Laboratories Offering Clinical Testing for *UGT1A1*

Laboratory	Test name/disorder tested	Method
Genzyme Genetics[a]	*UGT1A1* Molecular Assay	Invader (Third Wave)
Mayo Medical Labs[b]	*UGT1A1* TA Repeat Genotype	Invader (Third Wave)
LabCorp[c]	*UGT1A1* Irinotecan Toxicity	PCR / Capillary electrophoresis
Quest Diagnostics[d]	UGT1A1 Gene Polymorphism (TA repeat)	Fluorescent PCR
Molecular Diagnostics Laboratories[e]	*UGT1A1* (irinotecan/Camptostar) Metabolism, Gilbert syndrome	PCR / PAGE
Children's Hospital Boston DNA Diagnostic Laboratory[f]	Gilbert syndrome	Targeted mutation analysis
University of Chicago Genetics Laboratory[g]	Gilbert syndrome, Irinotecan metabolism	PCR / Capillary electrophoresis

[a] http://www.genzymegenetics.com/testmenu/tests/cancer/gene_p_testmenu_can_test_ugt1a1.asp, accessed 12/2006.
[b] http://www.mayoclinic.org/news2005-rst/3166.html, accessed 12/2006.
[c] http://www.labcorp.com/dos/index.html, accessed 12/2006.
[d] http://www.questdiagnostics.com/hcp/intguide/jsp/showintguidepage.jsp?fn=TS_UGT1A1.htm, accessed 12/2006.
[e] http://www.mdl-labs.com/UGT1A1.htm, accessed 12/2006.
[f] GeneTests, http://www.genetests.org/, accessed 12/2006.
[g] http://www.genes.uchicago.edu/lab.html, accessed 12/2006.

The FDA approved Invader® UGT1A1 Molecular Assay is based on a patented methodology that utilizes PCR with "specific Invader® DNA probes, a structure-specific cleavage enzyme and a universal fluorescent resonance energy transfer (FRET) system combined with interpretive software and third party microtiter plate reader instrumentation." [49] The assay uses four wells per sample (one well for each of the four PCR reactions) to make a genotype call. Each well contains a TATA box specific probe and an internal control. If acceptable quality criteria are not met, the software identifies the result as either "Low Signal" or "Equivocal" and the sample must be retested. After two unacceptable results a new sample must be obtained.

The Invader proprietary software reports genotypes as *6/6 (*1/*1), 6/7 (*28/*1), 7/7 (*28/*28),* and *"other".* A study from Japan noted that the Invader® UGT1A1 Molecular Assay is designed for detecting *UGT1A1*28* and the single nucleotide polymorphisms *UGT1A1*6* and *UGT1A1*27.* The capacity of this assay to test for four alleles (*UGT1A1*1, UGT1A1*28, UGT1A1*6,* and *UGT1A1*27*) was confirmed by the test manufacturer (personal communication, Dr. Amy Brower, Third Wave Technologies, Inc.). However, the FDA approved intended use for this test covers detection of only the **1* and *28 alleles. Consequently, Invader software must currently mask the presence of **6* and **27* polymorphisms and report them as "Other".

The Genetic Testing Reference Materials Coordination Program (GeT-RM) provides a set of *UGT1A1* control samples that were assessed using Invader and by sequencing (http://wwwn.cdc.gov/dls/genetics/rmmaterials/pdf/UGT1A1.pdf).

Analytic Sensitivity and Specificity

Table KQ2-2 summarizes the results of published and grey (*i.e.*, FDA submission) literature relating to the analytic sensitivity and specificity of *UGT1A1* assays. No false positive or false negative results were reported in four studies of three test methodologies that all used sequencing as the "gold standard" referent method. Results from the four studies were combined to provide estimates of analytic sensitivity and specificity for testing of **28* genotypes. Overall, genotypes for 190 of 190 samples homozygous or heterozygous for **28* were correctly identified for an analytic sensitivity of 100% (95% CI 98 -100%). The **1/*1* genotype was correctly identified in 131 of 131 samples, for an analytic specificity of 100% (95% CI 97-100%). All studies reported on testing of DNA samples extracted from EDTA anticoagulated whole blood; use of other sample types (*e.g.*, buccal, saliva) requires independent validation.

One study[59] reported a comparison of the performance of the Invader test versus sequencing with restriction fragment length polymorphism (PCR-RFLP) analysis in identifying the **6* and **27* alleles. Two inconsistent results were found between PCR-RFLP and Invader; retesting on both systems revealed a clear mix-up of the two samples in the first Invader test. With that

Table KQ2-2. Analytic Validity of *UGT1A1* Testing for *UGT1A1 *1* and **28* Genotypes

Reference	N	Assay Method	Referent Method	Analytic Sensitivity (test result / referent result)		Analytic Specificity
				***28/*28**	***28/*1**	***1/*1**
Monaghan G. et al, 1996[40]	12	Radioactive PCR	Sequencing	4/4	5/5	3/3
Pirulli D et al., 2000[56]	40	DHPLC	Sequencing	19/19	8/8	13/13
Hasegawa. et al., 2004[59]	60	Invader (RUO)	Sequencing	4/4	11/11	42/42[a]
Invader FDA 510(k) Summary, 2005[47]	212	Invader (IVD)	Sequencing	30/30	109/109	73/73
	324		**Total**	**57/57**	**133/133**	**131/131**
All				**100%** [98–100%]		**100%** [97–100%]

[a] 3 sample failures

exception, genotype results for **6* were consistent between the two methods. Estimates of analytic sensitivity and specificity were 100% (12 of 12; 95% CI 73.5-100%) and 100% (37 of 37 **1/*1*; 95% CI 90.5-100%). Genotype results were also consistent between sequencing/PCR-RFLP and Invader for two **27* heterozygotes. The two incorrect *6 results attributed to sample mix-up were not counted here as the focus is on errors in the analytic phase. However, this finding does again emphasize the need to consider pre- and post-analytic errors.

Update: Targeted review of the published literature between January and December 2007 identified two additional method comparison studies that are relevant to the above analysis. Huang et al. reported full concordance for 32 samples with genotypes that covered all $(TA)_{5-8}$ polymorphisms, comparing the Invader assay with their laboratory developed PCR/capillary electrophoresis method.[62] Baudhuin et al. reported on 119 samples tested for $(TA)_{5-8}$ polymorphisms by sequencing, the Invader assay, and a laboratory developed "size-based" method (PCR/capillary electrophoresis).[63] They reported 100% concordance between sequencing and PCR/capillary electrophoresis for all 119 samples, and 100% concordance between sequencing and the Invader assay for the 88 of 94 samples with **1/*1, *28/*1,* and **28/*28* genotypes for which results were obtained. The Invader software correctly identified 22 samples with non-**1/*1, *28/*1,* and **28/*28* genotypes as "Other".

Test Robustness

Robustness measures how resistant the assay is to changes in pre-analytic and analytic variables (*e.g.*, sample type and quality, reagent lot changes). Sample failures result in the need to repeat the test on the same sample and/or obtain a second sample for repeat testing, impacting turn-around time and the number of tested individuals who get a usable result. If a successful test result cannot be obtained, this can impact on clinical sensitivity. Good agreement between different lots of reagents was reported for the Invader assay.[47]

Test Reproducibility and Failure Rates

Data on reproducibility of the Invader® UGT1A1 Molecular Assay was reported as part of the Premarket Submission to the FDA for 510(k) approval.[47] Twenty blood samples were each tested five times at three different sites (N=300): six **1/*1* (N=90 = 5 x 3 x 6); five **28/*1* (N=75); four **28/*28* (N=60); five undisclosed genotypes (N=75).[59,60] Of the 49 initial sample failures, Third Wave reported that 40 were due to invalid results for positive or negative controls (*i.e.,* a quality control failure), and the remaining nine were due to a "Low Signal" result (*e.g.,* a likely reagent or equipment problem). Failure rates on a first run were 9.3% and 7.0% for sites 1 and 3. Site 2 did not report any sample failures, raising the question of whether failures were actually reported, or if the laboratory was generally more experienced and/or proficient with the assay. A second test resolved most problems and provided reportable sample results. However, genotype reactions for six samples were still reported as "Low Signal". The failure rate after two runs at the two sites was 1% (6 of 600; 95% CI 0.4-2.2%). In these studies, incorrect results were reported for 11 samples, all from Site 1, for an overall correct call rate of

98.8% (883/894; 95% CI 97.8-99.4%). Nine of these 11 incorrect results were explained as sample mix-ups, another example of pre-analytic errors that also occur in clinical practice.

Assuming two unexplained errors, the overall correct analytic call rate was 99.8% [95% CI 99.2-99.9%]. Review of this data by genotype did not provide any indication that failures or incorrect results were more likely in samples with specific genotypes. In another report of Invader testing for *UGT1A1*6* and *UGT1A1*27* , Hasegawa et al. observed failure rates of 10% (6 of 60) in *UGT1A1*6* testing (5 **1/*1*, 1 **6/other*), and 21.7% (13 of 60) in **27* testing.[59] These reported sample failures appear to relate most strongly to samples with DNA concentrations less than 6 ng/ul (≤ 2.1 ng/ul for 3 **28* failures, ≤4.6 ng/ul for 6 **6* failures, ≤5.9 ng/ul for 13 **27* failures). The Invader package insert recommends using DNA extracted and purified from blood samples at concentrations of 10-70 ng/ul. Therefore, fewer failures might be anticipated in practice if laboratories optimize testing protocols for DNA concentration.

Update: A 2007 study using the Invader assay reported that 9 of 119 samples failed on the first run due to "Low signal"; all failed on repeat analysis (1 low signal, 8 due to a control-based run failure), and insufficient sample remained for a third run.[63] The authors compared this rate to first run failure rates in the same study of 5.0% for sequencing and 1.7% for the size-based (PCR / capillary electrophoresis) method, with all failures resolved by repeat analysis with these methods. In this study, the authors concluded that Invader samples did not fail due to insufficient quantity or poor quality of DNA. The author of this study also provides a good discussion of other advantages and disadvantages of the different methods based on factors that include cost, turn-around time, reportable genotypes, need for PCR, sample requirements, assay robustness, and needed instrumentation.

Limitations of Studies

- data on the range of test methodologies currently used for clinical testing are limited.
- the data reflect only the analytic phase of testing, likely resulting in an overestimation of analytic performance. More generalizable estimates of analytic sensitivity and specificity, such as those derived from proficiency testing schemes (see below), also account for errors in the pre-analytic (*e.g.*, sample handling or labeling errors) and post-analytic (*e.g.*, data entry or interpretive/reporting errors) phases.[64,65]
- a large proportion of the data was reported by early Invader investigators[59] or the manufacturer (Third Wave FDA submission)[47]. The reported FDA multi-site study may

contain data previously published in the Hasegawa et al., 2004 report, but not identified as such. In addition, the performance of these assays is likely to be optimal in the hands of the test developers.

- in some method comparison studies, assays or samples that fail may not always be reported, or may be repeated until an answer is obtained.
- only one published study (Pirulli *et al.*)[56] reported that the samples were blinded to those performing the assays, in order to rule out retesting to get the "right" answer. Third Wave reported that the studies conducted as part of the FDA submission were blinded (personal communication, Dr. Amy Brower, Third Wave Technologies, Inc.).

Published data on test performance in clinical laboratories currently performing *UGT1A1* testing using Invader or in-house methodologies were not identified.

Proficiency Testing

In 2007, external proficiency testing became available for *UGT1A1* testing through the College of American Pathologists (CAP)/American College of Medical Genetics Pharmacogenetics (PGx) Survey for 2007 (www.cap.org). The PGx Survey consists of two challenges per year. Both analytical and interpretive challenges are included in this survey format. Results will be presented with peer comparisons and additional educational information. These external challenges address the pre- and post-analytic phases of testing, and, when carefully analyzed, provide a better estimate of inter-laboratory variation than individual method comparison studies, and are likely to provide a more useful assessment of analytic validity in routine practice.

Confirmatory Testing

Confirmatory testing has been suggested for some tests (*e.g.*, for *HFE C282Y* homozygotes) as a way to resolve false positives.[65] However, *UGT1A1*28* heterozygotes and homozygotes genotypes are much more common than *HFE C282Y* homozygotes (in Caucasians, about 45% and 11%, as compared to about 0.4%). Since strength of evidence for analytic sensitivity and specificity is relatively weak, benefits of confirmatory testing cannot be confidently estimated at this time. Monitoring the analytic sensitivity and specificity in actual clinical practice, including the types, rates, and causes of error, will be important to clarify the potential need for confirmatory testing of *UGT1A1* genotypes.

Analytic Validity Summary: While the data reported here are likely to overestimate analytic sensitivity and specificity, there is a reasonable expectation that laboratory results will, as for other single marker DNA tests studied through external proficiency testing surveys, be highly concordant.[64,65]

Key Question 3: What is the clinical validity of *UGT1A1* testing?

Key Question 3a: How well does *UGT1A1* testing predict phenotypic markers (*e.g.*, increased plasma SN-38 levels or decreased enzyme activity) and associated adverse drug reactions (*e.g.*, diarrhea, neutropenia)?

In the introduction, polymorphisms in the *UGT1A1* gene were described (see Table 2). These include the four polymorphisms in the TATA box in the promoter region (**1, *28, *36, *37*), as well as two additional polymorphisms in *UGT1A1* exon 1 (**6* and **27*) (Table 2, Figure 1). The analyses contained in this clinical validity section are generally restricted to the most common of the promoter region TATA repeat polymorphisms, **1* and **28*. They account for 98-99% of the polymorphisms in the Caucasian population, and are the focus of most studies. Homozygosity for **28* is specified as the primary risk factor in the Camptosar (irinotecan) package insert. Information on rare polymorphisms found in other racial/ethnic groups (*e.g., UGT1A1*6* and **27* in Asians) is more limited, so the decision was made to focus this report on the common alleles for which testing is broadly available.

The terminology used will be as follows: **1/*1* for the homozygous wild type genotype, **1/*28* for the heterozygous genotype, and **28/*28* for the homozygous variant genotype. Based on the frequency data presented later (Table KQ3-8), the expected distribution of these genotypes in the general Caucasian population is about 42%, 45% and 11%, respectively.

Some studies combine diarrhea and neutropenia (and, in some cases, other severe adverse drug reactions) into a single category.[66] There are, however, sufficient numbers of studies that report rates for single events (*e.g.*, severe neutropenia) to allow the analyses to focus on severe neutropenia (grade 3-4) alone and severe diarrhea (grade 3-4) alone as the two adverse drug reactions of interest. Descriptions of these adverse reactions can be found in the Introduction.

UGT1A1 genotypes and SN-38 levels

As previously described, the prodrug irinotecan is converted to SN-38, the most active form of the drug, by carboxylesterase enzymes. SN-38 is subsequently conjugated by UGT enzymes to form the inactive SN-38 glucuronide (SN-38G) metabolite in the liver. SN-38G is then excreted into bile and urine.[42] Individuals vary in their ability to convert SN-38 to SN-38G due to varying UGT phenotypes. The most widely studied is *UGT1A1*. One way to assess the 'exposure' to SN-38 is to compare the *area under the curve* (AUC) for SN-38G with that for SN-38. Essentially, this compares the integrated time dose exposure (AUC) for the inactive form (SN-38G) to that of the active form (SN-38). High values indicate that most exposure is to the inactive form; low values indicate increased exposure to the active form. In general, the AUC ratios would be expected to be lower for **1/*28* heterozygotes than for **1/*1* individuals, and the **28/*28* homozygotes would be expected to be even lower. This would indicate that the highest exposure to the active form of irinotecan occurs among the **28/*28* homozygotes. Table KQ3-1 and Figure KQ3-1 present data from six studies reporting AUC ratios[42,67-71], and results are consistent with expectations. In all of the studies, the highest exposure is in the **28/*28* individuals. The AUC ratios are lower for individuals heterozygous for **28* (**1/*28*) than for wild type individuals (**1/*1*). The AUC ratios are even lower for individuals homozygous for **28* (**28/*28*). This indicates that the highest relative exposure to the active form of irinotecan, SN-38, occurs among the individuals homozygous for **28 (*28/*28*). The SN-38 to SN-38G AUC ratios should be viewed as an intermediate outcome.

A more appropriate measure of exposures would include the irinotecan dose. The biliary index (BI) is the irinotecan AUC times the ratio of the SN-38 to SN38G AUCs. Two studies provided the BI in cancer patients stratified by *UGT1A1* genotype. One studied 71 colorectal cancer patients[71], the other reported on 20 cancer patients, 4 of whom had colorectal cancer.[67] Both found a significant and consistent dose response in the BI from the wild type, through the heterozygotes and homozygotes. These data strongly indicate the highest time-weighted exposure to the active form of irinotecan occurs in individuals homozygous for **28 (*28/*28*).

A more formal analysis can be made using the data from Table KQ3-1 by expressing the AUC ratios for **28* heterozygotes and homozygotes as proportions of that same ratio in **1/*1* individuals. When this is done, the ratios are 0.81 (a 19% reduction) in **1/*28* heterozygotes compared to **1/*1* individuals, and 0.43 (a 57% reduction) among **28/*28* homozygotes. This indicates, for example, that **28/*28* homozygotes have about twice the exposure to SN-38 than

*1/*1 individuals. The study by Toffoli et al.[71] reported molar ratios and these data could not be combined.

Table KQ3-1. Ratio of *Area Under the Curve* (AUC) for SN-38G to SN-38 by *UGT1A1* Genotype in Six Studies

				***UGT1A1* Genotype**					
				***1/*1**		***1/*28**		***28/*28**	
Author	**Country**	**CRC (%)**	**Dose* (mg/m^2)**	**N**	**Ratio**	**N**	**Ratio**	**N**	**Ratio**
Iyer 2002[67]	US	30	300 IV	9	9.28	7	4.04	4	2.41
Mathijssen 2003[68]	NL	80	200-350 IV	32	6.60	19	6.60	2	3.70
Mathijssen 2004[69]	NL	38	600 IV	13	9.27	12	5.79	3	3.48
Paoluzzi 2004[70]	Italy	100	600 IV	44	7.00	37	6.26	5	2.51
Sai 2004[42]	Japan	25	60-150 IV	23	6.38	15	3.45	3	3.57
Toffoli 2006[71]	Italy	100	180 IV	31	3.75	32	3.33	8	1.86
Total				144		122		25	

CRC – colorectal cancer; NL – Netherlands

*Dose in mg/m^2 administered via infusion

When examining the actual distribution of AUC ratios, however, it is clear that they are not Gaussian distributions. For three of the studies,[42,69,70] it was possible to generate individual data points from figures included in the publications. These three studies have similar mean AUC ratios compared to the remaining three studies (0.76 vs 0.87 for heterozygotes and 0.42 vs 0.44 for homozygotes), so the sub-analysis is likely to be representative of the finding for all six sudies. Figure KQ3-1 shows the digitized data for the three studies on a logarithmic y-axis. After transformation, the data appear to fit a Gaussian distribution more closely (Figure KQ3-2). Analysis of variance (ANOVA) indicates that the differences in mean levels are statistically significant ($p < 0.001$), but that the differences in variances are not. Overall, the pooled variance on the logarithm scale is 0.246, after accounting for the varying mean levels by *UGT1A1* genotype (*e.g.*, horizontal lines, Figure KQ3-1).

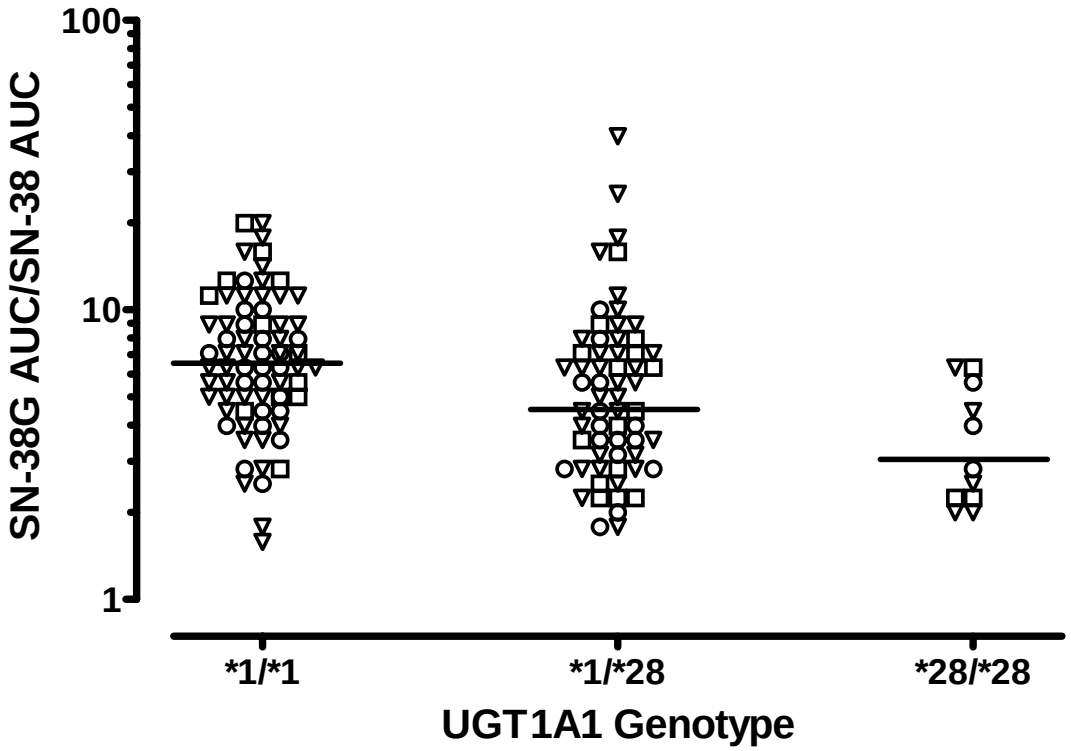


Figure KQ3-1. SN-38G AUC to SN-38 AUC ratio by *UGT1A1* Genotype. Each symbol corresponds to one of three studies from which the data were digitized (Paoluzzi 2004 ∇, Sai 2004 O, and Mathijssen 2004 □).[70,42,69] The horizontal lines indicates the median levels of 7.2, 5.0 and 3.4 for **1/*1*, **1/*28* heterozygous and **28/*28* homozygous individuals, respectively).

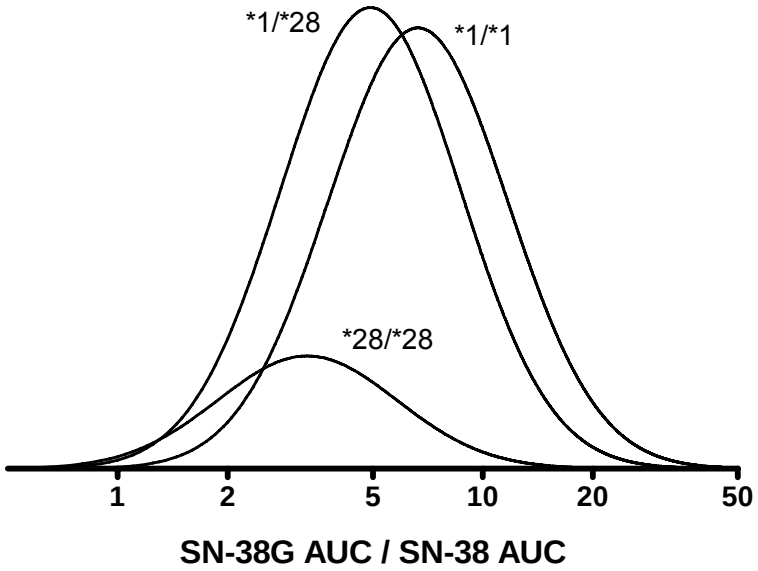


Figure KQ3-2. The overlapping distributions of the AUC ratios for SN-38G to SN-38 by *UGT1A1* genotypes. Absolute frequency distributions are plotted on a horizontal logarithmic scale with each curve having the same logarithmic standard deviation. Mean values for each are based Figure KQ3-1 data. Since curves represent absolute frequency distributions, no vertical axis or scale has been presented.

Using untransformed data, the earlier analysis estimated reductions in the AUC ratios to be 19% and 57%, for heterozygotes and homozygotes, respectively. The transformations shown in Figure KQ3-2 allows for a more reliable comparison of the SN-38 exposure. On average, heterozygotes have an average reduction in the AUC ratios of 31% (1 – 5.0/7.2), while the average reduction in homozygotes is 53% (1– 3.4/7.2). Using these latter estimates of effect, the pooled logarithmic standard deviation of 0.246, and the genotype frequencies in Caucasians (Appendix D, Table D-2; 42%, 45% and 11% for **1/*1*, **1/*28* heterozygous and **28/*28* homozygous individuals, respectively), the overlapping AUC ratios for the three *UGT1A1* genotypes can be plotted. Figure KQ3-2 shows these three curves in absolute frequency, with the area of the curves in the ratio described above (since curves in Figure KQ3-2 represent absolute frequency distributions, no vertical axis or scale has been presented.). One can easily see the reductions in the average AUC ratios indicating differing exposures to SN-38, but the overlap is still considerable. This analysis shows that homozygous *28/*28 individuals have about twice the SN-38 (active form) exposure than do **1/*1* individuals receiving the same dose of irinotecan. The SN-38 to SN-38G AUC ratios are an intermediate outcome.

Chemotherapy treatment regimens utilized in studies for clinical validity

The studies selected for evaluating clinical validity (and clinical utility) did not use standardized treatment regimens. Table KQ3-2 provides a brief description of each study's treatment protocol(s).[14,67,71-77] In several studies, multiple treatment protocols were evaluated. It was not possible to account for treatment regimens in subsequent analyses. Rather, we examined homogeneity of results to rule out any large effect of the varying treatments. In other words, if the analysis of a clinical validity measure (*e.g.*, severe neutropenia) was found to be homogeneous within a comparison group (*e.g.*, **28* homozygotes compared to **1/*1* wild type), it was assumed that a given treatment regimen did not have a significant impact on that measure.

Table KQ3-2. Chemotherapy treatment regimens used in studies selected for analysis

Study (reference)	Description
Carlini et al., 2005[72]	• Group 1 (15 patients) received 1,000 mg/m^2 Capectabine orally twice daily on days 2-15 of 3 wk cycle with 125 mg/m^2 of irinotecan (90 min IV infusion) on days 1 and 8 of each cycle. • Group 2 (52 patients) received 900 mg/m^2 Capectabine orally twice daily for the same period with 100 mg/m^2 of irinotecan (90

	min IV infusion) on days 1 and 8 of each cycle.
Font et al., 2003[76]	• 70 mg/m^2 of irinotecan (90 min IV infusion) + 25 mg/m^2 docetaxel (30 min IV infusion) on days 1, 8, and 15 followed by a 1 wk rest (28 day cycles)
Innocenti et al., 2004[14]	• 350 mg/m^2 of irinotecan (90 min IV infusion) once every 3 wks
Iyer et al., 2002[67]	• 300 mg/m^2 of irinotecan (90 min IV infusion) once every 3 wks
Marcuello et al., 2004[73]	• Regimen A: 350 mg/m^2 of irinotecan (45 min IV infusion) once every 3 wks • Regimen B: Regime A + 3 mg/m^2 Tomudex in 15 min IV every cycle • Regimen C: 80 mg/m^2 of irinotecan (45 min IV infusion) every wk + 1 dose 2250 mg/m^2 5-FU (48 min continuous infusion) every cycle • Regimen D: 180 mg/m^2 of irinotecan (45 min IV infusion) every 2 wks + 5-FU and leucovorin
Massacesi et al., 2006[77]	• 80 mg/m^2 of irinotecan (30 min IV infusion) on days 1, 8, 15, 22, and 36, 43, 50 and 57 days. 3 mg/m^2 of raltitrexed 2 to 4 hours later (15 min IV infusion) on days 1, 22 and 45.
Rouits et al., 2004[74]	• IRIFUFOL (28 patients): 85 mg/m^2 of irinotecan (90 min IV infusion) + 1200 mg/m^2 5-FU (7 hour IV infusion) and 100 mg/m^2 bolus L-folinic acid, each week. • FOLFIRI (47 patients): 180 mg/m^2 of irinotecan (90 min IV infusion) + 2500 mg/m^2 5-FU (continuous infusion) and 400 mg/m2 bolus L-folinic acid, biweekly.
Soepenberg et al., 2005[75]	• 70 or 80 mg/m^2 of irinotecan given orally to fasted patients once daily for 5 days.
Toffoli et al., 2006[71]	• Modified FOLFIRI (90% of patients): 180 mg/m^2 of irinotecan (2 hr IV infusion) on day 1 + 400 mg/m^2 of 5-FU bolus followed by 2,400 mg/m^2 of 5-FU (46 hr IV infusion) + 200 mg/m^2 of LV on day 1 every 2 wks. • FOLFIRI (10% of patients): 180 mg/m^2 of irinotecan (2 hr IV infusion) on day 1 + 400 mg/m^2 bolus of 5-FU followed by 600 mg/m^2 of 5-FU (22 hr IV infusion) on days 1 and 2 + 200 mg/m^2 of LV on days 1 and 2 every 2 wks.

UGT1A1 genotypes and severe neutropenia

Eight published reports contained sufficient information to report (or estimate) the rate of severe neutropenia (grade 3-4).[14,67,71-75,77] Table KQ3-3 shows that the overall observed rate of severe neutropenia (grades 3 and 4 combined) from the studies selected for analysis was 16% (95% CI 13% to 19%). When stratified by *UGT1A1* genotypes, the rates were 9.8% (6.8% to 14%), 18% (14% to 23%) and 38% (22% to 57%) for wild type (**1/*1*) individuals, individuals heterozygous

for **28* (**1/*28*) and individuals homozygous for **28* (**28/*28*), respectively. The results were homogeneous within genotype (Q values of 4.5, 7.1 and 7.9 and p values of 0.7, 0.4 and 0.2, respectively) Analysis was performed using a random effects model using appropriate software (Comprehensive Meta-Analysis, Version 2, Englewood, NJ). A summary of the raw data and analysis is contained in Appendix D (Figure D-1).

Table KQ3-3. Summary of severe neutropenia rates in eight studies[14,67,71-75,77], stratified by *UGT1A1* genotype

UGT1A1 Genotype	Number	Neutropenia (%)	95% CI	Heterogeneity
**1/*1* (wild type)	25/294	9.8	6.8% to 14%	p=0.7
**1/*28* (heterozygote)	49/287	18	14 to 23%	p=0.4
**28/*28* (homozygote)	18/56	38	22% to 57%	p=0.2
Overall		16	13% to 19%	

Figure KQ3-3 shows the corresponding risk ratios from eight studies, with the risk in individuals with the wild genotype as the referent category. The summary risk ratios (point estimates and 95% confidence intervals) were computed using original data and a random effects model. The risk ratio for individuals heterozygous for **28 (*1/*28*) was 1.82 (95% CI 1.16 to 2.85), and the risk ratio for **28* homozygotes (**28/*28*) was 3.51 (95% CI 2.03 to 6.07). There was no significant heterogeneity between studies within the two comparison groups (Q values of 1.2 and 5.2, p values of 0.9 and 0.5). The difference in the risk ratios of 1.82 and 3.51 is not statistically significant (p=0.1), but because of the relatively large observed difference, and the biological plausibility, these differences are likely to be clinically important. Future analyses will use these point estimates.

UGT1A1 genotypes and severe diarrhea

Table KQ3-4 shows that the overall observed rate of severe diarrhea (grades 3 and 4) from the six studies selected for analysis[67,72-74,76,77] is 24% (95% CI 19% to 30%). When stratified by *UGT1A1* genotypes, the rates of severe diarrhea are 18% (95% CI 11% to 28%), 27% (95% CI 20% to 36%) and 27% (95% CI 12% to 48%) for individuals with the wild type, those heterozygous for **28 (*1/*28*) and those homozygous for **28* (**28/*28*), respectively. The analysis showed the rates between studies to be homogeneous within genotype (Q values of

10.4, 7.1 and 9.5; p values of 0.1, 0.3 and 0.2). Figure KQ3-4 shows the corresponding risk ratios from these studies, using the risk in the 154 study participants with wild genotype as the referent category. The summary risk ratios were computed using original data and a random effects model. The risk ratio for 155 individuals heterozygous for **28 (*1/*28*) was 1.40 (95% CI 0.94 to 2.08). The risk ratio for the 41 individuals homozygous for *28 (*28/*28) was 1.63 (95% CI 0.64 to 4.14). The results were homogeneous within groupings (Q values of 3.0 and 8.5, p values of 0.7 and 0.1, respectively). The point estimate for the risk ratio in homozygotes is higher than for heterozygotes, which meets expectation, though neither group shows a significant increase over the rate in **1/*1* individuals. Larger studies may find these differences to be significant.

Table KQ3-4. Summary of diarrhea rates in six clinical trials, stratified by *UGT1A1* genotype [67,72-74,76,77]

UGT1A1 Genotype	Number	Diarrhea (%)	95% CI	Heterogeneity
**1*1* (wild)	32/190	18	11% to 28%	p=0.1
**1/*28* (heterozygote)	47/179	27	20% to 36%	p=0.3
**28/*28* (homozygote)	14/48	27	12% to 48%	p=0.2
All		24	19% to 30%	

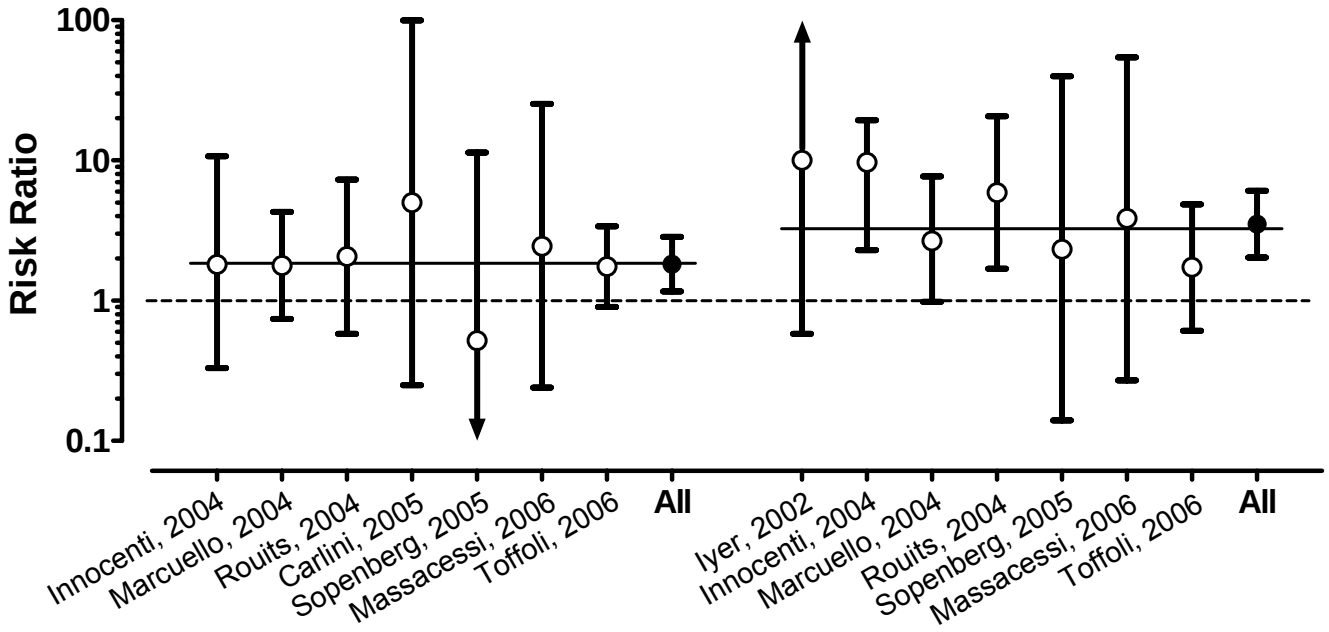


Figure KQ3-3. Risk ratios for grade 3 and grade 4 severe neutropenia by *UGT1A1* genotype from eight published studies.[14,67,71-75,77] The studies are listed on the x-axis, stratified by heterozygote individuals (**1/*28*) versus wild type (**1/*1*) on the left hand side and homozygote individuals (*28/*28) versus wild type on the right hand side. Two results (Iyer 2002 for heterozygotes, Carlini 2005 for homozygotes) are not shown as the risk ratio could not be computed due to no observations in one or more groups. The bars indicate the 95% confidence interval with the consensus estimate (All) for the two comparison groups. The dotted line indicates a risk ratio of 1.00 (no difference). The two thin solid lines indicate the consensus estimates for the two groups of 1.82 (95% CI 1.16 to 2.85) and 3.51 (95% CI 2.03 to 6.07), respectively.

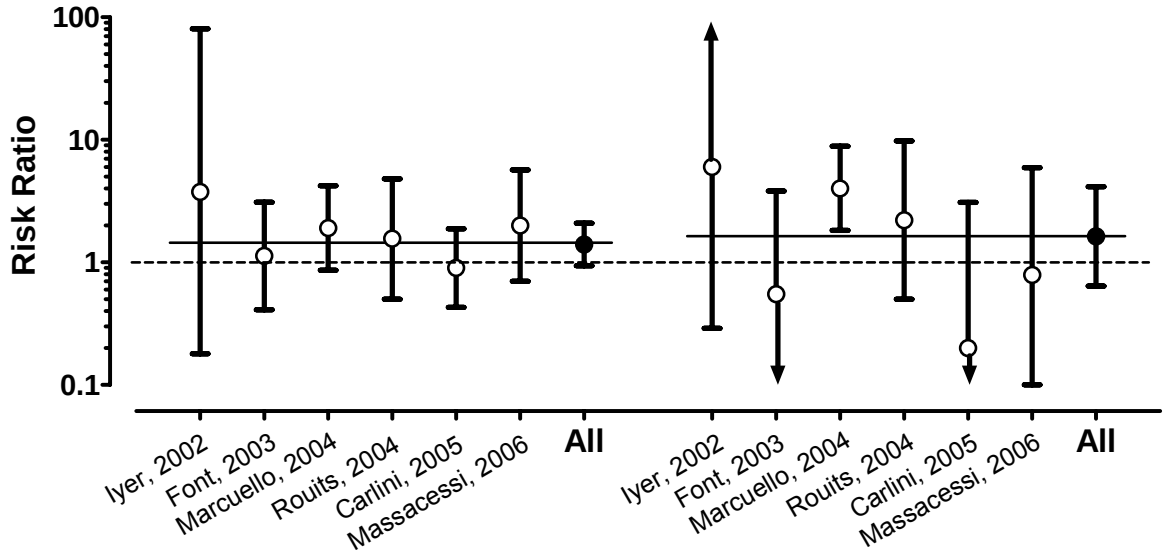


Figure KQ3-4. Risk ratios for grade 3 and grade 4 severe diarrhea by *UGT1A1* genotype from six published studies. The studies are listed on the x-axis, sorted by the risk in heterozygotes (**1/*28*) versus wild type (**1/*1*) on the left hand side and the risk is homozygotes (**28/*28*) versus wild type on the right hand side. The bars indicate the 95% confidence interval with the consensus estimate (**All**) for the two comparison groups. The dotted line indicates a risk ratio of 1.00 (no difference). The two thin solid lines indicate the consensus estimates for the two groups of 1.40 (95% CI 0.94 to 2.08) and 1.63 (95% CI 0.64 to 4.14), respectively.

Clinical sensitivity and specificity

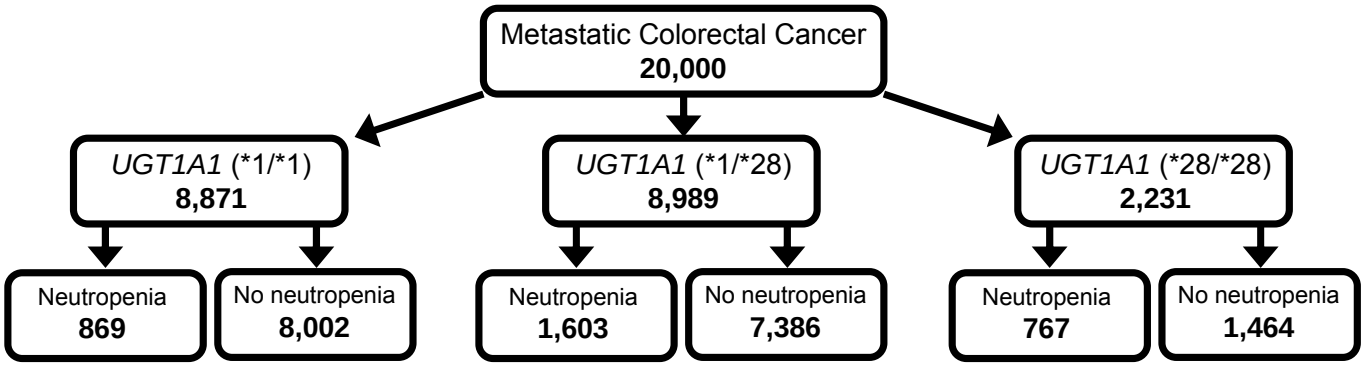
This section focuses on severe neutropenia (grade 3 and 4) because it is most strongly associated with *UGT1A1* genotype. A positive *UGT1A1* test is defined as a individuals homozygous for **28* (**28/*28*). The outcome of interest is the finding of severe (grade 3 or 4) neutropenia. Thus, the clinical sensitivity is defined as the proportion of individuals with severe neutropenia who are homozygous for **28*, while the clinical specificity is the proportion of individuals without severe neutropenia that are not homozygous for **28*. Table KQ3-5 shows the eight studies from which estimates of clinical sensitivity and specificity for severe neutropenia could be derived. [14,67,71-75,77] The overall clinical sensitivity estimate is 23% (95% CI 15% to 34%), with an associated clinical specificity of 92% (95% CI 90% to 94%) (estimates computed using a random effects model). In this analysis, sensitivity and specificity were considered to be independent (*i.e.*, ROC analysis was not performed).

Table KQ3-5. Clinical sensitivity and specificity of *UGT1A1* genotyping for severe neutropenia

Study	True Positive	False Negative	True Negative	False Positive	Sensitivity	Specificity
Carlini 2005[72]	0	2	59	5	0%	92%
Innocenti 2004[14]	4	5	48	2	44%	96%
Iyer 2002[67]	2	0	18	2	100%	90%
Marcuello 2004[73]	4	18	73	6	18%	92%
Massacesi 2006[77]	1	3	52	6	25%	90%
Rouits 2004[74]	4	10	59	3	29%	95%
Soepenberg 2005[75]	0	1	21	1	0%	95%
Toffoli 2006[71]	4	33	195	18	11%	92%
All (95% confidence intervals)					**23% (15-34%)**	**92% (90-94%)**

In these small genotyping studies, this simplification is likely to be acceptable. Analysis shows the results to be homogeneous (Q=9.0, p=0.2 for sensitivity; Q =2.8, p = 0.9 for specificity) and, therefore, no further stratified analyses were performed (*e.g.,* by study quality*)*.

It is also possible to compute the expected clinical sensitivity and specificity from parameters obtained earlier in this review as shown in Figure KQ3-5. A theoretical population of 20,000 Caucasians is first stratified by *UGT1A1* genotypes. Using the Hardy-Weinberg principal and the consensus estimate of the **28* allele frequency (0.334 from Table KQ3-8), the expected number of **1/*1, *1/*28,* and **28/*28* individuals can be derived. In the 8,871 wild-type individuals, the baseline rate of severe neutropenia (9.8%) would result in 869 wild-type individuals experiencing this adverse drug reaction. Using the risk ratios of 1.82 and 3.51, the numbers of adverse reactions in the individuals heterozygous and homozygous for **28* can also be computed.



Clinical sensitivity = 767 / (869 + 1,603 + 767) = **23.6%**
Clinical specificity = (8,002 + 7,386) / (8,002 + 7,386 + 1,464) = **91.3%**

Figure KQ3-5. Flow diagram showing the derivation of clinical sensitivity and specificity in a hypothetical cohort of 20,000 Caucasian individuals with metastatic colorectal cancer. The clinical sensitivity and specificity are derived using previously reported parameters (e.g., allele frequency, risk ratios), stratified by *UGT1A1* genotype. Overall, the clinical sensitivity is 24% with a specificity of 91% (false positive rate of 9%).

Estimates of expected clinical sensitivity and specificity of 24% and 91%, respectively, were derived. This agrees closely with the observed rates of 23% and 92% computed from published

observations (Table KQ3-5). The corresponding positive predictive value derived from Figure KQ3-5 is 52% (767/1,464) and the negative predictive value is 86% (1 - (869 + 1603) / (8871 + 8898)).

The estimates derived from Figure KQ3-5 are likely to be more reliable, as they are derived from an analysis that more correctly combines data from multiple studies (random effects model). Other possible reasons for discrepancies might include: some studies having Gilbert's syndrome as an explicit or implicit exclusion criteria (nearly all Gilbert syndrome patients are **28/*28*), exclusion criteria based on bilirubin levels, and chance.

Rating the quality of studies used for this key question

RTI reviewers rated studies based on a numerical ranking system (see Appendix C); EGAPP reviewers generated descriptive summaries of the studies that included specific elements (e.g., study design, population studied and inclusion/exclusion criteria, statistical analyses, potential biases) and concluded with a subjective rating and the key criteria that support the rating. There is no "correct" answer and various approaches have strengths and weaknesses. For example, number ratings may not assess or properly weight certain characteristics. In addition, RTI provided a single rating for each study, whereas the EGAPP approach rated each study with regard to the specific key question addressed. The studies and rankings are summarized in Table KQ3-6; detailed information can be found in Appendix C and Appendix E (RTI tables).

Table KQ3-6. Quality of Studies for Answering Key Question 3a

Study	RTI Rating	EGAPP Rating
Carlini et al, 2005[65]	*Poor*	*Fair*
Font et al, 2003[68]	*Poor*	*Marginal*
Innocenti et al, 2004[16]	*Poor*	*Marginal*
Iyer et al, 2002[58]	*Poor*	*Marginal*
Marcuello et al, 2004[63]	*Poor*	*Fair*
Massacessi et al, 2006[67]	*Poor*	*Fair*
Rouits et al, 2004[64]	*Poor*	*Fair*

Soepenberg et al, 2005[66]	*Poor*	*Marginal*
Toffoli et al, 2006[62]	*Not reviewed*	*Fair*

Key Question 3b: How well does *UGT1A1* testing in patients with metastatic colorectal cancer predict morbidity and mortality?

The *UGT1A1* genotypes of interest will again be limited to the wild type, heterozygous and homozygous genotypes (*e.g.*, **1/*1, *1/*28 and *28/*28,* respectively). In the following summaries, morbidity will be addressed by such factors as tumor response stratified by genotype. Mortality will be addressed by 1 or 2 year survival, or survival analysis. Reports that include cancers other than colorectal will be considered.

UGT1A1 genotypes and tumor response

Three studies provided information on tumor response, stratified by *UGT1A1* genotype.[71,72,76] One study found a higher rate of stable or partial responsive tumors among **28* heterozygotes and homozygotes combined, compared to wild type individuals (risk ratio of 1.6, 95% CI 0.8 to 3.0).[76] The two other studies defined a responsive tumor as 'partial or compute response' and provided sufficient data to examine response rates by *UGT1A1* genotype.[71,72] Among wild type, heterozygous and homozygous individuals, the tumor response rates were 41% (95% CI 33% to 40%), 47% (95% CI 33 to 63%) and 70% (95% CI 40 to 84%), respectively. The results were homogeneous within genotype (Q values of 0.2, 2.2 and 0.6, respectively; p values of 0.6, 0.1 and 0.4, respectively). Figure KQ3-6 shows analyses of the tumor response rate (as defined in the studies) versus *UGT1A1* genotype, with the **1/*1* wild individuals used as the referent category for those same two studies. Overall, the heterozygotes have a non-significantly higher response rate (risk ratio 1.09, 95% CI 0.83 to 1.43). The response rate in homozygotes is higher and reaches statistical significance (risk ratio 1.70, 95% CI 1.24 to 2.33; p=<0.001). The studies were homogeneous within genotype (Q values of 0.4 and 0.8, and p values of 0.6 and 0.8, respectively).

UGT1A1 genotypes and mortality

Two of the three studies providing information on tumor response also provided some information about mortality.[71,76] The other study provided information only on survival.[73] It was not possible to combine the information from these three studies in a formal analysis. Instead, the findings are summarized in tabular form in Table KQ3-7. The data from Font and colleagues[76] are for combined **28* heterozygotes and homozygotes, and all patients had lung cancer. The data from Toffoli and colleagues compare **28* heterozygotes and homozygotes with wild type (**1/*1)* individuals, and all patients had CRC.[71] The data from Marcuello and colleagues[73] include 95 patients with CRC, and represent combined heterozygotes and homozygotes.[73] None of the differences were statistically significant. Findings from two studies[71,76] were in the direction of improved survival for homozygotes versus non-wild type individuals, while the third reported a survival advantage for the wild-type individuals.

Rating the quality of studies used for this key question

RTI reviewers rated studies based on a numerical ranking system (see Appendix C); EGAPP reviewers generated descriptive summaries of the studies that included specific elements (e.g., study design, population studied and inclusion/exclusion criteria, statistical analyses, potential biases) and concluded with a subjective rating and the key criteria that support it. Both RTI and EGAPP reviewers rated Font et al[76] as *Poor/Marginal*; Carlini et al[72] was considered *Fair* by EGAPP reviewers, but *Poor* by RTI. Toffoli et al[71], identified in the later EGAPP search and not reviewed by RTI, was ranked as *Good* by EGAPP reviewers. Detailed information can be found in Appendix C.

Limitations of Studies on Clinical Validity – Key Questions 3a and 3b

- Nearly all of the clinical validity information was collected from populations consisting of non-Hispanic Caucasians. When studies did include other races/ethnicity, the clinical results were not stratified.
- Treatment protocols varied widely both within- and between-studies. Dosage, method of delivery and frequency of treatment might impact both the overall rate of adverse events as well as the relative risk of specific adverse events between *UGT1A1* genotypes. We did not find strong evidence of such an impact, but most studies contained too few study subjects to be confident.

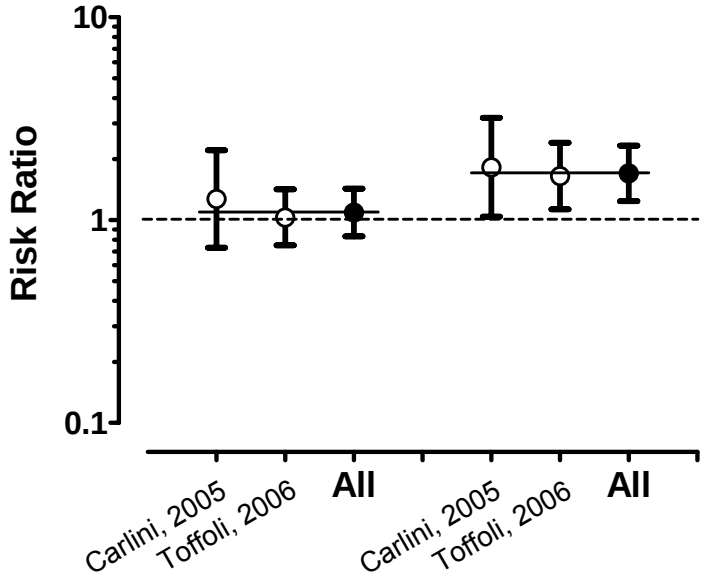


Figure KQ3-6. Risk ratios for tumor response by *UGT1A1* genotype

The studies are listed on the x-axis, stratified by heterozygote individuals (**1/*28*) versus wild type (**1/*1*) on the left hand side and homozygote individuals (**28/*28*) versus wild type on the right hand side. The bars indicate the 95% confidence interval with the consensus estimate (All) for the two comparison groups. The dotted line indicates a risk ratio of 1.00 (no difference). The two thin solid lines indicate the consensus estimates for the two group of 1.09 (95% CI 0.83 to 1.43) and 1.70 (95% CI 1.24 to 2.33), respectively.

Table KQ3-7. Mortality in cancer patients treated with irinotecan, stratified by *UGT1A1* genotype

Study	Outcome measure	Finding
Font et al, 2003[68]	Time to progression	3 months (*1/*1) vs. 4 months (other)
	Median survival	8 months (*1/*1) vs. 11 months (other)
	1 year survival	21% (*1/*1) vs. 41% (other)
	2 year survival	14% (*1/*1) vs. 31% (other)
Marcuello et al, 2004[73]	Median survival	32 mo (*1/*1) vs. 24 mo (other)
Toffoli et al, 2006[71]	Hazard ratio	0.81 (95% CI 0.45 to 1.44) (*28/*28 vs. *1/*1)
	Hazard ratio	0.84 (95% CI 0.58 to 1.21) (*1/*28 vs. *1/*1)
	Median survival	613 days (*1/*1) vs. 686 days (*28/*28)
	Median survival	613 days (*1/*1) vs. 669 days (*1/*28)

- Some studies reported adverse events after the first cycle of treatment, others after the completion of treatment, and others provided both. There was some evidence that the adverse events among individuals homozygous for the **28* allele most often occurred in the first cycle.[71] However, not enough studies provided clinical outcomes at both times to allow for a meaningful sub-analysis.
- Several studies included patients that had cancer at sites other than the colon. The impact of including these studies could not be determined, as none stratified their results by cancer site.
- Several studies identified individuals with less common genotypes. However, they were always included in larger groupings and, therefore, it was not possible to combine results for these genotypes across studies.

Key Question 3c: Do other factors (*e.g.*, race, ethnicity, other medications) affect clinical validity?

In June, 2005, the FDA changed the labeling for irinotecan to add homozygosity for *UGT1A1*28* as a risk factor for severe toxicity to the drug, along with pelvic/abdominal irradiation, poor performance status, and age greater than 65 years.[29,78] *Performance status* is a measure that uses scoring systems to quantify the general well-being or quality of life of cancer patients, in order to determine whether they are candidates for chemotherapy, whether dose adjustment is necessary, and what level of palliative care is needed.[c] Patients having prior *pelvic/abdominal irradiation* are at increased risk of severe myelosuppression. *Patients greater than 65 years of age* are at increased risk of late diarrhea. *UGT1A1* testing is an early example of genetic testing to identify a subpopulation of patients at increased risk of toxicity. The pharmacokinetics of irinotecan does not appear to differ based on gender or race. However, *UGT1A1* genotype frequencies do differ by race/ethnicity as discussed in the next section.

Genotype and Allele Frequencies for *UGT1A1* Polymorphisms

Caucasians (non-Hispanic)

Eleven published studies[40,52,79-87] contained sufficient information to report observed *UGT1A1* allele and genotype frequencies in Caucasian subjects (N=2,517) for the **1*, **28*, **36, *37, *6*

[c] Wikipedia, http://en.wikipedia.org/wiki/Performance_status, accessed 12/2006.

and **27* alleles. Allele frequencies are summarized in Table KQ3-8 (more detail and genotype frequencies can be found in Appendix D, Tables D-2 to D-4). Given the countries in which the studies were performed, it is likely that few Hispanic Caucasians would have been included. Thus, these estimates are likely to only be applicable to non-Hispanic Caucasians. No studies were identified that were performed in populations known to be all, or mostly, Hispanic Caucasians.

Table KQ3-8. *UGT1A1* allele frequencies stratified by race

Race	Studies (patients)	Allele Frequencies (95% confidence interval)				
		***28 (TA7)**	***36 (TA5)**	***37 (TA8)**	***6 (211G>A)**	****27* (686C>A)**
Caucasian [40,52,79-85,87,88]	11 (2,517)	0.334 (.309 - .361)	0.003 (.001 - .008)	0.002 (.001 - .009)	0.005 (.001 – 0.03)	no data
Asian/Asian American [79,85,89,90]	4 (454)	0.139 (.112 - .171)	0.000 (.00 - .09)	0.000 (.00 - .09)	0.13* (.10 -.17) *Refs: 89,90,96	0.023 (.014 - .035)
African/African American [23,79,91]	3 (411)	0.404 (.358 - .452)	0.058 (.039 - .085)	0.043 (.026 - .070)	no data	no data

Despite the fact that the 2,517 control or general population samples were obtained from a number of different sources (*e.g.*, randomly selected healthy unrelated subjects from a workplace or clinic, anonymous banked DNA samples) and assayed using variations of three methodologies, the reported frequencies of the promoter region TATA alleles and common *UGT1A1* genotypes are consistent. The consensus genotype frequencies for **28/*28* homozygotes, **1/*28* heterozygotes, and **1/*1* homozygotes were 0.108 (95% CI 0.90-0.130), 0.454 (95% CI 0.429-0.479), and 0.423 (95% CI 0.391-0.456), respectively. Analysis relied on a random effects model. There was significant heterogeneity found among the studies for **28* and **1* homozygotes (Q values of 21 and 22, p values of 0.02, 0.02), mostly due to the low rate for **28* homozygotes found by Danoff *et al*.[83] There was no heterogeneity for the **1/*28* heterozygote estimates (Q value of 14, p value of 0.2).

The consensus *UGT1A1*28* allele frequency in Caucasians is 0.334 (95% CI 0.309-0.361) (Table KQ3-8). Raw data that includes the point estimates for the individual studies and the overall estimates and 95% confidence intervals (random effects model) can be found in Appendix D, Table D-2a. The commonly quoted allele frequency estimate for **28* of 0.39 (provided in the Invader package insert)[47,49] is based on one of these 11 studies, with a total N of only 77.[79] This point estimate from the larger sample falls within the 95% confidence interval from Beutler et al.[79] of 0.31-0.47.

Asians

Four studies[79,85,89,90] were initially identified that provided data on observed frequencies of the *UGT1A1* promotor TATA genotypes in mixed Asian subjects (N=545; 68 Japanese, 441 Taiwanese, 6 Chinese). The *UGT1A1** 28 (TA7) allele is much less common in Asians, with an estimated allele frequency of 0.139 (95% CI of 0.112 to 0.171) in this diverse population (see Table KQ3-8 and Appendix D, Table D-2b). Observed frequency of **28* heterozygotes was 0.263, and **28* homozygotes were rare. The **36* and **37* alleles were not observed in two studies (N=77) that tested for these variants. The allele frequency estimate for **28* in Asians of 0.16 provided in the Invader package insert [47,49] is based on one of these two studies (N=47)[79] and is consistent.

The *UGT1A1*6 (211G>A*) and *UGT1A1*27 (*686C>A) alleles, also associated with reduction in UGT activity, have been identified only in Asian individuals, with allele frequencies of 0.11 (95% CI 0.09-0.15) and 0.023 [0.014-0.035], respectively. An association between heterozygosity or homozygosity for these alleles and severe toxicity has been suggested, but not clearly established, and may be more likely in combination with a *UGT1A1*28* variant.[60]

Update: A 2004 report from Sai et al.[42] and two subsequent studies from the National Cancer Center in Japan[44,45] have shown that *UGT1A1*60* is also a common variant in Japanese populations. It is difficult to assess potential overlap in these study populations, but reported estimates of allele frequencies for **6, *28,* and **60* range from 0.151 - 0.213, 0.113 - 0.138, and 0.136 - 0.160, respectively. Another 2007 report from Sandanaraj et al. estimated the **6* allele frequency in 279 "pooled healthy Asians" to be 0.08, with estimates ranging from 0.15 in 90 Chinese individuals to 0.03 in 85 Malaysians[46]

Africans/African Americans

Two studies[37,76] were identified that provide data on observed frequencies of the *UGT1A1* promotor TATA genotypes in African/African American subjects (N=301). The *UGT1A1** 28 allele frequency is similar to that in Caucasians; the consensus estimate from the two studies is 0.40 (95% CI 0.34-0.45) (see Table KQ3-8 and Appendix D, Table 2c). The allele frequency estimate for **28* of 0.426 provided in the Invader package insert [47,49] is based on one of these 2 studies (N=101)[79] and is consistent with the second study. Consensus estimates of allele frequencies for the **36* and **37* alleles in this population are 0.06 (95% CI 0.03-0.10) and 0.04 (95% CI 0.01-0.13), respectively. One other study provided only allele frequencies for a cohort study control group (n=117); the allele frequency estimates were consistent (0.42, 0.05, 0.04 for *28, *36, and *37, respectively).[92]

Rating the quality of studies used for this key question

EGAPP reviewers generated descriptive summaries of the studies that included specific elements (e.g., study design, population studied and inclusion/exclusion criteria, statistical analyses, potential biases) and concluded with a subjective rating and the key criteria that support it. Of the 14 studies identified that provided information on genotype and allele frequencies, four were ranked as *Good*[37,72,75,79], 10 as *Fair*[32,34,46,70,71,73,74,76-78], and none as *Marginal*. Summaries can be found in Appendix C.

Limitations of Studies on *UGT1A1* Genotype and Allele Frequencies – Key Question 3c

- Allele frequencies provided without supporting data
- Genotype frequencies were not provided or data were incomplete
- Specific selection criteria for study participants were not always clear, so estimates may not be truly population-based
- Small sample sizes
- No studies addressed frequencies in non-Hispanic Caucasian populations
- Limited data for Asian/Asian American and Africans/African American populations

Key Question 4: What are the benefits and harms (clinical utility) related to *UGT1A1* testing for patients with metastatic CRC treated with irinotecan?

Based on the data presented in KQ3 (clinical validity) and additional information on the pharmokinetics of irinotecan, it is biologically plausible that a reduced initial dose in

homozygotes would result in a reduction in neutropenia. However, there are no studies that have genotyped patients prior to first dosage, modified starting dosages related to the genotype, and then compared the outcomes based on these modified dosages.

Will reduced dose result in a reduction in severe adverse drug events?

Based on the clinical validity and additional information on the pharmacokinetics of irinotecan, it is biologically plausible that a reduced initial dose in homozygotes (**28/*28*) would result in a reduction in severe neutropenia. However, no studies (with or without randomization) have genotyped patients prior to first dosage, modified starting dosages, and then compared the clinical outcomes (e.g., severe neutropenia, tumor response), based on these modified dosage. Reduced dosage in subsequent cycles is the current method of avoiding additional instances of neutropenia. For example, Toffoli and colleagues[71] have shown that their management techniques (reducing dosage from 180 mg/m^2 to between 90 and 150 mg/m^2 in all individuals having neutropenia) have the effect of reducing the rate of neutropenia in homozygotes in subsequent cycles. Their reported odds ratio for neutropenia in homozygotes relative to wild type dropped from 8.6 (95% CI 1.3 to 57) after the first cycle, to 2.0 (95% CI 0.6 to 7) after the end of therapy (2 to 6 cycles). The data show, however, that the point estimates for tumor-related morbidity and mortality are lower among homozygotes (and to a lesser extent heterozygotes), possibly due to the effects of 'over-dosing'. Thus, the reduced drug metabolism (i.e. reduced inactivation of SN-38) in these two groups that may cause the increased rate of severe adverse drug events (harm) is possibly also responsible for the apparent increase in tumor response and improved survival (benefit).

Comparing benefits and harms

The benefit of testing metastatic CRC patients for *UGT1A1* genotype comes from reducing adverse drug events (e.g., severe neutropenia) by modifying initial and/or subsequent doses of irinotecan based on genotype. The concomitant harm can come from reduced effectiveness of the chemotherapy in tumor suppression and long-term survival. The current analyses will provide a preliminary comparison of these competing interests, but should be considered preliminary and are being undertaken only to try to place into context the competing benefits and harms and to identify clear gaps that need to be addressed by additional studies prior to creating more reliable models.

The model begins by estimating the number of severe neutropenia episodes avoided by reducing initial dosage in **28* homozygotes. It then models the number of additional colorectal cancer (CRC) tumors that are non-responsive to treatment. The numbers are based on the hypothetical population shown in Figure KQ3-5. The effectiveness of an irinotecan dose reduction can vary from 20% to 100%. A 100% effectiveness means that the rate of severe neutropenia among homozygotes *(*28/*28*) receiving the reduced dose will be equivalent to that among individuals with the wild type. From the literature, that rate is expected to be about 9.8%. The number needed to test (NNT) indicates the total number of cancer patients that need to be genotyped (and have reduced dose in all found to be homozygous for **28*) in order to avoid a case of severe neutropenia among a homozygous patient. Our calculations assume that the reduced dose will cause homozygotes to have the same tumor response rate as individuals with the wild type. This may be an oversimplification of the model, as response rates may also be dose dependent.

As an example, consider the instance when the effectiveness is 100%, indicating that all excess neutropenia among homozygous individuals (**28/*28*) is removed when the irinotecan dose is reduced (Table KQ4-1, row 1). Referring to Figure KQ3-5, there are 2,231 such homozygotes (**28/*28*) with a 3.51 fold risk ratio (Figure KQ3-3) above the 9.8% rate of severe neutropenia in the referent category*(*1/*1*). Thus, the expected background number of homozygous individuals with severe neutropenia (Table KQ4-1, column 2) would be 219 (0.098 * 2,231). The number of severe neutropenia events attributable to *UGT1A1* genotype (and possibly avoidable) would then be 767 (Figure KQ3-5) minus 219, or 548 (Table KQ4-1, column 3). The number needed to test to avoid one individual with severed neutropenia, in this case, is 20,000 divided by 548, or 36 (Table KQ4-1, column 4). The number of non-responsive CRC tumors among homozygous individuals *(*28/*28*) receiving a reduced dose is considered a constant and is computed as follows: the baseline response rate in wild-type individuals is 41%, and the observed response rate for homozygotes is 1.70 times higher, or 69% (Figure KQ3-6). Thus, there were originally 1,539 responsive tumors among the homozygotes (0.69 * 2,231), but only 892 will be responsive with a reduced dose (0.40 * 2,231). This is a drop of 647 responsive tumors. Comparing these 647 additional non-responsive tumors to the avoidance of 548 cases of neutropenia results in an odds of 647:548 or 1.2:1 (Table KQ4-1, column 5).

Table KQ4-1. Preliminary estimates of the clinical utility of testing metastatic colorectal cancer (CRC) patients for *UGT1A1* polymorphisms: Benefits and harms among homozygotes

Effectiveness of irinotecan dose reduction in preventing neutropenia	Total number of cases with neutropenia	Cases of neutropenia avoided	Number needed to test to avoid 1 neutropenia	Additional non-responsive CRC tumors : case of neutropenia avoided
100%	219	548	36	1.2:1
90%	274	493	41	1.3:1
80%	328	439	46	1.5:1
70%	383	384	52	1.7:1
60%	438	329	61	2.0:1
50%	493	274	73	2.4:1
40%	548	219	91	2.9:1
30%	603	164	122	3.9:1
20%	658	109	183	5.9:1
10%	713	54	370	12:1

An alternative approach would be to compare additional non-responsive CRC tumors to deaths resulting from severe neutropenia. An estimated 1 in 110 cases of severe neutropenia might result in death in individuals receiving irinotecan as a first line treatment (See Background, page 19), and the last column in Table KQ4-1 could be converted to this measure by dividing the right-hand side by 110. For example, using the numbers in row 1, the odds of non-responsive tumor versus death resulting from severe neutropenia change from 647:494 to 647:(494/110), or about 140:1. According to the very preliminary analysis reported in Table KQ4-1, it appears that at high rates of effectiveness (70% to 100%), each avoided case of neutropenia is associated with one non-responsive tumor. At lower rates of effectiveness (20% to 50%), there are likely to be 2 to 5 times as many non-responsive tumors as avoided cases of severe neutropenia.

Table KQ4-2. Preliminary estimates of clinical utility of testing metastatic colorectal cancer patients for *UGT1A1* polymorphisms: Benefits and harms among heterozygotes

Effectiveness of irinotecan dose reduction in preventing neutropenia	Total number of cases with neutropenia	Cases of neutropenia avoided	Number needed to test to avoid 1 neutropenia	Additional NR CRC tumors: case of neutropenia avoided
100%	872	776	2.9	0.5:1
90%	950	698	3.2	0.5:1
80%	1,027	621	3.6	0.6:1
70%	1,105	543	4.1	0.7:1
60%	1,182	466	4.8	0.8:1
50%	1,260	388	5.7	0.9:1
40%	1,338	310	7.2	1.1:1
30%	1,415	233	9.6	1.5:1
20%	1,493	155	14	2.3:1
10%	1,570	78	29	4.6:1

Table KQ4-2 shows the same analysis, except that it considers the benefits and harms among the **1/*28* heterozygotes. Because there are more heterozygotes, there are actually many more cases of severe neutropenia that might be avoided. In addition, the best estimate is that this group does not have much of an advantage in responsiveness (risk ratio of 1.09). Under the assumptions stated, reducing the dose of irinotecan in heterozygotes may have at least as favorable benefit/harm ratio as among homozygotes.

Might individuals with the wild-type be under-dosed?

Given some limited evidence that individuals homozygous for **28* (**28/*28*) have improved survival[16] (Table KQ3-8), it is possible that individuals with the wild type *(*1/*1*) are under-dosed. Original phase I studies did not stratify patients by *UGT1A1* genotype and, therefore, higher doses may be well tolerated by wild type individuals *(*1/*1*). Calls have been made for new phase I trials with patients stratified by genotype.[56] Preliminary results from Phase I dose-escalation trials, which account for differences in *UGT1A1* genotype, have recently begun to appear.

Update: Since the formal literature search, one additional trial[93] has been reported that would have been included in the analysis of clinical validity. Specifically, that study found a higher rate of severe neutropenia among individuals homozygous for **28* (risk ratio 5.4, 95% CI 2.4 to 12), but no difference among individuals heterozygous for **28* (**1/*28*) (risk ratio 0.8, 95% CI 0.2 to 2.8). Little or no relationship between *UGT1A1* genotype and severe diarrhea was reported. This study found improved survival for individuals homozygous for **28* (p=0.06). All of these findings are consistent with, and strengthen the findings of the existing evidence review.

Limitations of testing

In general, the same problems with studies of clinical validity are applicable to clinical utility. Populations of mainly non-Hispanic Caucasians, widely varying treatments protocols, grouping of less common genotypes with common ones, and inclusion of patients with cancers other than of the colon. The modeling of benefits (reduction in the proportion of responsive tumors and improved survival) is based on weak evidence. These limitations underscore the need for caution in interpreting the results, and indicate the need for further study.

Key Question 4a: Based on *UGT1A1* test results, what are the management options for patients?

Options for modifying patient care

There is insufficient information from the less common genotypes to provide clear options for patient management. For the common **28* polymorphism, the three main options for modifying patient care have been summarized and discussed.[16]

- The irinotecan regimen can be modified. In June, 2005, the FDA changed the labeling for irinotecan. The Camptosar (irinotecan) package insert provides suggested modified (reduced) dose levels (mg/m^2) for two single-drug regimens of Camptosar (125 mg/m^2 weekly and 350 mg/m^2 every 3 weeks).[29] It states that a reduction by one dose level may be considered for patients 65 years or older, having low performance status, or with increased bilirubin levels; reduction in starting dose by at least one level "should be considered for patients known to be homozygous for the *UGT1A1*28* allele". However, the package insert also notes that "the appropriate dose reduction in this patient population is not known."

- Other drugs can be used. Newer drugs (*e.g.*, cetuximab, bevacizumab) can be substituted in a variety of regimens that vary the combination of drugs, as well as the doses, schedules and duration of infusion for each drug. Physicians may choose these alternatives when the patient is found to be homozygous for **28 (*28/*28).*
- Pre-treatment with colony-stimulating factors. Prior to the onset of the first cycle of chemotherapy, individuals homozygous for **28* could be treated with colony-stimulating factors to prevent the occurrence of febrile neutropenia. Such treatments are currently recommended by the National Comprehensive Cancer Network for individuals with a 20% or greater risk of febrile neutropenia (nccn.org). Although individuals homozygous for **28* have a 36% risk of severe neutropenia, the proportion associated with fever is unknown. Treatment costs are two to three thousand dollars per dose. This suggests that monitoring white cell counts might be an acceptable indicator of acceptable dosing.

Additionally, treatment options need to be placed in the context of overall care. If a clinician has decided that a regimen including irinotecan is best, he/she may need to discuss with the patient what level of risk of side effects is acceptable. The *UGT1A1* test may be useful for patients electing risk for low toxicity in spite of potentially reduced efficacy, but may not be as useful for those seeking aggressive therapy and accepting higher toxicity.

Decisions about testing may also be based on the specific planned regimen and dosing. Reduced dosage in subsequent cycles is the current method of avoiding additional instances of neutropenia. For example, Toffoli and colleagues[71] have shown that their management techniques (reducing dosage from 180 mg/m^2 to between 90 and 150 mg/m^2 in all individuals having neutropenia) have the effect of reducing the rate of neutropenia in **28/*28* homozygotes in subsequent cycles. Their odds ratio for neutropenia in homozygotes relative to **1/*1* dropped from 8.6 (95% CI 1.3 to 57) after the first cycle, to 2.0 (95% CI 0.6 to 7) after the end of therapy (2 to 6 cycles).

In a recent published interview, McLeod proposed that, unless patients will receive irinotecan at a dose greater than 150 mg/m^2, either alone or in combination with a myelotoxic drug, or irinotecan at 100 mg/m^2 in combination with a myelotoxic agent, the increase in risk for toxicity is “neither statistically nor clinically significant” and testing may not be warranted.[19,94] Derivation of these dosage cutoffs will be described in a report submitted for publication, but not yet available.[95]

Key Question 4b: Do these options provide improved patient outcomes or management by patients or providers?

No data currently exist to directly answer this question. Randomized controlled trials are needed to define dosage by genotype for different regimens, measure the effect of dose reduction on outcomes, and determine acceptability and uptake of testing in practice.

Quality of evidence

Figure KQ4-1 summarizes the quality of evidence for key questions (Table 1). The quality of evidence is *fair* for the analytic validity of the common *UGT1A1* variant **28*, as there are two or more relatively high quality studies providing consistent results. However, the number of challenges do not allow for a confident estimate for analytic sensitivity and specificity, even though the point estimates are high. Lastly, the data are restricted mainly to the analytic phase of testing. There are little or no data to estimate the analytic validity of the less common *UGT1A1* variants.

The quality of evidence is *fair* for the association of the **28* variant with the active form of irinotecan (SN-38), severe diarrhea and severe neutropenia. For all three outcome measures, there was a systematic review of lower quality studies. Little or no data are available to examine these three outcomes with respect to the less common *UGT1A1* variants. Although plausible, little or no data are available to prospectively examine whether an initial reduction in irinotecan dosage in individuals homozygous for **28* does reduce severe neutropenia. Little or no data are available to allow a direct, prospective comparison of these possible benefits and harms.

Important gaps in knowledge

- There appears to be a clear relationship between *UGT1A1* genotype and severe neutropenia (and some evidence of a relationship with severe diarrhea), but there is no direct or indirect evidence (chain of evidence) to support the clinical utility of modifying an initial and/or subsequent dose of irinotecan in patients with metastatic colorectal cancer as a way to change the rate of adverse drug events (*e.g.*, severe neutropenia).
- Even if adverse drug events were reduced, this may come at the expense of a reduction in tumor responsiveness in **28* homozygotes, leading to an overall net harm.
- The data on clinical validity of *UGT1A1* variants other than **28* are limited.

- If the test were recommended for routine use in clinical practice, additional studies would be needed to understand the potential effects of alleles that are rare in Caucasians but more common in other racial/ethnic groups (*e.g., *6* in Asians), and testing panels would need to include all variants of clinical significance in the population to be tested.
- There are limited data on *UGT1A1* variants in Hispanic and African American populations.
- The analytic validity of *UGT1A1* testing in clinical practice is unknown. Laboratories offering such testing may include variants in addition to **28* for which little evidence is available.
- Pre- and post-analytic errors have not been reported, but these are likely to be similar to other genetic tests done in high-complexity laboratories (*CFTR, HFE*).[64,65] A new external proficiency testing program jointly offered by the American College of Medical Genetics (ACMG) and the College of American Pathologists (CAP) is likely to provide important evidence about the analytic validity of *UGT1A1* testing in a clinical setting.

Given these major gaps, a prospective trial (preferably a randomized controlled trial) may be warranted to determine whether or not *UGT1A1* genotyping to determine drug dose or selection results in net benefit to the patient.

Research agenda

Analysis of data from the ACMG/CAP proficiency testing program will provide needed information about the analytic validity of *UGT1A1* tests offered for clinical use. Additional information concerning the clinical validity of the less common *UGT1A1* variants is needed. Given the rarity of these genotypes, studies will need to include large numbers of subjects

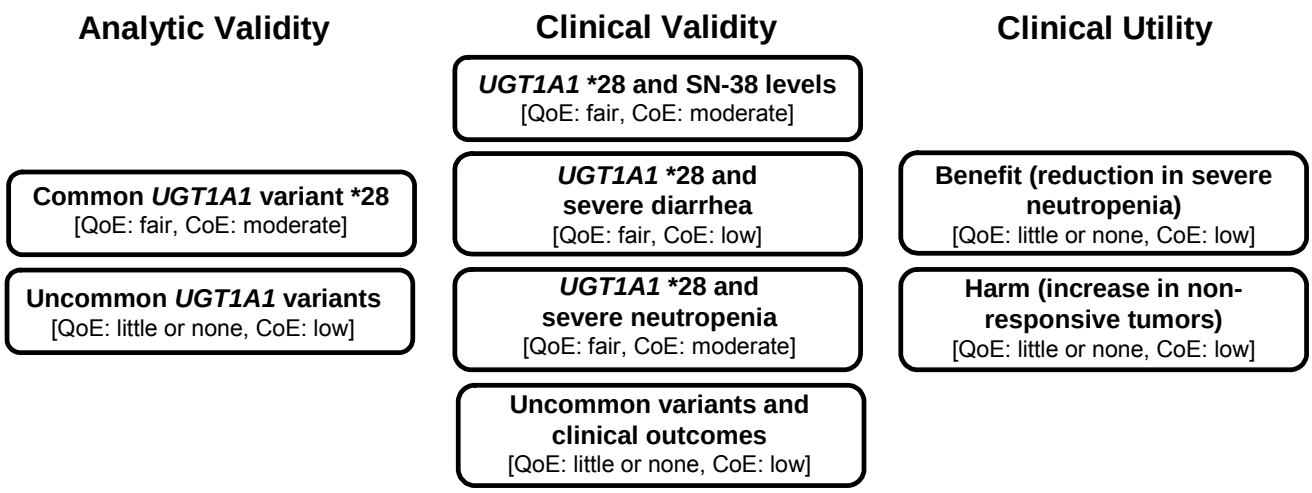


Figure KQ4-1. Graphic display of the quality of evidence (QoE) and certainty of evidence (CoE) for selected components of the current evidence review. For analytic validity, clinical validity and clinical utility, each of the main components of the evidence review is represented by the text within a box. The QoE and CoE for each component is indicated by the bracketed text.

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receiving treatment. This is feasible, however, because metastatic CRC is relatively common, as is chemotherapy with irinotecan. The most appropriate way to collect the evidence needed to document whether, or how, to modify dosage in the light of the *UGT1A1* genotype is to mount prospective studies (perferably including randomized trials) of targeted doses versus the current practice of irinotecan dosing. Such a study should be considered ethical, as it is not known whether the supposed benefits outweigh the possible harms. There are sufficient numbers of subjects for recruitment to be completed in a relatively short period of time.

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Appendix A

UGT1A1 Technical Expert Panel (TEP) and Report Peer Reviewers

Technical Expert Panel

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Appendix B

The ACCE Model Process - List of Targeted Questions

Element	Component	Specific Question
Disorder / Setting		
		1. What is the specific clinical disorder to be studied?
		2. What are the clinical findings defining this disorder?
		3. What is the clinical setting in which the test is to be performed?
		4. What DNA test(s) are associated with this disorder?
		5. Are preliminary screening questions employed?
		6. Is it a stand-alone test or is it one of a series of tests?
		7. If it is part of a series of screening tests, are all tests performed in all instances (parallel) or are only some tests performed on the basis of other results (series)?
Analytic Validity		
		8. Is the test qualitative or quantitative?
	Sensitivity	9. How often is the test positive when a mutation is present?
	Specificity	10 How often is the test negative when a mutation is not present?
		11. Is an internal QC program defined and externally monitored?
		12. Have repeated measurements been made on specimens?
		13. What is the within- and between-laboratory precision?
		14. If appropriate, how is confirmatory testing performed to resolve false positive results in a timely manner?
		15. What range of patient specimens have been tested?
		16. How often does the test fail to give a useable result?
		17. How similar are results obtained in multiple laboratories using the same, or different technology?
Clinical Validity		
	Sensitivity	18. How often is the test positive when the disorder is present?
	Specificity	19. How often is the test negative when a disorder is not present?
		20. Are there methods to resolve clinical false positive results in a timely manner?
	Prevalence	21. What is the prevalence of the disorder in this setting?
		22. Has the test been adequately validated on all populations to which it may be offered?
		23. What are the positive and negative predictive values?
		24. What are the genotype/phenotype relationships?
		25. What are the genetic, environmental or other modifiers?
Clinical Utility		
	Intervention	26. What is the natural history of the disorder?
	Intervention	27. What is the impact of a positive (or negative) test on patient care?
	Intervention	28. If applicable, are diagnostic tests available?
	Intervention	29. Is there an effective remedy, acceptable action, or other measurable benefit?
	Intervention	30. Is there general access to that remedy or action?

		31. Is the test being offered to a socially vulnerable population?
	Quality Assurance	32. What quality assurance measures are in place?
	Pilot Trials	33. What are the results of pilot trials?
	Health Risks	34. What health risks can be identified for follow-up testing and/or intervention?
		35. What are the financial costs associated with testing?
	Economic	36. What are the economic benefits associated with actions resulting from testing?
	Facilities	37. What facilities/personnel are available or easily put in place?
	Education	38. What educational materials have been developed and validated and which of these are available?
		39. Are there informed consent requirements?
	Monitoring	40. What methods exist for long term monitoring?
		41. What guidelines have been developed for evaluating program performance?
ELSI		
	Impediments	42. What is known about stigmatization, discrimination, privacy/confidentiality and personal/family social issues?
		43. Are there legal issues regarding consent, ownership of data and/or samples, patents, licensing, proprietary testing, obligation to disclose, or reporting requirements?
	Safeguards	44. What safeguards have been described and are these safeguards in place and effective?

From: http://www.cdc.gov/genomics/gTesting/ACCE.htm

Haddow JE, Palomaki GE. ACCE: A Model Process for Evaluating Data on Emerging Genetic Tests. In: Human Genome Epidemiology: A Scientific Foundation for Using Genetic Information to Improve Health and Prevent Disease. Khoury M, Little J, Burke W (eds.), Oxford University Press, pp. 217-233, 2003.

Appendix C - Detailed Methods

Analytic Validity

Literature Search Methods

In August 2006, EGAPP staff searched MEDLINE® for the key Medical Subject Heading (MeSH) terms to be used for obtaining available information from the published literature on analytic validity of tests for polymorphisms in the *UGT1A1* gene, as well as information on key genotype and allele frequencies in different populations. Since there is considerable variability in nomenclature related to DNA-based assays, multiple searches were conducted using different search terms (Table C-1). Articles were also identified by search of the references included in the selected articles. A targeted search of the grey literature was also conducted to obtain information on:

- Methodologies utilized by laboratories offering clinical testing – Review of: 1) websites identified through the Google search of laboratories offering clinical testing; and 2) information submitted by laboratories to GeneTests
- Data submitted by test manufacturers seeking FDA 510(k) premarket approval – Search of FDA website for 510(k) summaries and committee reports
- Information released on new tests by laboratories and/or manufacturers - Google searches for press releases, lay magazine/newspaper articles, and package inserts for tests
- Laboratories offering *UGT1A1* testing – General Google search and search of GeneTests website (http://www.genetests.org)

Table C-1. Search Terms and Results

MEDLINE® searches and search terms	Titles (unique results)	Abstracts Reviewed
UGT1A1 (or UDP-glucuronosyltransferase) or **UGT1A1 (or UDP-glucuronosyltransferase) test** and **analytic validity** or **method comparison**	0	0
UGT1A1 (or UDP-glucuronosyltransferase) and **test performance**	24	1
UGT1A1 (or UDP-glucuronosyltransferase) and **genetic test** or **genetic testing**	49	6
UGT1A1 (or UDP-glucuronosyltransferase) and **method** or **methodology**	480	27
UGT1A1 (or UDP-glucuronosyltransferase) and **molecular** or **molecular test**	711	27
UGT1A1 (or UDP-glucuronosyltransferase) and **assay**	1127	11
Total	2,391	72

Article Selection Process

Based on key questions and discussion with the *UGT1A1* Technical Expert Panel, a list of article inclusion and exclusion criteria was generated (Table C-2 below). Studies were excluded that: 1) did not report on testing of human samples; 2) were published in languages other than English; 3) did not report information pertinent to the key questions; 4) were not original studies; and 5) did not provide an adequate description of the study design and conduct. The search was not restricted by the setting of the study or the date of publication. Sequencing was considered the gold standard, but comparisons with other referent methods were also included.

Table C-2. Inclusion Criteria for Studies Used to Estimate Analytic Validity

Number	Category	Inclusion Criteria
1	Study population	Human samples from all races/ethnicities
2	Study settings	All settings (e.g., research, development/validation, clinical)
3	Time period	All dates
4	Publication languages	English only
5	Study design and conduct	Peer-reviewed method comparisons or FDA submissions that provide: - adequate technical descriptions of the *index* and *referent* or *gold standard* tests - sufficient detail regarding methods and results to enable use of the data (e.g., alleles tested, sample types, source and utilization of control samples, quality control measures, reproducibility) - adequate description of the basis for the "right answer" - appropriate information to avoid bias (e.g., test failure rates, variants of unknown significance) - sufficient information to allow calculation of analytic sensitivity and specificity with 95% confidence intervals Studies that provide information on genotype and allele frequencies of *UGT1A1* variants in control groups that are likely to represent a specific racial/ethnic population – adequate description of study and criteria for selection of controls – representative of population

- ideally genotype frequencies provided

Since identification of limited information on analytic validity and allele/genotype frequencies required the use of a larger number of search terms, a slightly different approach was used for this section. Two EGAPP staff members (Linda Bradley, PhD and Michael Douglas, MS) reviewed the summary lists of article titles from each search, eliminating those titles already identified in previous searches and articles clearly unrelated to the search objectives. One search term, **UGT1A1 (or UDP-glucuronosyltransferase)** and **assay,** yielded more than 1,100 articles; review of the titles showed that this was due to identification of molecular and biochemical assays for UGT in a variety of settings, so identification of relevant abstracts by title and abstract search was feasible. For the subset of titles selected, each abstract and selected full article was systematically reviewed against the *a priori* criteria by two reviewers to determine inclusion in the review; an article was retained if one reviewer concluded it should be included in the review.

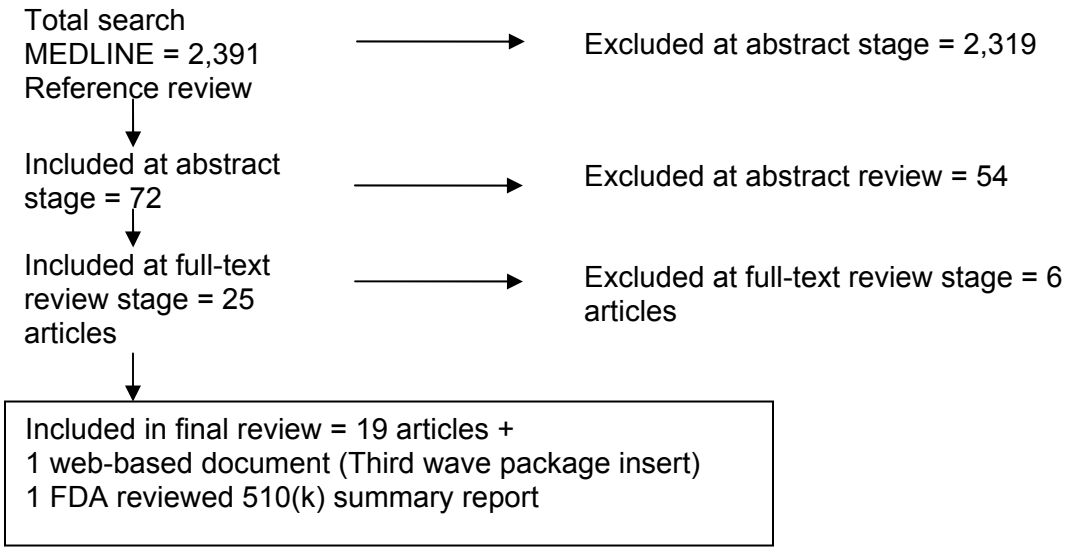
From the review of 2,391 titles, 72 abstracts were selected for review. Full-text articles of 18 selected abstracts were then obtained and reviewed for inclusion in the review.[1-18] Seven additional sources (5 journal articles, 2 sources on an FDA 510(k) submission) were identified by search of the grey literature and from references included in the articles reviewed.[19-25] Two additional articles on allele frequencies were suggested by peer reviewers.[26,27]

Twenty-five journal articles, one web-based document, and a FDA 510(k) summary addressed key questions: 5 on analytic validity (KQ2)[7,13,14,19,25], and 17 on allele/genotype frequencies in subpopulations (KQ3c).[1,2,5,6,11-13,15,17,20-24,26-28] Six articles on allele/genotype frequencies were excluded (see Articles Excluded on Analytic Validity and Allele/genotype Frequencies). [3,8-10,16,18]

Development of Tables

The EGAPP staff members who conducted the systematic review abstracted the data and developed summary tables on analytic sensitivity/specificity and allele and genotype frequencies. Particular emphasis was given to essential information related to the key questions. The studies used to estimate analytic sensitivity and specificity and allele/genotype frequencies are described and the quality of the studies is considered using criteria of the EGAPP Working Group.

Figure C-1. Final Disposition of Abstracts and Articles on Analytic Validity



Rating the Quality of Individual Studies on Analytic Validity

Studies were rated for quality based onan EGAPP checklist for reporting studies of analytic validity; studies should ideally include:

- Adequate description of the *index* test: specific alleles tested, technical specifications of the method(s), sample types, sources and use of positive and negative control materials, reproducibility, quality control
- Adequate description of the *referent* test - basis for the "right answer" (e.g., comparison to a 'gold standard' or referent test, consensus in an external quality assessment scheme, validation with characterized control materials)
- Avoidance of biases - blinded testing and interpretation of results, with reporting of test failures and uninterpretable or indeterminate results
- Analysis of data – includes point estimates of analytic sensitivity and specificity with 95% confidence intervals (or sufficient data to calculate)

The EGAPP Working Group discussed strengths and weaknesses of data sources or study designs that have been (or could be) used to obtain unbiased and reliable information about analytic validity (Table C-3 below). The highest quality data (Level 1 studies) are likely to come from collaborative studies using a single large, carefully selected panel of well-characterized control samples that are blindly tested and reported, with the results independently analyzed; at this time, such studies are largely hypothetical. Data from proficiency testing schemes have both strengths and weaknesses, but, if available and carefully analyzed, are likely to contain good information about analytic validity in routine practice, are likely to be more generalizable, and include the pre- and post-analytic phases of

testing.[29] Data from proficiency testing schemes, well-designed peer–reviewed publications (e.g., method comparisons, validation studies), and expert panel-reviewed FDA submissions for approved products, if appropriately performed and reported are considered Level 2 quality. Level 3 quality studies include less well-designed peer-reviewed studies, unpublished and/or non-peer reviewed research, and clinical laboratory or manufacturer data. Unpublished, non-peer reviewed research, clinical laboratory, or manufacturer data is considered lowest quality (Level 4).

Table C-3. Suggested Hierarchy of Data Sources for Analytic Validity

Level	Description
Level 1	Multi-site collaboration using a single, large and comprehensive panel of well-characterized samples
Level 2	Data from external proficiency testing schemes or inter-laboratory comparison programs
Level 3	Peer-reviewed studies (method comparisons, validation studies) and FDA submissions
Level 4	Unpublished and/or non-peer reviewed research, clinical laboratory or manufacturer data

Rating the Quality of Individual Studies

Two or three EGAPP reviewers (Glenn Palomaki, Linda Bradley, Michael Douglas) independently reviewed the articles identified and generated descriptive summaries of the studies that included specific elements, including (e.g., data source, population studied and inclusion/exclusion criteria, statistical analyses, potential biases) and concluded with a subjective rating and the key criteria that support it.

Four studies provided information on the performance of three specific assays (KQ2), all using sequencing as the "gold standard" referent method. The overall quality of the evidence was rated as *Fair* based on data sources, consistency and generalizabilty. Brief summaries of the studies are provided below:

- Monaghan G. *et al*.[13] obtained blood samples from healthy staff volunteers at a teaching hospital in Dundee, Scotland. Testing was done on 12 of these samples by both direct sequencing and radioactive PCR (PCR with ^{32}P-end labeling, PAGE, autoradiograph) as part of a study of *UGT1A1* genotypes and serum bilirubin levels in patients with Gilbert syndrome in the Scottish population. *Quality: Small peer-reviewed report of an adequately performed and described comparison of methods conducted as part of a larger study (Level 3).*

- Pirulli D. *et al*.[14] tested blood samples from 20 healthy controls and 20 clinically diagnosed Gilbert syndrome patients by sequencing and denaturing high performance liquid

chromatography (DHPLC) to demonstrate that DHPLC provides a rapid and low cost alternative to sequencing in testing for Gilbert syndrome. *Quality: Small peer-reviewed report of an adequately conducted and described method comparison (Level 3).*

- Hasegawa Y. *et al.* [7] genotyped blood samples from 60 patients who had received irinotecan-containing chemotherapy, in order to evaluate the use of a newly developed assay for rapid detection of four *UGT1A1* polymorphisms (**1, *28, *6, and *27*). Not all subjects received a complete genotype, due to failures of the assay for one or more polymorphisms. *Quality: Moderate sized peer-reviewed report of an adequately conducted and described method comparison (Level 2).*

- The Invader® UGT1A1 Molecular Assay Premarket Notification Summary (K043576) is publicly available on the US Food and Drug Administration website.[19] The information presented in the summary was submitted to the FDA by the manufacturer, Third Wave Technologies, and describes a device classified as a Drug Metabolizing Enzyme Genotyping System. The application for 510(k) status was reviewed by an FDA Center for Devices and Radiological Health review committee and approved in July, 2005 *Quality: Large (N=212) FDA-reviewed report of an adequately conducted and described method comparison (Level 2).*

Genotype and allele frequencies (KQ3c)

The overall quality of the evidence was rated as *Fair* based on data sources, consistency and generalizabilty. Brief summaries of the studies are provided below:

- Monaghan G. *et al.*, 1996.[13] tested 77 healthy volunteers recruited from staff of a teaching hospital in Dundee, Scotland. Selection criteria relate to biochemical testing: non-smokers, drug and alcohol-free 5-7 days, overnight fast. Quality assessment: *Level 3* – peer-reviewed, small N, race not specified (Caucasian assumed based on demographics of Scotland), adequate description, and complete genotype data.

- Beutler E *et al.*,1998[1] tested anonymous DNA samples from 71 U.S. Caucasians of European ancestry, 47 Asians (41 Chinese, 6 Japanese), and 101 individuals from "North and Central America with varying degrees of African ancestry"; genotype frequencies were reported for *UGT1A1 *1*, **28*, **36*, and **37* polymorphisms. Quality assessment: *Level 3*- small to moderate N, African ancestry not well-described, complete genotype data. Unclear if population based, as selection criteria for subjects providing anonymous DNA samples is not described; study is quoted in the Invader package insert.

- Sampietro M *et al.*, 1998[23] tested 44 individuals "from the general population of Milan, Italy". Observed genotype frequencies were not significantly different from expected based on Hardy Weinberg. Quality assessment: *Level 3* - small N, race not specified (Caucasian

assumed in Milan), selection criteria for control subjects not provided, complete genotype data.

- Lampe J. *et al.*,1999[11] genotyped 202 Caucasian and 30 Asian non-smokers, aged 20-40, from Seattle as part of a cross-sectional study of diet and enzymes. Observed genotype frequencies were reported to be in Hardy Weinberg equilibrium. Quality assessment: *Level 2* - large N for US Caucasians, race specified, detailed selection criteria, complete genotype data.

- Borlak J. *et al*., 2000[2] tested 265 healthy, unrelated individuals participating in clinical pharmacology phase I trials in Germany. Quality assessment: *Level 2* – large N, specific selection criteria for clinical trials not specified, race specified, complete genotype data.

- Guillemette C *et al.*,2000[21] genotyped 200 African Americans randomly selected from a North Carolina population-based case/control study on breast cancer. Quality assessment: Level 2 - large N, race specified, complete genotype data and includes less common alleles.

- Rauchschwalbe S*. et al.*, 2002[15] genotyped 302 Caucasian Germans recruited from Bayer AG employees/family members/friends. Quality assessment: Level 3 – large N, but proportion of family members participating was not provided and could introduce bias.

- Sugatani J *et al.*, 2002[24] genotyped by sequencing 27 blood samples from unrelated Japanese with no history of jaundice. The allele frequencies for the *UGT1A1* polymorphisms (*6/6, 6/7,* and *7/7* genotypes and 211G>A mutation) in this population based study were calculated. Quality assessment: *Level 3* - small sample size, peer-reviewed report of an adequately conducted and described method comparison.

- Kohle C *et al.*, 2003[22] genotyped 100 randomly selected healthy Caucasians (50 males, 50 females) from a university hospital in Germany. Observed genotype frequencies are consistent with Hardy Weinberg. Quality assessment: *Level 3* - moderate N, specific selection criteria not described*.*

- Cecchin E. *et al.*, 2004[5] tested 205 consecutive female blood donors in the same geographical area in Italy. Observed frequencies were not different from expected based on Hardy Weinberg equilibrium. Quality assessment: *Level 2* - large N, population-based, race specified, complete genotype data.

- Danoff T *et al.,* 2004[6] genotyped 909 US Caucasians selected as controls in a phase III controlled drug trial. Quality assessment: *Level 3* – large N, limited description regarding selection criteria for controls.

- Haverfield EV *et al.*, 2005[26] genotyped 111 Jamaicans enrolled as controls in the Jamaican Sickle Cell Cohort Study (all phenotype AA). Quality assessment: *Level 3* - limited description regarding selection criteria for controls, and not all genotype data provided.

- Kaniwa *et al.*, 2005[27] genotyped peripheral blood from 150 healthy individuals in three populations to assess differences in haplotype frequencies among the three groups. African-American and Caucasian samples were obtained from the Tennessee Blood Service, and Japanese samples from local volunteers. Japanese populations. Quality assessment: *Level 3* - moderate N, race specified, limited description regarding selection criteria, complete genotype data not provided.

- Tang K. *et al.*,2005[17] genotyped 441 healthy Taiwanese seen for a physical exam in a specific time period (included alleles 211G>A, and 686C>A); observed frequencies followed Hardy-Weinberg equilibrium. Quality assessment: *Level 2* - large N, race specified, and complete genotype data.

- Bosch TM. *et al*., 2006[20] tested 93 healthy Caucasian volunteers in a Dutch population. Quality assessment: *Level 3* - small N, and specific selection criteria for study subjects not provided.

- Goldberg et al.,[28] 2006 genotyped 117 African-Americans as part of the Intergroup N9741 study. Quality assessment: *Level 3* – abstract from American Society of Clinical Oncology 2006 Annual Meeting (included because a reputable study and little data on African-Americans), and specific selection criteria for study subjects not provided.

- Mercke Odeberg *et al.*, 2006[12] genotyped 248 healthy Swedish subjects participating in clinical trials at a university hospital. Observed frequencies of 7 polymorphisms were in Hardy-Weinberg equilibrium. Quality assessment: *Level 3* - race not specified (assumed Caucasian) and specific selection criteria for study subjects not provided.

Articles Excluded on Analytic Validity and Allele/genotype Frequencies

1. Bosma PJ, Chowdhury JR, Bakker C, Gantla S, de BA, Oostra BA, Lindhout D, Tytgat GN, Jansen PL, Oude Elferink RP, . The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *N.Engl.J.Med.* 1995;333:1171-1175.
   Notes: No selection criteria for subjects; unclear if allele/genotype frequencies are representative of population.
2. Huang, CS, Luo GA, Huang ML., Yu SC, Yang SS. Variations of the bilirubin uridine-diphosphoglucuronosyl transferase 1A1 gene in healthy Taiwanese. *Pharmacogenetics* 2000;10: 539-44.
   Notes: Overlap of allele/genotype frequency data with Tang et al., 2005 cannot be ruled out.
3. Innocenti F, GrimsleyC, Das S, Ramirez J, Cheng C, Kuttab-Boulos H, Ratain MJ, Di Rienzo A. Haplotype structure of the UDP-glucuronosyltransferase 1A1 promoter in different ethnic groups. *Pharmacogenetics* 2002;12:725-733.
   Notes: Genotyped livers from US Liver Tissue and Procurement System; no information on donors and unclear if observed allele/genotype frequencies are representative of population.
4. Iyer L, Hall D, Das S, Mortell MA, Ramirez J, Kim S, Di RA, Ratain MJ. Phenotype-genotype correlation of in vitro SN-38 (active metabolite of irinotecan) and bilirubin glucuronidation in human liver tissue with UGT1A1 promoter polymorphism. *Clin.Pharmacol.Ther.* 1999;65:576-582.
   Notes: No data on banked liver samples tested; unclear if observed allele/genotype frequencies are representative of population.
5. Skarke C, Grosch S, Geisslinger G, Lotsch J. Single-step identification of all length polymorphisms in the UGT1A1 gene promoter. *Int.J.Clin.Pharmacol.Ther.* 2004;42:133-138.
   Notes : No selection criteria for subjects; unclear if observed allele/genotype frequencies are representative of population.
6. von Ahsen N, Oellerich M, Schutz E. DNA base bulge vs unmatched end formation in probe-based diagnostic insertion/deletion genotyping: genotyping the UGT1A1 (TA)(n) polymorphism by real-time fluorescence PCR. *Clin.Chem.* 2000;46:1939-1945.
   Notes: Not population based for allele/genotype frequencies; insufficient detail on methods comparison.

Clinical Validity and Utility – RTI and EGAPP

Literature Search Methods

In May, 2006, RTI staff searched MEDLINE® for the key Medical Subject Heading (MeSH) terms to be used for clinical validity and utility, irinotecan and UDP-glucuronosyltransferase 1A1 or *UGT1A1* (Table C-5). Articles were also identified by search of the references included in the 72 selected articles.

Table C-5. RTI Search Results

Search no.	Search terms	Results
1	Search **irinotecan**	2989
2	Search **UDP-glucuronosyltransferase 1A1** or ***UGT1A1***	491
3	Search #**1** and #**2**	72

Article Selection Process

Based on key questions and discussion with the UGT1A1 Technical Expert Panel, a list of article inclusion and exclusion criteria was generated (Table C-6 below). Studies were excluded that: 1) did not report on humans; 2) were published in languages other than English; 3) did not report information pertinent to the key questions; and 4) were not original studies. Due to the limited literature, studies of patients with tumors other than CRC were included. Criterion 1 was interpreted as excluding studies based solely on cell lines (human or nonhuman). The search was not restricted by the setting of the study or the date of publication.

Each abstract and article was systematically reviewed against these *a priori* criteria to determine inclusion in the review. The RTI review group included an evidence-based practice researcher, Meera Viswanathan, PhD (Task Leader), two genetic epidemiologists, Nedra Whitehead, PhD (Senior Advisor), and Eric Gillis, MS (Research Analyst).

- Two reviewers separately evaluated each abstract for inclusion or exclusion (Appendix A). If one reviewer concluded that the abstract should be included in the review, it was retained.
- Articles for all included abstracts were then obtained and dually and independently reviewed each for inclusion in the review. Each excluded article was assigned a reason for exclusion (Appendix B in the RTI Preliminary Report).
- From the review of 72 abstracts, 19 articles on 15 studies were identified that addressed the key questions.[30-48]

Table C-6. Inclusion/Exclusion Criteria

Number	Category	Inclusion Criteria
1	Study population	Humans, all races, ethnicities, and cultural groups

2	Study geography and settings	All settings
3	Time period	All dates
4	Publication languages	English only
5	Admissible evidence (study design and other criteria)	Original research studies that: - provide sufficient detail regarding methods and results to enable use of the data. - allow abstraction of relevant outcomes from data presented in the papers. (Single case reports, letters, editorials, and comments excluded)
6	Tumor type	CRC and other

Development of Evidence Tables and Data Abstraction Process

The staff members who conducted this systematic review jointly developed the data abstraction tables (Appendix B in the RTI Preliminary Report) and evidence tables (Appendix C in the RTI Preliminary Report). Tables were designed to provide sufficient information to enable readers to understand the studies and to determine the quality of the studies. Particular emphasis was given to essential information related to the key questions. The format of the evidence tables was based on successful designs used by RTI for prior systematic reviews.

All RTI team members shared the task of entering information into the data abstraction forms. Another member of the team also reviewed the articles and edited all initial entries for accuracy, completeness, and consistency. The two abstractors reconciled all disagreements concerning the information reported in the abstraction forms.

After entering the data from the abstraction forms into evidence tables, the data was again checked for consistency and accuracy. The final RTI evidence tables are presented in their entirety in Appendix E. Studies are presented in the evidence tables alphabetically by last name of the first author.

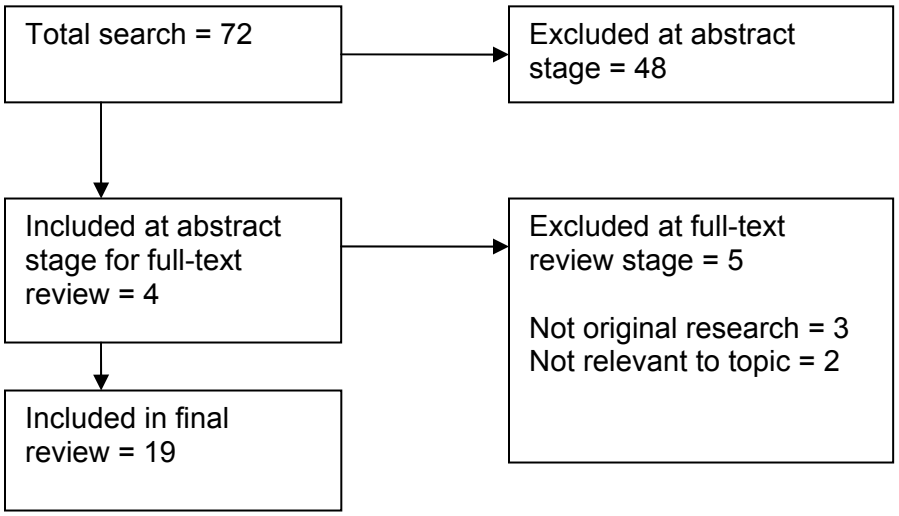
Rating the quality of individual articles – RTI International reviewers

Studies were rated for quality, recording and ranking them for: 1) study design; 2) study population; 3) comparability of subjects; 4) statistical analyses; and 5) measure of effect and loss to follow-up. RTI developed the approach for assessing the quality of individual articles based on the domains and elements for randomized clinical trials and nonrandomized observational studies recommended in the evidence report prepared by West and colleagues.[49] The following criteria were used to rate the quality of nonrandomized observational studies:

1. *Study design:* We assigned prospective cohort studies a higher score.

Approach: To receive a rating of *Fair* for this component of the study design, a study was required to have prospectively ascertained genotype and to have either enrolled patients in the study based on genotype or used expected genotype frequencies to determine the number of patients enrolled (*Fair* is maximum possible rating).

Figure C-2. Disposition of abstracts and articles in Preliminary RTI Report



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2. *Study population:* Documentation was sought of the degree to which the study population was representative of all patients with the cancers of interest in the study facilities or the broader population sampled.

   Approach: To receive a rating of *Good* for this component of study design and conduct, the study was required to describe clearly: 1) the base population from which cohort participants were sought; 2) the number of patients in that base population (a denominator); and (3) the proportion of eligible patients who were ultimately enrolled in the cohort.

   Studies meeting all three criteria were rated as *Good*; studies lacking information on one criterion were classified as *Fair* and studies lacking information on two or more criteria were rated as *Poor*.
3. *Comparability of subjects:* For cohort studies, five tiers of documentation were sought to show that the study had: 1) specific inclusion/exclusion criteria for all groups; 2) applied criteria equally to all groups; 3) comparable study groups at baseline with reference to variables other than genotype; 4) study groups comparable to non-participants with regard to confounding factors; and 5) study groups comparable with regard to follow-up.

In addition, for case-control studies, we sought documentation on whether the study had: 1) explicit case definition; 2) case ascertainment not influenced by exposure status; and 3) controls similar to cases with the exception that they did not have the condition of interest and did have an equal opportunity for exposure.

Approach: A cohort study was considered to have *Good* comparability of subjects if at least four of five elements were present. Studies were rated as having *Fair* comparability if two or three elements were present. Studies with one comparable element were rated as *Poor*.

Case-control studies were required to have all three elements of the case-control rating to rate a *Good* for the overall category. Case-control studies were rated as *Fair* if they that were missing one element for the case-control rating, and those missing two or more elements were rated as *Poor*.

4. *Statistical analyses:* Documentation was sought on whether the study reported on the following aspects of statistical analyses: 1) appropriate statistical tests; 2) modeling and multivariate techniques or multiple comparisons; 3) power calculations and achieved sample size; 4) assessment of confounding by bivariate analyses, stratified analyses, or multivariable modeling; 5) reporting of adjusted estimates for main effects that took into account identified confounding or modifying factors (stratified or separate analyses were acceptable for simple constructs); and 6) presentation of adjusted results with a measure of statistical precision such as a confidence interval or *P*-value.

   Approach: A rating of *Good* was assigned for the category of statistical analyses if studies provided at least five of the six elements above. A rating of *Fair* was assigned if studies reported on three or four elements and a rating of *Poor* if studies reported on fewer than three elements.

5. *Result and loss to follow-up:* For all studies, documentation was sought on whether the study reported a measure of effect for outcomes and provided an appropriate measure of precision. In addition, for panel studies, documentation was sought on two follow-up measures: 1) analyses of how respondents differed from non-respondents if loss exceeded 20%; and 2) if absolute loss to follow-up exceeded 25%.

   Approach: For studies with cross-sectional measures, a rating of *Fair* was assigned if the study reported a measure of effect with an appropriate measure of precision; studies without a measure of effect were rated *Poor* (*Fair* is maximum possible rating). Panel studies needed to have an absolute loss to follow-up at or below 25%. If the differential loss to follow-up from panel studies exceeded 20%, the investigators needed to report on bias from follow-up to receive a good rating. A study was rated as *Poor* for this component if it had more than 25% loss to follow-up or more than 20% loss without comparison for response bias.

Based on the lack of randomized controlled trials, studies could receive a maximum rating of *Fair* for categories 1 and 5. For categories 2, 3, and 4, studies could receive a maximum rating of *Good*. Studies were given one point for each *Fair* score on categories 1 and 5 and each *Good* score on categories 2, 3, and 4. Ratings were summed across all five categories to assign an overall rating as follows:

- ***Good***, if the study received a total score of 5;
- ***Fair***, if the study received a score of 3 or 4; or
- ***Poor***, if the study received a score of 2 or lower.

Clinical Validity

The following short summaries, prepared by EGAPP reviewers, provide information for overall quality assessment for the articles as they relate to each different key questions. Assessment is based on issues such as study design, study population, generalizability, statistical analysis, and follow-up activities. Information on the studies can also be found in Appendix D (Tables and Figures) and Appendix E (RTI Evidence Tables).

Neutropenia and diarrhea (KQ3a)

- Ando et al., 2000[31] was a case-control, retrospective review of clinical records of Japanese cancer patients treated with varying dosages and schedules of irinotecan-containing chemotherapy. 26 of these patients experienced severe toxicity (leukopenia grade 4, or diarrhea grade 3 or worse) and 92 patients did not; all had received previous chemotherapy regimens. Explicit inclusion and exclusion criteria were provided, as were patient characteristics at baseline. Blood sampling and genetic analyses took place after the administration of irinotecan. 5 variant alleles *(*28, *6, *27, *29 and *7*) were researched. The multivariate analysis suggested that patients with a **28* allele would be seven times as likely to encounter severe toxicity from irinotecan as those without that genotype. Other factors such as female gender and use of other anticancer drugs were important variables for the occurrence of severe toxicity. Quality assessment: *Level 3* - retrospective case-control study design, moderate N, previous chemotherapy regimens and small percentage of primary cancers were CRC. RTI quality assessment: *Poor*

- Carlini LE et al., 2005[33] reported on a convenience sample of 67 adult patients with metastatic CRC (66 genotyped) recruited at Fox Chase Cancer Center, Philadelphia, as part of a multicenter phase II trial of capecitabine/irinotecan combination therapy. Enrollment and treatment were not dependent on the *UGT1A1* genotype. Explicit inclusion and exclusion (including Gilbert syndrome) criteria were provided. Median age was 61. Irinotecan dose was 125 mg/m2 or 100 mg/m2. A total of 36 males and 30 females (55 Caucasian) were followed for adverse drug reactions (severe neutropenia and severe diarrhea identifiable) over multiple treatment cycles (median 9). Outcome information included whether the tumor was completely, or partially, responsive to therapy. Response

was not available for 10 patients (5 wild and 5 heterozygotes). Nearly all raw numbers were available. Analysis was descriptive with some confidence intervals; no multivariate analysis was undertaken. Four individuals were identified with 'other' genotypes (i.e., **1/*36, *36/*37, *1/*37, *28/*37*); these are likely to have been found among the 9 African Americans included in the study. We relied on the author's assignment to genotype (*i.e.,* wild, heterozygous, homozygous); in a few analyses, one or more of these individuals are not counted. Quality assessment : *Level 3* - convenience sample, exclusion of individuals with Gilbert syndrome, inclusion of some African Americans without ability to stratify results, and relatively small sample size. RTI quality assessment: *Poor*

- Font et al., 2003[34] identified 51 metastatic non-small cell lung carcinoma patients in Madrid, Spain undergoing a second-line irinotecan/docetaxel regimen; 47 were genotyped (4 died prior to testing). Median age is 55 and 85% are males. Enrollment and treatment were not dependent on *UGT1A1* genotype. Explicit inclusion and exclusion criteria were provided. Median number of cycles was 3, and irinotecan dose was 3 x 70 mg/m^2 over 3 weeks. All genotyped patients received evaluation for adverse drug reactions, including severe diarrhea, but it was not possible to separate out neutropenia. Analysis was descriptive with some confidence intervals; no multivariate analysis of adverse drug events was undertaken. Quality assessment: *Level 3* - convenience sample, previous chemotherapy regimes, small sample size, and no CRC cases studied. RTI quality assessment: *Poor*

- Innocenti F et al., 2004[35] recruited 66 patients from an unspecified cohort with solid tumors (*e.g.*, lung, colorectal, gastroesophageal) or lymphoma in the Chicago area; 65 had *UGT1A1* genotyping. Median age was 60. Enrollment and treatment were not dependent on *UGT1A1* genotype. Readers were referred to inclusion/exclusion criteria published elsewhere (Iyer et al, 2002[36]). A total of 39 males and 27 females (50 Caucasian) were followed for adverse outcomes; specifically diarrhea (grade 3-4) and neutropenia (grade 4). Three were not assessed for adverse drug events (1 wild, 1 heterozygote and 1 **28/*37*). All had received previous chemotherapy regimens. Irinotecan dose was 350 mg/m^2. Adverse drug events are those occurring during the first cycle of irinotecan therapy. Analysis was descriptive with some confidence intervals; no multivariate analysis was undertaken. Four individuals (probably among the 10 African Americans) were identified with 'other' genotypes (two **1/*37*, and one each of **1/*35* and **28/*37*). We relied on the author's assignment to one of the three genotype groups. Quality assessment: *Level 3* - small sample size, previous chemotherapy regimens, mostly non-CRC, and adverse drug events considered only during the first cycle. RTI quality assessment: *Poor*

- Iyer et al., 2002[36] identified 20 patients (10 women, 10 men) in the Chicago area with solid tumors (7 lung, 3 colon, 3 liver, 7 other); all had *UGT1A1* genotyping. Enrollment and treatment were not dependent on *UGT1A1* genotype. All but 2 of the 20 were Caucasian. Explicit inclusion and exclusion criteria were provided; many had previous cycles of chemotherapy and/or irradiation. Median number of cycles was 3 and starting dose of

Irinotecan was 300 mg/m^2. All genotyped patients received evaluation for adverse drug reactions, including severe diarrhea and/or neutropenia. Analysis was descriptive only; no multivariate analysis was undertaken. Quality assessment: *Level 3* - very small sample size, previous chemotherapy regimes, and few CRC cases studied. RTI quality assessment: *Poor*

- Marcuello et al., 2004[38] identified 95 patients (35 women, 60 men) with metastatic CRC; all had *UGT1A1* genotyping. Enrollment and treatment was not dependent on *UGT1A1* genotype. Inclusion and exclusion criteria were provided. Most had previous cycles of chemotherapy. Four regimens were used: A) irinotecan alone, 350 mg/m^2 every 3 weeks; B) irinotecan 350 mg/m^2 every 3 weeks plus Tomudex; C) irinotecan 80 mg/m^2 every week plus 5-FU; D) irinotecan 180 mg/m^2 every 2 weeks plus 5-FU and leucovorin. All received evaluation for adverse drug reactions, including severe diarrhea and/or severe hematological toxicity (grade 3-4 neutropenia, anemia, or thrombocytopenia). Quality assessment: *Level 3* - inability to differentiate toxicity rates between regimens; cannot specifically identify grade 3-4 neutropenia. RTI quality assessment: *Poor*

- Massacessi et al., 2006[39] identified 56 patients (27 women, 29 men) with metastatic or locally advanced CRC; all had *UGT1A1* genotyping. Enrollment and treatment was not dependent on *UGT1A1* genotype; genotype was blinded to investigators researching toxicities. Explicit inclusion and exclusion criteria were provided, including at least one previous cycle of chemotherapy for advanced disease. Irinotecan dose was 80 mg/m^2 administered weekly, plus raltitrexed. All genotyped patients received evaluation for adverse drug reactions, including severe diarrhea and/or neutropenia. Analysis included univariate and multivariate analysis of toxicities. Quality assessment: *Level 3* - small sample size, convenience sample, and inclusion of localized CRC. RTI quality assessment: *Poor*

- Rouits et al., 2004[43] identified 75 patients (23 women, 52 men; ethnicity not provided) undergoing irinotecan based treatment for metastatic CRC; all had *UGT1A1* genotyping. Enrollment and treatment was not dependent on *UGT1A1* genotype. Explicit inclusion and exclusion criteria were provided. Most had previous cycles of chemotherapy. Regimens were IRIFUFOL (irinotecan 85 mg/m^2 weekly plus 5-fluorouracil and L-folinic acid) or FOLFIRI regimen (irinotecan 180 mg/m^2 biweekly plus 5-fluorouracil and L-folinic acid). All genotyped patients received evaluation for adverse drug reactions, including severe diarrhea and/or neutropenia. Two patients with rare genotypes *(*28/*36, *36/*1*) were excluded from analysis. Analysis was descriptive only. Quality assessment: *Level 3* - small sample size, convenience sampling, and ethnicity assumed to be Caucasian. RTI quality assessment: *Poor*

- Soepenberg et al, 2005[45] identified 25 patients (10 men, 15 women; race not provided) among patients with conformed diagnosis of a malignant solid tumor refractory to

chemotherapy; 23 had *UGT1A1* genotyping. Enrollment and treatment was not dependent on *UGT1A1* genotype. Explicit inclusion and exclusion criteria were provided; most had previous chemotherapy, but not with irinotecan. Dosages were 70 and 80 mg/m^2, given daily for 5 days and repeated every 3 weeks. All genotyped patients received evaluation for adverse drug reactions, including grade 3-4 neutropenia and diarrhea. Levels of plasma and urine SN-38 were quantified. Quality assessment: *Level 3* - small sample size, previous chemotherapy regimens, and mostly non-CRC. RTI quality assessment: *Poor*

- Toffoli et al., 2006[50] identified 250 metastatic CRC patients from 13 centers in northeast Italy; all had *UGT1A1* genotyping. Enrollment and treatment was not dependent on *UGT1A1* genotype. Explicit inclusion and exclusion criteria were provided. Patients underwent a modified FOLRIRI regimen (irinotecan 180 mg/m^2 plus 5-fluorouracil and leucovorin) or FOLFIRI regimen as first-line treatment. All genotyped patients received evaluation for adverse drug reactions, including severe diarrhea and/or neutropenia; plasma levels of irinotecan, SN38 and SN38G were measured in 71 patients on the modified FOLFIRI regimen. Multivariate analyses were performed. The reported category "Hematologic" includes anemia, neutropenia, and leucopenia, but is mostly neutropenia. Quality assessment: *Level 3* - necessary to estimate the rate of severe neutropenia. RTI quality assessment: *Not reviewed*

Morbidity and Mortality (KQ3b)

- Carlini LE et al., 2005[33] studied 66 mainly Caucasian individuals with CRC. He defined a positive responder as 'complete or partial objective response' based on pre- and post-treatment tumor measurement. Ten patients were not followed up for tumor response (five wild and five heterozygotes). Results were separated into the three genotypes. Quality assessment: *Level 3* - small sample size and selected lost to follow-up. RTI quality assessment: *Poor*

- Toffoli et al., 2006[50] studied 250 Italians with CRC, all genotyped. Two definitions of response were made. We chose to use the following: 'partial and complete response' to be consistent with Carlini et al. [33] The second, and unused definition, included stable response. Results were reported separately for the three genotypes. The actual criteria for partial and complete response were not provided. Quality assessment: *Level 2*. RTI quality assessment: *Not reviewed*

- Font *et al.*, 2003[34] studied 51 lung cancer patients in Spain. This study was not included in the analysis. However, it did report improved response among the heterozygotes and homozygotes combined. Of the 47 genotyped patients, all were assessed for tumor response. A positive response was defined as 'disease control of either stable disease or partial response'. No positive responses were noted. Results were only stratified into wild versus heterozygotes and homozygotes combined. Quality assessment: *Level 3* - lack of

CRC patients and the inability to separate out the response in homozygotes. RTI quality assessment: *Poor*

- Marcuello et al., 2004[38] identified 95 patients (35 women, 60 men) with metastatic CRC; all had *UGT1A1* genotyping. Enrollment and treatment was not dependent on *UGT1A1* genotype. Inclusion and exclusion criteria were provided. Most had previous cycles of chemotherapy. Four regimens were used: A) irinotecan alone, 350 mg/m^2 every 3 weeks; B) irinotecan 350 mg/m^2 every 3 weeks plus Tomudex; C) irinotecan 80 mg/m^2 every week plus 5-FU; D) irinotecan 180 mg/m^2 every 2 weeks plus 5-FU and leucovorin. All received evaluation for adverse drug reactions, including severe diarrhea and/or severe hematological toxicity (grade 3-4 neutropenia, anemia, or thrombocytopenia). Quality assessment: *Level 3* –unable to differentiate toxicity rates between regimens; cannot specifically identify grade 3-4 neutropenia. RTI quality assessment: *Poor*

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Appendix D – Analytic and Clinical Validity Evidence Tables and Figures

Table D-1. Inter-laboratory Reproducibility of the Invader® UGT1A1 Molecular Assay[1]

Genotype[a]	1st run tests per site	Site	Results of 1st Run		Results of 2nd Run			
			Invalid calls (failures)	Correct calls (%) [95% CI]	Also invalid on repeat	Correct after 2nd run (%) [95% CI]	Invalid after 2 runs (%)	Overall Correct Calls (%) [95% CI]
*1/*1 $(TA)_6/(TA)_6$	90	1	8	79/82 (96.3)	2/8	6/6	2/90 (2.2)	85/88
		2	0	90/90 (100)	0/0	--	0	90/90
		3	6	84/84 (100)	1/6	5/5	1/90 (1.1)	89/89
*1/*28 Hz $(TA)_7/(TA)_6$	75	1	6	69/69 (100)	0/6	6/6	0	75/75
		2	0	75/75 (100)	0/0	--	0	75/75
		3	5	70/70 (100)	1/5	4/4	1/75 (1.3)	74/74
*28/*28 Homozygote $(TA)_7/(TA)_7$	60	1	7	50/53 (94.3)	1/7	6/6	1/60 (1.7)	56/59
		2	0	60/60 (100)	0/0	--	0	60/60
		3	5	55/55 (100)	0/5	5/5	0	60/60
Other	75	1	7	63/68 (92.7)	1/7	6/6	1/75 (1.3)	69/74
		2	0	75/75 (100)	0/0	--	0	75/75
		3	5	70/70 (100)	0/5	5/5	0	75/75
Total	**300**	**1**	**28/300 (9.3)**	**261/272 (96.0) [92.9-98.0]**	**4/28 (14.3)**	**24/24 (100)**	**4/300 (1.3)**	**285/296 (96.3) [93.5-98.1]**
	300	**2**	**0**	**300/300 (100) [98.8-100]**		**--**	**0/300**	**300/300 (100) [98.8-100]**
	300	**3**	**21/300 (7.0)**	**279/279 (100) [98.7-100]**	**2/21 (9.5)**	**19/19 (100)**	**2/300 (0.7)**	**298/298 (100) [98.8-100]**
	900	**All**	**49 (5.4)**	**840/851 (98.7) [97.7-99.4]**	**6/49 (12.2)**	**43/43 (100)**	**6/900 (0.7)**	**883/894 (98.8) [97.8-99.4]**

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[a] All genotypes based on bi-directional sequencing.

Table D-2a. *UGT1A1* Promoter (TATA) Genotype[a] and Allele Frequencies in Caucasians

Study	Population	N	Assay Method	7/7	7/6	7/5	6/6	6/5	6/8	7/8	8/8	Allele Frequencies 7,5,8
Monaghan G et al, 1996[2]	Caucasian; Scottish	77	Radioactive PCR	0.12	0.48	--	0.40	--	--	--	--	0.36, ND, ND
Beutler E et al, 1998[3]	Caucasian; European	71	Radioactive PCR	0.11	0.56	0	0.34	0	0	0	0	0.39, 0, 0
Sampietro M et al, 1998[4]	Italian; not specified	44	PCR-PAGE	0.20	0.48	--	0.32	--	--	--	--	0.44, ND, ND
Lampe J et al, 1999[5]	Caucasian; US	202	Radioactive PCR	0.11	0.37	--	0.51	0.01	0.005	--	--	0.29, 0.005, 0.003
Borlak J et al, 2000[6]	Caucasian; German	265	FRET	0.12	0.46	--	0.42	--	--	--	--	0.35, ND, ND
Ranchschwalbe S et al, 2002[7]	Caucasian; German	303	PCR-PAGE	0.12	0.46	0	0.42	0.003	--	--	--	0.35, 0.002, ND
Kohle C et al, 2003[8]	Caucasian; Germany	100	FRET LightCycler	0.08	0.41	--	0.50	--	--	--	--	0.29, ND, ND
Danoff T et al, 2004[9]	Caucasian; US/UK	909	Bi-directional sequencing	0.07	0.44	--	0.49	--	--	--	--	0.29, 0.001, 0.001
Cecchin E et al, 2004[10]	Caucasian; Italy	205	Sequencing	0.14	0.47	--	0.39	--	--	--	--	0.38, ND, ND
Bosch TM et al, 2006[11]	Caucasian; Dutch	93	Sequencing	0.09	0.54	--	0.37	--	--	--	--	0.36, ND, ND
Mercke-Odeberg J et al, 2006[12]	Caucasian; Swedish	248	Pyro-sequencing	0.10	0.45	--	0.45	--	--	--	--	0.32, ND, ND
All		**2,517**		**0.108** [0.90-0.130]	**0.454** [0.429-0.479]		**0.423** [0.391-0.456]					

Allele	Freq	95% CI
7	0.334	[0.309-0.361]
5	0.003	[0.001-0.008]
8	0.002	[0.001-0.009]

[a] Genotype terminology : TA 7/7 or *28/*28; TA 7/6 or *28/*1; TA 6/6 or *1/*1; TA 7/5 or *28/*36; TA 6/5 or *1/*37; TA 6/8 or *1/*37; TA 7/8 or *28/*37; TA 8/8 or *37/*37.

Table D-2b. *UGT1A1* Promoter (TATA) Genotype[a] and Allele Frequencies in Asians

Asian	Population	N	Assay Method	7/7	7/6	7/5	6/6	6/5	6/8	7/8	8/8	Allele Frequencies 7, 5, 8
Beutler E et al,1998[3]	41 Japanese, 6 Chinese	47	Radioactive PCR	0.02	0.28	0	0.70	0	0	0	0	0.16, 0, 0
Lampe J et al, 1999[5]	Asian; US	30	Radioactive PCR	0	0.27	0	0.73	0	0	0	0	0.13, 0, 0
Sugatani J et al, 2002[13]	Asian; Japan	27	Sequencing	0	0.04	--	0.96	--	--	--	--	0.02, ND, ND
Tang KS et al., 2005[14]	Asian; Taiwan	441	PCR/RFLP	0	0.29	--	0.71	--	--	--	--	0.14, ND, ND
All		**545**			**0.263**		**0.732**			**Allele**	**Freq**	**95% CI**
										7	0.139	[0.112-0.171]
			0.01							5	0.00	[0.00 – 0.09]
										8	0.00	[0.00 – 0.09]

[a] Genotype terminology : 7/7 or *28/*28; 7/6 or *28/*1; 6/6 or *1/*1; 7/5 or *28/*36; 6/5 or *1/*37; 6/8 or *1/*37; 7/8 or *28/*37; 8/8 or *37/*37.

Additional reported estimates of allele frequencies (no genotype frequencies provided):

Healthy Asians:	Kaniwa N et al., 2005[15] (N=150 Japanese)	TA7: 0.097 [0.064-0.130] TA5: 0.000 TA8: 0.000
Healthy/control African-Americans: See Table D-2c below	Kaniwa N et al., 2005[15] (N=149)	TA7: 0.446 [0.390-0.502] TA5: 0.044 [0.021-0.067] TA8: 0.064 [0.000-0.092]
	Haverfield et al., 2005[16] (N=117 Jamaicans)	TA7: 0.423 TA5: 0.054 TA8: 0.041

Table D-2c. *UGT1A1* Promoter (TATA) Genotype[a] and Allele Frequencies in Africans/African-Americans

Study	Population	N	Assay Method	7/7	7/6	7/5	6/6	6/5	5/5	5/8	6/8	7/8	8/8	Allele Freq. 7, 5, 8
Beutler E et al, 1998[3]	African ancestry; North & Central America	101	Radioactive PCR	0.19	0.37	0.05	0.26	0.02	0	0	0.04	0.06	0.02	0.43, 0.035, 0.07
Guillemette C et al., 2000[17]	African Americans; US	200	Sequencing	0.165	0.36	0.05	0.28	0.095	0.005	0	0.02	0.025	0	0.38, 0.068, 0.02
All		**301**		**0.17**	**0.36**	**0.05**	**0.27**	**.07**	**<.01**	**<.01**	**.03**	**.04**	**0.01**	

Allele	Freq	95% CI
7	0.40	[0.34-0.45]
5	0.06	[0.03-0.10]
8	0.04	[0.01-0.13]

Includes Goldberg et al with no information on alleles 5 and 8 (5 and 8 alleles in other studies reclassified as wild)

Study	Population	N	Assay Method	7/7	7/6	7/5	6/6	6/5	5/5	5/8	6/8	7/8	8/8	Allele Freq. 7, 5, 8
Beutler E et al, 1998[3]	African ancestry; North & Central America	101	Radioactive PCR	0.27	0.46	-	0.28	-	-	-	-	-	-	0.43, ND, ND
Guillemette C et al., 2000[17]	African Americans; US	200	Sequencing	0.19	0.43	-	0.38	-	-	-	-	-	-	0.38, ND, ND
Goldberg RM et al., 2006[18]	African Americans; US	117	Not provided (abstract)	0.21	0.62	-	0.17	-	-	-	-	-	-	0.52, ND, ND
All		**418**												

Allele	Freq	95% CI
7	0.44	[0.36-0.53]

[a] Genotype terminology : 7/7 or *28/*28; 7/6 or *28/*1; 6/6 or *1/*1; 7/5 or *28/*36; 6/5 or *1/*37; 6/8 or *1/*37; 7/8 or *28/*37; 8/8 or *37/*37.

Table D-3. *UGT1A1*6 (c.211G>A,* point mutation) Genotype and Allele Frequency

Study	Population	N	Assay Method	*6/*6	*1/*6	*1/*1 (Wild)	Allele Frequency *6
Kaniwa et al., 2005[15]	**African American**; US	150	Sequencing	0	0	1.00	0.000
Bosch T et al, 2006[11]	**Caucasian**; Dutch	93	Sequencing	0	0.01	0.99	0.005 [0.0001-0.03]
Sugatani et al, 2002[13]	**Asian**; Japan	27	Sequencing	0.04	0.26	0.70	0.17
Tang KS et al., 2005[14]	**Asian**; Taiwan	441	PCR/RFLP	0.007	0.21	0.78	0.11
Kaniwa et al., 2005[15]	**Asian;** Japan	150	Sequencing	0.04	0.23	0.73	0.157 [0.116-0.198]
	Asian[a]	618		0.02	0.23	0.75	0.13 [0.10-0.17]

Table D3-a Targeted update on *UGT1A1*6*: Genotype and Allele Frequencies in Asians

Study	Population	N	Assay Method	*1/*1 %	*6/*1 %	*6/*6 %	*6 Allele Frequency [95% CI]
Sandanaraj et al., 2007[19]	"Pooled healthy Asians"	269			0.13	0.011	0.08 [0.055-0.102]
	Chinese	96	0.86	0.71	0.280	0.011	0.150 [0.102-0.211]
	Malays	90		0.92	0.071	0.012	0.047 [0.021-0.091]
	Indians	93		0.95	0.043	0.011	0.032 [0.012-0.068]
Liu et al., 2007[20]	"Healthy Asians"; CA	82		0.76	0.20	0.05	0.15 [0.096-0.21]
	Chinese	37		0.78	0.14	0.08	0.15 [0.077-0.25]
	Filipino	37		0.76	0.24	0.00	0.12 [0.057-0.22]

Table D-4. *UGT1A1*27 (c.*686C>A, point mutation) Genotype and Allele Frequencies

Study	Population	N	Assay Method	**27/*27*	**1/*27*	**1/*1* (Wild)	Allele Frequency **27*
Kaniwa et al., 2005[15]	**Caucasian**; US	150	Sequencing	0	0	150	0.000
Kaniwa et al., 2005[15]	**African American**; US	149	Sequencing	0	0	149	0.000
Tang KS et al., 2005[14]	**Asian;** Taiwan	441	PCR/RFLP	0	0.05	0.95	0.023 [0.014-0.035]
Kaniwa et al., 2005[15]	**Asian;** Japan	150	Sequencing	0	1	149	0.003 [0.000-0.009]

Figure D-1 Rate of severe neutropenia stratified by *UGT1A1* genotypes

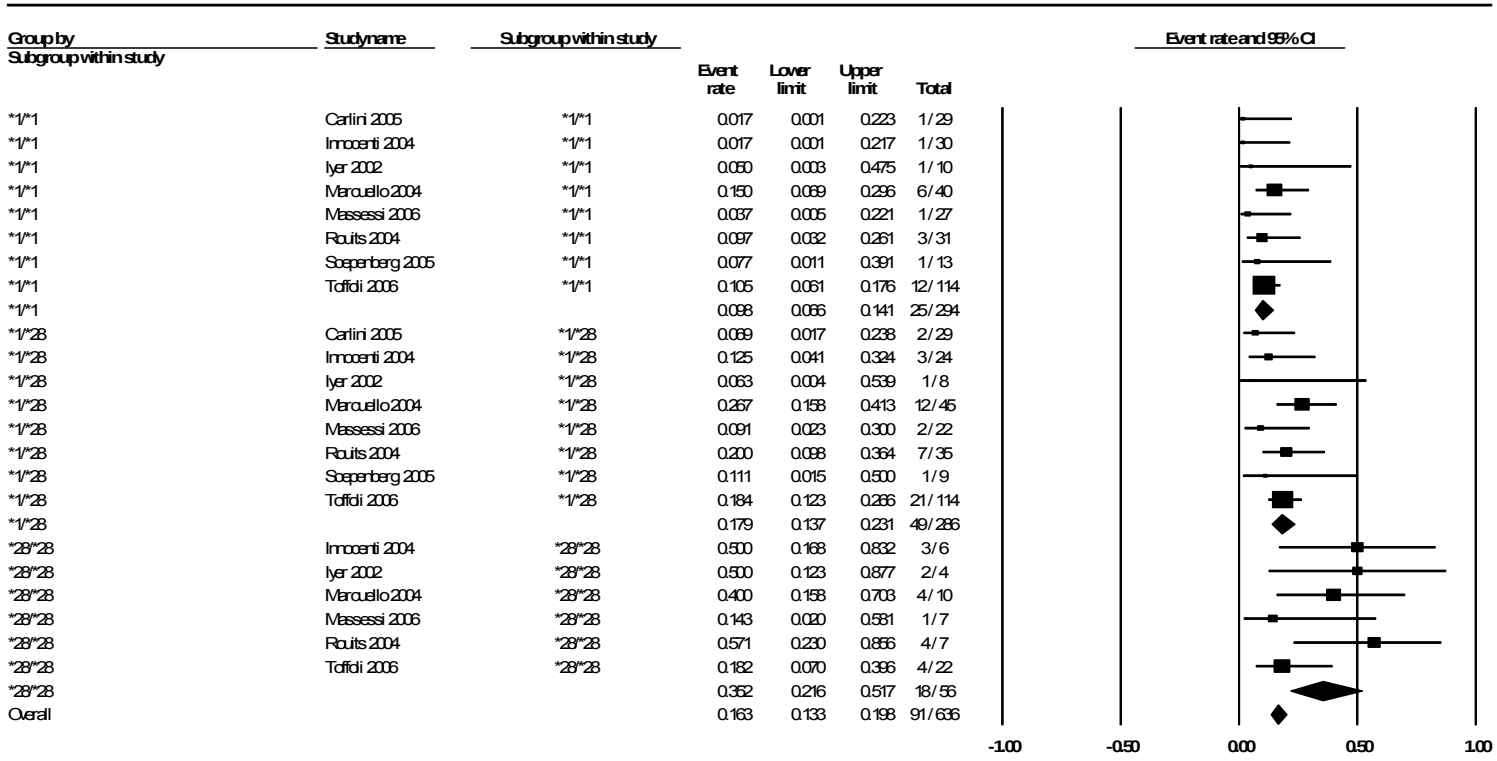


Figure D-2. Risk ratios for severe neutropenia stratified by UGT1A1 genotypes.

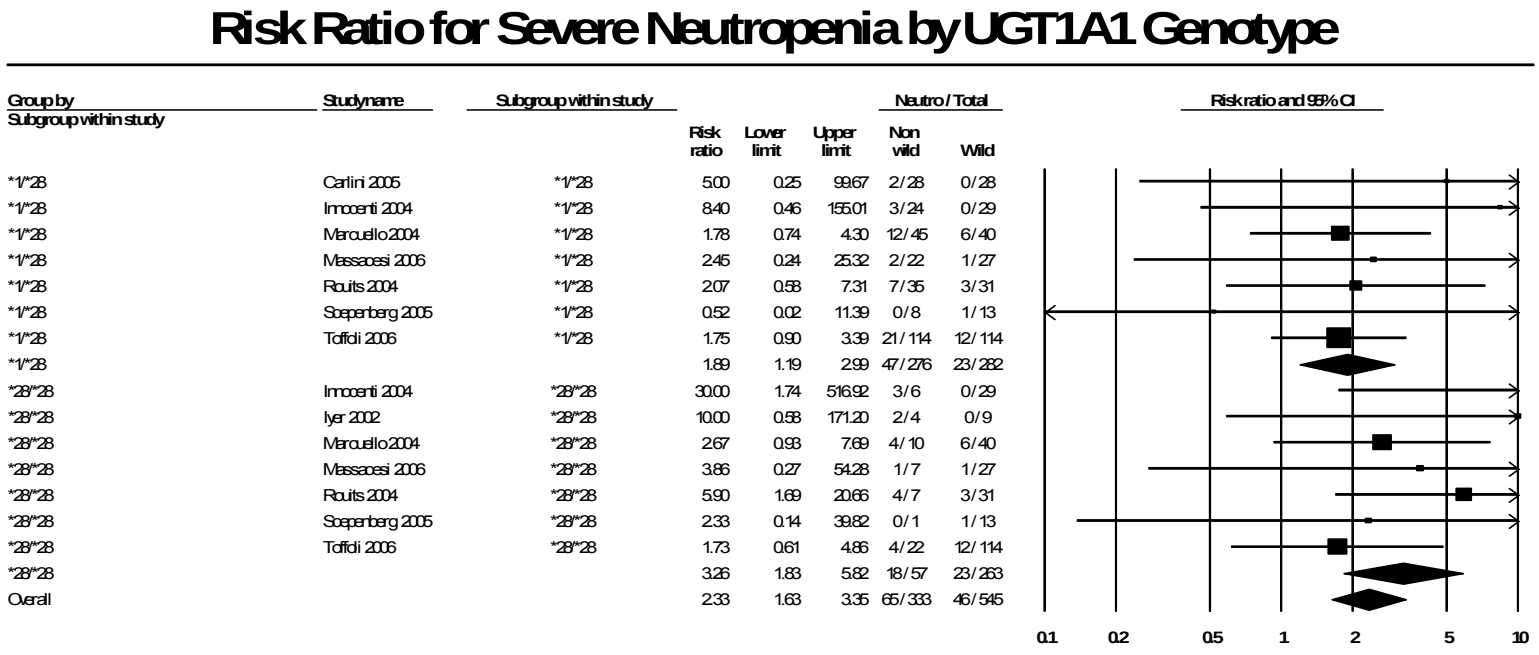


Figure D-3. Rate of severe diarrhea stratified by *UGT1A1* genotypes.

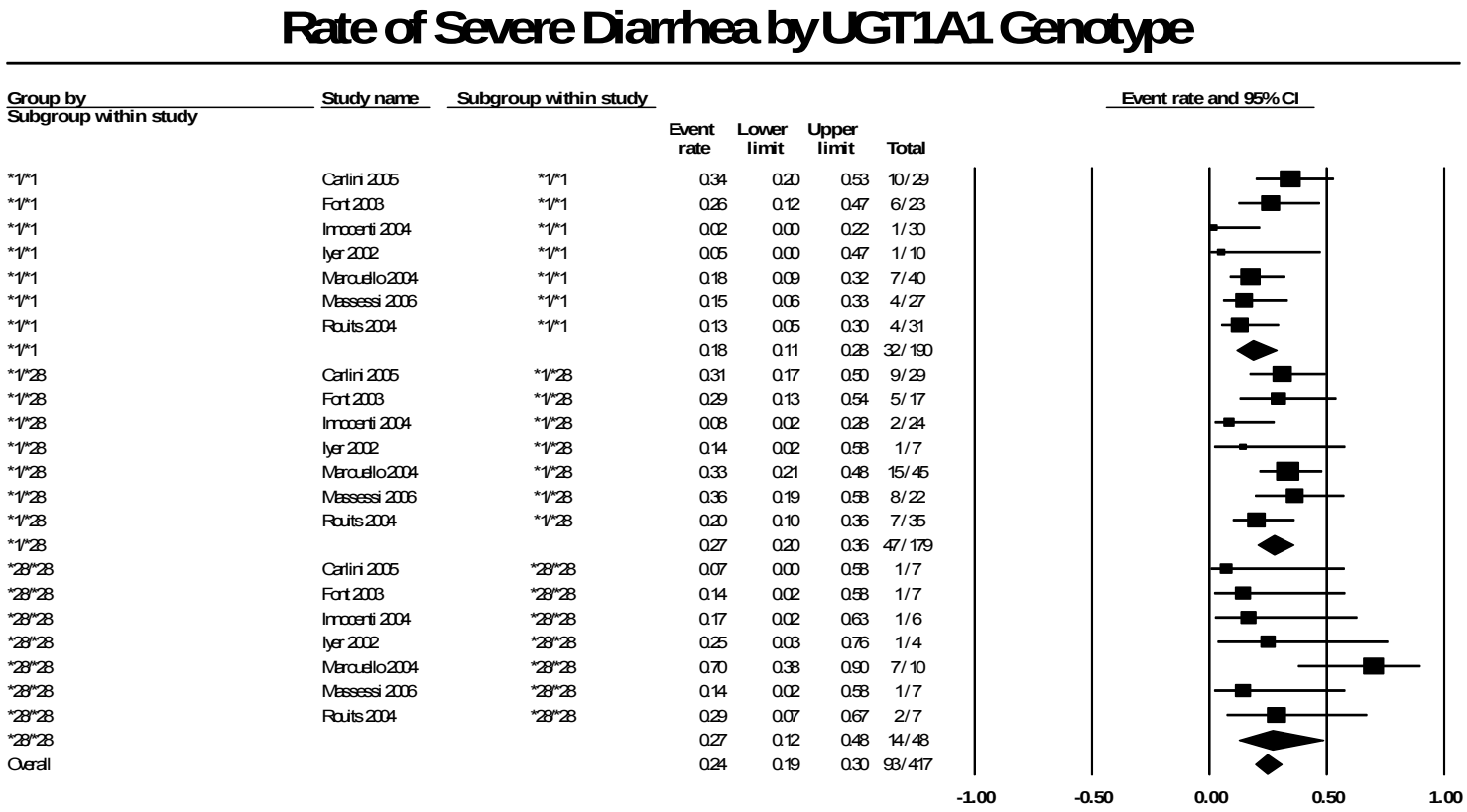


Figure D-4. Risk ratio for severe diarrhea stratified by *UGT1A1* genotype.

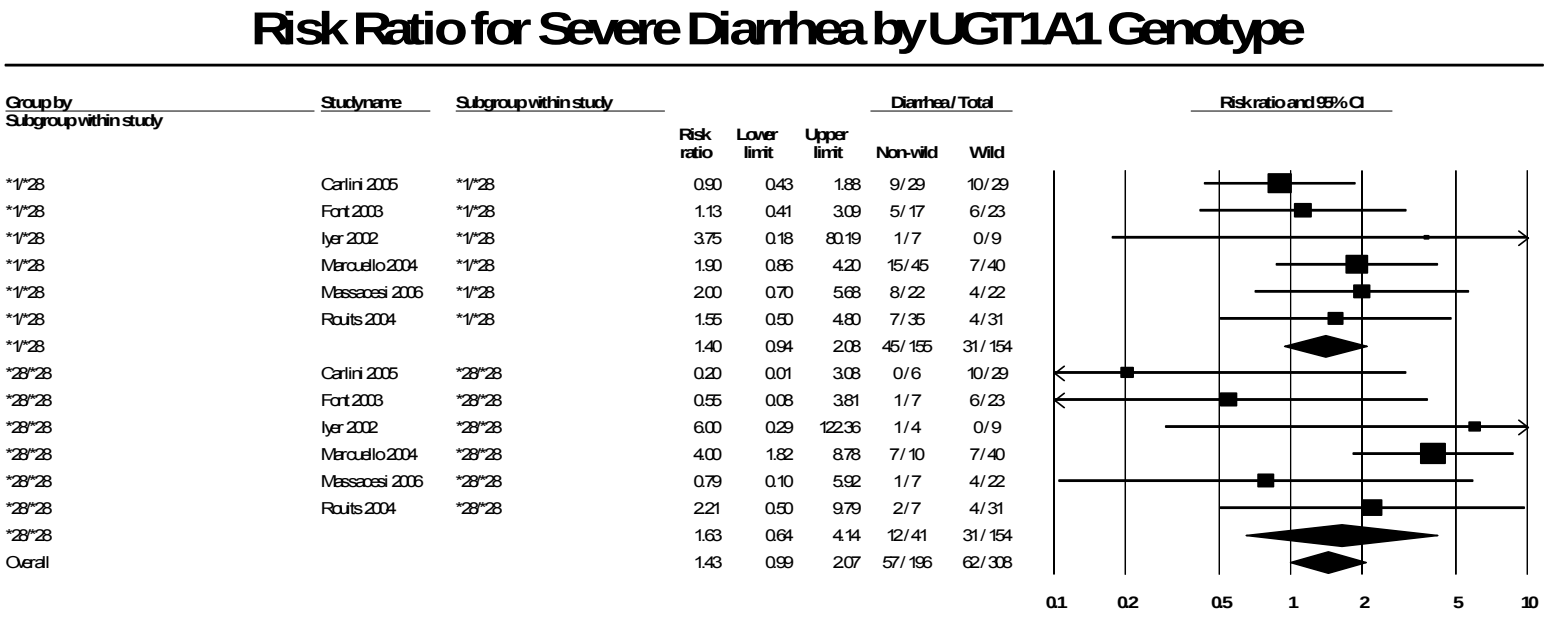
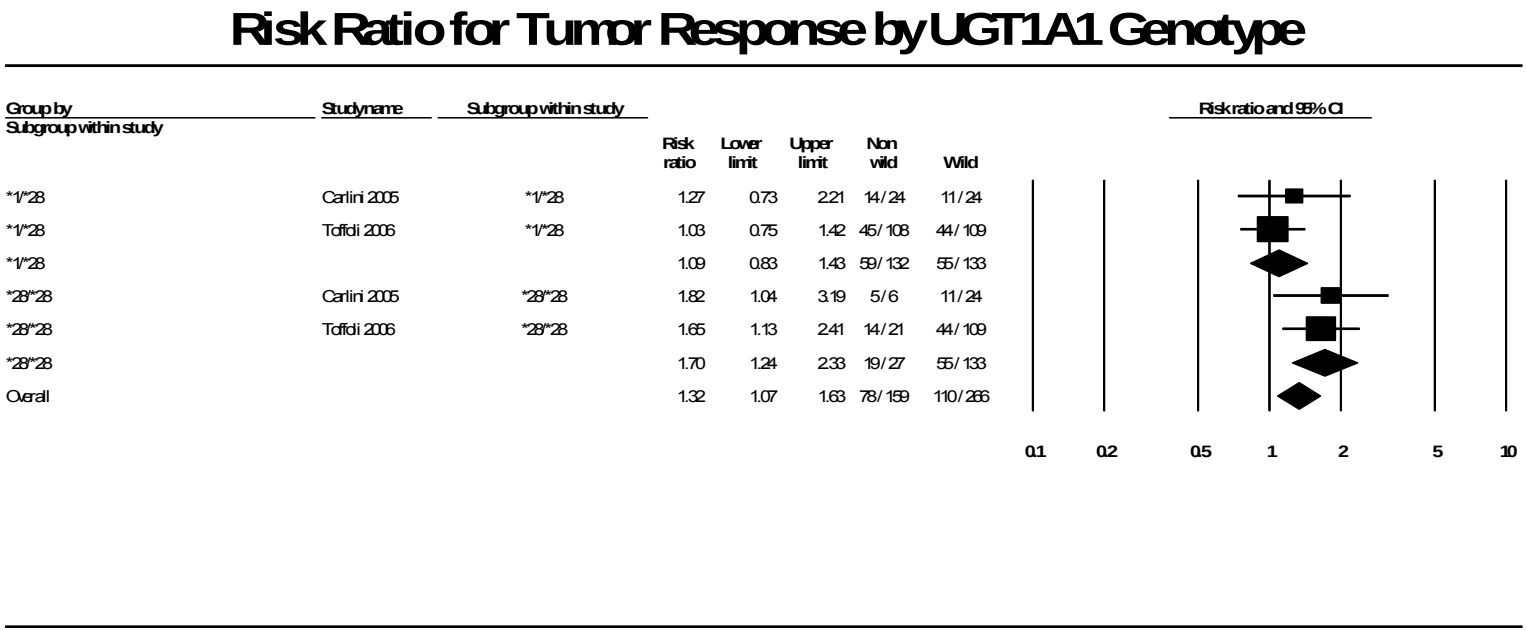


Figure D-5. Risk ratios for tumor response stratified by UGT1A1 genotypes.



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Appendix E:

Final RTI Report

Evidence Tables

Summary Table

Data Abstraction Tables

Summary Table.

Summary of studies of UGT1A1 genotypes and measures of irinotecan metabolism and toxicity

Source Country Patient Population Number of Patients Quality Score	Intermediate outcomes	Toxicity	Health outcomes
Carlini et al., 2005[21] USA Hospital-based, colorectal cancer N=66 Quality: Poor		**Severe Toxicity** 5(TA)/*1 or *1/*1: 10/29 *1/*28: 11/29 *28/*28or*28/8(TA): 0/6 *P* = 0.28	**Responder** 5(TA)/*1 or *1/*1: 11/24 *1/*28: 14/24 *28/*28 or *28/8(TA): 5/6 *P* = 0.25
Font et al., 2003[18] Spain Hospital-based, non small cell lung cancer N = 51 Quality: Poor		**Diarrhea Grade 3-4** ***28/*28: 1/7 ***1/*28: 5/17 *1/*1: 6/23 *P* = 0.84 (for grade distribution)	**Disease Control** *28/*28 +*1/*28: 13/24 *1/*1: 8/23 *P* = 0.36 **Progressive Disease** *28/*28 +*1/*28: 7/24 *1/*1: 11/23 *P* = NS **Mean Time to Progression (months)** *28/*28 +*1/*28: 4 *1/*1: 3 *P* = 0.08 **Median Survival (months)** ***28/*28 +*1/*28: 11 *1/*1: 8 *P* = 0.27 **1 year survival** ***28/*28 +*1/*28: 10/24 *1/*1: 5/23 *P* = NS **2 year survival** ***28/*28 +*1/*28: 7 *1/*1: 3 *P* = 0.27
Innocenti et al., 2004[4] USA Hospital-based, mixed tumors N=65 Quality: Poor	**Pretreatment total bilirubin** *28/*28: .80 +/-.12 *1/*28: .48+/-.03 *1/*1: .48+/-.03 *P* <0.01	**Neutropenia** *28/*28: 3/6 *1/*28: 3/24 *1/*1: 0/29 *P* = 0.001 **ANC nadir** *28/*28: <500 *1/*28: 1500 *1/*1: 2000 *P* = 0.02	1 death (full TA genotype not given but either *1/*28 or *28/*28)

Summary of studies of UGT1A1 genotypes and measures of irinotecan metabolism and toxicity

Source Country Patient Population Number of Patients Quality Score	Intermediate outcomes	Toxicity	Health outcomes
Iver et al., 2002[9] USA Hospital-based, mixed cancer N=20 Quality: Poor	**SN-38G/SN 38 AUC ratio** *28/*28: 2.41 *1/*28: 4.04 *1/*1: 9.28 *P* =0.001	**Diarrhea >2** *28/*28: 1 *1/*28: 1 *1/*1: 0 *P* = 0.289 **Neutopenia > 1** *28/*28: 2 *1/*28: 0 *1/*1: 0 *P* = 0.242 **Absolute neutrophil count** *28/*28: <2000 *1/*28: 2500 *1/*1: 3750 *P* < .0001	
Kitagawa et al., 2005[22] Other reports of same patients: Ando et al 1998,[23] Ando et al 2000,[24] Ando et al 2002[25] Japan Hospital-based, mixed cancer N=119 Quality: Poor		**Severe toxicity** Adj OR (*1/*1, -/*28) = 6.84 (95% CI. 1.86 – 25.2)	
Marcuello et al., 2004[16] Spain. Hospital-based, colorectal cancer N = 95 Quality: Poor	**Pretreatment bilirubin** ***28/*28: 15 (6-28) *1/*28: 8.7 (4-18) *1/*1: 8.3 (4-22) *P* <0.001 **Highest post treatment bilirubin** *28/*28: 22 (6-65) *1/*28: 13 (5-37) *1/*1: 9.9 (4-25) *P* = 0.001	**Diarrhea** ***28/*28: 7/10 *1/*28: 15/45 *1/*1: 7/40 *P* = 0.005 **Asthenia** *28/*28: 10/40 *1/*28: 17/45 *1/*1: 7/10 *P* = 0.03 **Hemotological** *28/*28: 4/40 *1/*28: 12/45 *1/*1: 6/10 *P* = 0.2	**Median survival (months)** *28/*28 + *1/*28: 21 *1/*1: 33 *P* = 0.09 Regression *P* = 0.8 **Clinical response** *P* = 0.3 Regression *P* = 0.1

Summary of studies of UGT1A1 genotypes and measures of irinotecan metabolism and toxicity

Source Country Patient Population Number of Patients Quality Score	Intermediate outcomes	Toxicity	Health outcomes
Marcuello et al., 2004 (continued)		**Nausea** ***28/*28: 5/40 *1/*28: 10/45 *1/*1: 5/10 *P* = 0.4 **Mucositis** ***28/*28: 0/40 *1/*28: 2/45 *1/*1: 1/10 *P* = 0.4 **Infection** ***28/*28: 0/40 *1/*28: 2/45 *1/*1: 7/10 *P* = 0.13	
Massacesi et al., 2006[19] Italy Hospital based, colorectal cancer N = 56 Quality: Poor		**Toxicity-related mortality (patients)** *28/*28: 0/7 *1/*28: 1/22 *1/*1: 0/27 *P* : NS **Diarrhea 2-4 (cycles)** *28/*28: 4/19 *1/*28: 23/60 *1/*1: 6/90 *P* <0.0001 Adj: UGT1A1 (*1/*1 vs *1/*28 vs *28/*28) *P* <0.00005 **Diarrhea 3-4 (cycles)** *28/*28: 1/19 *1/*28: 8/60 *1/*1: 4/90 *P* =0.009 Adj: UGT1A1 (*1/*1 vs *1*28/ vs *28/*28) *P* = 0.012 **Neutrophil Grade 3-4 (cycles)** *28/*28: 1/19 *1/*28: 2/60 *1/*1: 1/90 *P* = NS	

Summary of studies of UGT1A1 genotypes and measures of irinotecan metabolism and toxicity

Source Country Patient Population Number of Patients Quality Score	Intermediate outcomes	Toxicity	Health outcomes
Massacesi et al., 2006 (continued)		**Nausea 2-4 (cycles)** *28/*28: 5/19 *1/*28: 20/60 *1/*1: 7/90 *P* <0.0001 Adj: UGT1A1 (*1/*1 versus *1*28/ versus *28/*28) *P* = 0.001 **Nausea 3-4 (cycles)** *28/*28: 1/19 *1/*28: 3/60 *1/*1: 1/90 *P* =0.009 Adj: UGT1A1 (*1/*1 vs *1*28/ vs *28/*28) *P* = NS **Asthenia Grade 2-3 (cycles)** ***28/*28: 4/19 *1/*28: 13/60 *1/*1: 2/90 *P* = <0.0002 Adj: UGT1A1 (*1/*1 vs *1*28/ vs *28/*28) *P* = 0.0065 **Hepatic toxicity 3-4 (cycles)** ***28/*28: 1/19 *1/*28: 3/60 *1/*1: 8/90 *P* = NS Adj: UGT1A1 (*1/*1 vs *1/*28 vs *28/*28) *P* = NS	
Mathijssen et al., 2003[10] The Netherlands Hospital-based, mixed tumors N = 65 Quality: Poor	**SN-38G/SN 38 AUC ratio** *28/*28: 2.2 +/- 5.2 *1/*28: 7.1 +/- 3.6 *1/*1: 7.6 +/- 4.1 *P* = 0.221		

Summary of studies of UGT1A1 genotypes and measures of irinotecan metabolism and toxicity

Source Country Patient Population Number of Patients Quality Score	Intermediate outcomes	Toxicity	Health outcomes
Mathijssen et al., 2004[11] The Netherlands. Hospital-based, mixed tumors N = 30 Quality: Poor	**AUC of concentration vs time curve for SN-38** *28/*28: 1343 *1/*28**:** 631 *1/*1: 435 ng*h/mL *P* = 0.006 **SN-38/irinotecan AUC Ratio** *28/*28: 0.042 *1/*28: 0.030 *1/*1: 0.018 *P* < .001 **SN-38G/SN 38 AUC Ratio** ***28/*28: 3.48 *1/*28**:** 5.79 *1/*1: 9.27 *P* < 0.010	Comparisons below appear to be –/*28 to *1 but it is not clear: NCI neutropenia *P* = 0.02 Absolute neutrophil count *P* = 0.26 Percent decrease in absolute neutrophil count: 0.024	
Paoluzzi et al., 2004[12] Site of study not stated Hospital-based, mixed tumors N = 94 Qualtiy: Poor	**AUC of concentration vs time curve for:** **Irinotecan** *28/*28: 20,071 *1/*28: 17,167 *1/*1: 18,957 *P* = 0.422 **SN-38** ***28/*28: 600 *1/*28: 600 *1/*1: 508 *P* = 0.253 **SN-38G** *28/*28: 2450 *1/*28: 3157 *1/*1: 3363 *P* = 0.467 **SN-38/irinotecan AUC Ratio** *28/*28: 3.08 *1/*28: 2.97 *1/*1: 2.67 *P* = 0.055 **SN 38G/SN-38 AUC Ratio** *28/*28: 2.51 *1/*28: 6.26 *1/*1: 7.00 *P* = 0.022	Diarrhea graded 2- 4 not significantly associated with UGT1A1 genotype *P* =0.74	

Summary of studies of UGT1A1 genotypes and measures of irinotecan metabolism and toxicity

Source Country Patient Population Number of Patients Quality Score	Intermediate outcomes	Toxicity	Health outcomes
Rouits et al., 2004[20] France Hospital-based, colorectal cancer N = 75 Quality: Poor		**Toxicity-related mortality** ***28/*28: 1/7 *1/*28: 0/35 *1/*1: 0/31 *P* : NS **Diarrhea Grade 4** ***28/*28: 2/7 *1/*28: 2/35 *1/*1: 3/31 *P* = 0.001 **Neutopenia 3-4** *28/*28: 4/7 *1/*28: 14/35 *1/*1: 3/31 *P* = NS **Mucositis 3-4:** *28/*28: 0/7 *1/*28: 0/35 *1/*1: 1/31 *P* = NS **Treatment Postponement** ***28/*28: 5/7 *1/*28: 21/35 *1/*1: 10/31 *P* = NS **Toxicity Related Hospitalization** *28/*28: 5/7 *1/*28: 3/35 *1/*1: 0/31 *P* = NS	
Sai et al., 2004[13] Japan. Hospital-based, mixed cancers N = 88 Quality: Poor	**SN 38G/38 AUC ratio** *28/*28: 3.57 (n=3) *1/*28: 3.45 *1/*1: 6.36 *P* = 0.0014 Regression coefficient = -1.666 *P* = 0.0012 **Total Bilirubin** *28/*28: 1.1 *1/*28: 0.7 *1/*1 0.50 *P* = 0.007 Regression coefficient = -0.213 ; *P* = <0.0001		

Summary of studies of UGT1A1 genotypes and measures of irinotecan metabolism and toxicity

Source Country Patient Population Number of Patients Quality Score	Intermediate outcomes	Toxicity	Health outcomes
Soepenberg et al. 2005[26] Country not stated Hospital based, mixed tumors N = 23 Quality: Poor	**Dose-normalized peak SN-38 concentration** G1: Not stated G2: Not stated G3: Not stated *P* = 0.026	Stated UGT1A1 not associated with toxicity	
Tobin et al., 2006[17] Australia Hospital-based, colorectal cancer N = 20 Quality: Poor	**Bilirubin** No relationship of *28/*1 genotype with SN39G /SN-38 ratio or to total plasma bilirubin *P* = NS	**Diarrhea** No relationship of *28/*1 genotype with diarrhea *P* = NS	
Wright et al., 2005[14] USA Mixed tumors, multiple hospitals N = 30 Quality: Poor	**SN 38/SN-38G AUC ratio** –/*28: 0.31 (.15 - .62) *1/*1: 0.2 (.06 - .38) *P* = 0.03		
Zhou et al., 2005[27] Singapore Hospital-based, nasopharyngeal cancer N = 29 Quality: Poor	**Relative extent of glucuronidation** ***28/*28: 33.8 +/- 2.5 *1/*28: 36.8 +/- 1.7 *1/*1: 33.4 +/- 2.5 *P* = NS		

Genotype notation: *1, UGT1A1*1 – 6 TA repeats; *28, UGT1A1*28 – 7 TA repeats; 5(TA) – 5 TA repeats; 8(TA) – 8 TA repeats

–, denotes unstated allele. For exampled, –/*28 denotes either *1/*28 or *28/*28 genotype.

NS, Not Stated

Data Abstraction Tables

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Ando 1998 [23] **Setting** Japan Hospital-based **Study design** Retrospective case series **Inclusion criteria** Lung cancer patients treated with irinotecan **Exclusion criteria** None stated **Groups** **G1:** *28/*28 (n = 1) **G2:** *28/*1 (n = 1) **G3:** *1/*1 (n = 7) Note: All patients in this paper also included in 2000 paper **Quality rating** Poor	**Objective of the study** Investigate impact of UGT1A1 on likelihood of severe toxicity in patients receiving irinotecan	**AUC or Biliary index** **G1:** 7390 **G2:** 4900 **G3:** 3450-6160 P not reported	**Toxicity-related mortality** **G1:** 0 **G2:** unclear **G3:** unclear Article states severe toxicity not found in 'some' other patients (i.e., other than 7/7 patient) *P* not reported	Not reported

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Ando 2000 Ando 2000 [24] **Setting** Japan Hospital-based **Study design** Retrospective case-series **Inclusion criteria** • Cancer patients treated with irinotecan **Exclusion criteria** • Active infection • Watery diarrhea • Paralytic ileus • Pneumonia • Fibrosis • Ascites • Pleural effusion • Jaundice • Hypersensitivity to Irinotecan **Groups** **G1:** *28/*28 (n = 7) **G2: *28/*1** (n = 18) **G3A:** UGT1A1*1/*1 (n = 67) **G3B:** UGT1A1*1/*6 (n = 25) Patients without *28 mutation were split into two groups due to other tested genotypes **Quality rating** Poor	**Objective of the study** Explore a clinical advantage of determining UGT1A1 polymorphism prior to irinotecan chemotherapy	**Bilirubin after infusion** **G1:** 34.2 (22.2-42.8) **G2:** 18 (12-23.1) **G3A:** 13.7 (10.3-17.1) **G3B:** 15.4 (11.1-25.7) *P < 0.001*	**Severe toxicity*** **G1:** 4/7 **G2:** 8/18 **G3: 14/93** *P < 0.001* **aOR (G1 & G2 compared to G3) = 7.23 (2.52 – 22.3)** Adjusted for regimen and sex. *Grade 4 neutropenia and/or Grade 3 diarrhea	Not reported

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Ando, 2002[25] **Setting** Japan Hospital-based **Study design** Retrospective case series **Inclusion criteria** • Adequate bone marrow and organ function **Exclusion criteria** • Apparent jaundice **Groups** **G1:** UGT1A1*28/*28 (2) **G2:** *28/*1(n = 2**)** **G3:** *6/*1 (n = 1) **G3:** *1/*1 (n = 9) Note: all genotyped patients report in previous study **Quality rating** Poor	**Objective of the study** To explore frequency distribution of AUC ratios of SN-38 to SN-38G using pooled pharmacokinetic data	**AUC or Biliary index** **G1:** 0.6, 1.1 **G2:** 0.5, 0.8 **G3:** 0.3 **G4:** 0.2 - 0.6 *P* not reported	Not reported	Not reported

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Carlini 2005[21] **Setting** USA Hospital-based **Study design** Prospective cohort **Inclusion criteria** • 18 yrs of age • Histologically confirmed colorectal adenocarcinoma • No previous cytotoxic chemo • Ambulatory • Karnofsky performance status > 70% • Neutrophil.1.5x10*9 • Platelet ct.100x10*9/L • Creatinine clearance .50ml/min • Bilirubin < 1.25xupper limit normal • ALAT/ASAT < 2.5x upper limit normal • Alkaline phosphatase < 2.5 upper limit normal	**Objective of the study** Correlate efficacy and toxicity of capecitabine/CPT-11 with genetic variation in genes important in metabolism of CPT-11	Not reported	**Severe Toxicity** **G1:** 10/29 **G2:** 11/29 **G3:** 0/6 *P* = 0.28	**Tumor Response** **G1:** 11/24 **G2:** 14/24 **G3:** 5/6 *P* = 0.25

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Carlini (2005) (continued) **Exclusion criteria** • Gilbert's disease • Pregnancy • Central nervous system metastasis • Active cardiac disease or MI within 12m • Active infection • Physical gastrointestinal disorder • Malabsorption disorder **Groups** **G1:** 5/6 & 6/6 (n = 1/28) **G2:** 6/7 (n = 29) **G3:** 7/7 & 7/8 (n = 5/1) **Quality** Poor				

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Font 2003[18] **Setting** Spain Hospital-based **Study design** Prospective cohort **Inclusion criteria** • Patients with histologically confirmed metastatic NSCLC • ECOG 0-2 • Life expectancy > 12wks • Adequate hematologic,renal, and hepatic function • Received at least one chemotherapy regime **Exclusion criteria** • Symptomatic peripheral neuropathy • Unstable cardiac condition • > 3wks since last chemo or radiotherapy. **Groups** **G1:** *28/*28 (n = 7) **G2: ***1/*28 (n = 17) **G3:** *1/*1 (n = 23) **Quality** Poor	**Objective of the study** To examine activity of irinotecan/docetaxel in NSCLS patients, and secondarily to correlate UGT1A1 polymorphisms with toxicity profile and activity	Not reported	**Diarrhea Grade 3-4** **G1:** 1/7 **G2:** 5/17 **G3:** 6/23 *P* = 0.84 (for grade distribution)	**Disease Control (stable disease or partial response)** **G1 +G2:** 13/24 **G3:** 8/23 *P* = 0.36 **Progressive Disease** **G1 +G2:** 7/24 **G3:** 11/23 *P* = Not stated **Mean Time to Progression (months)** **G1 +G2:** 4 **G3:** 3 *P* = 0.08 **Median Survival (months)** **G1 +G2:** 11 **G3:** 8 *P* = 0.27 **1 year survival** **G1 +G2:** 10/24 **G3:** 5/23 *P* = Not stated **2 year survival** **G1 +G2:** 7 **G3:** 3 *P* = 0.27

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Innocenti 2004[4] **Setting** US Hospital-based **Study design** Prospective cohort **Inclusion criteria** • Patients with confirmed solid tumors or lymphoma **Exclusion criteria** • None described **Groups** **G1:** *28/*28 (n = 6) **G2:** *28/*1 (n = 24) **G3:** *1/*1 (n = 29) **Quality** Poor	**Objective of the study** To study association between UGT1A1 genetic variants and severe toxicity and identify other factors contributing to toxicity	**Pretreatment Bilirubin** **G1:** .80 +/-.12 **G2:** .48+/-.03 **G3:** .48+/-.03 *P* < 0.01	**Toxicity-related mortality** 1 death TA genotype not given **Neutropenia** **G1:** 3/6 **G2:** 3/24 **G3:** 0/29 *P* : 0.001 **Absolute Neutrophil count** **G1:** < 500 **G2:** 1500 **G3:** 2000 *P* : 0.02	Not reported

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Iyer 2002[9] **Setting** USA Hospital-based **Study design** Prospective cohort **Inclusion criteria** • > 70% Karnofsky score • Evaluable disease • WBC > 3500 • ANC > 1500 platelet/ul • Creatinine < 1.5mg dl • Normal conjugate bilirubin levels **Exclusion criteria** • Biological or chemotherapy within 4 weeks prior to irinotecan treatment • Prior radiation treatment within 2 weeks • Post transplant patients • Women of childbearing potential not on birth control • Pregnant or lactating women • Paralytic ileus • Inflammatory bowel disease requiring therapy **Groups** **G1:** *28/*28 (n = 4) **G2:** *28/*1 (n = 7) **G3:** ***1/*1 (n = 9) **Quality** Poor	**Objective of the study** Pharmacogenetic investigation of influence of UGT1A1*28 on disposition and toxicity of irinotecan	**AUC ratio** **G1:** 2.41 **G2:** 4.04 **G3:** 9.28 *P* = 0.001	**Diarrhea > 2** **G1:** 1 **G2:** 1 **G3:** 0 *P* = 0.289 **Neutopenia (> 1)** **G1:** 2 **G2:** 0 **G3:** 0 *P* = 0.242 **Absolute neutrophil count** **G1:** < 2000 **G2:** 2500 **G3:** 3750 *P* < 0.0001	Not reported

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Kitagawa Kitagawa 2004[22] **Setting** Japan Hospital-based **Study design** Retrospective case-series **Inclusion criteria** • Cancer patients treated with Irinotecan **Exclusion criteria** • None stated **Groups** **Groups** **G1:** *28/*28 (n = 7) **G2: ***28/*1 (n = 17) **G3:** *1/*1 (n = 95) Note: All patients in Ando 2000 paper are also included in this paper **Quality** Poor	**Objective of the study** Examine influence of T-3279G polymorphism in addition to UGT1A1	**AUC or Biliary index** **G1:** *28/*28 **G2:** *28/*1 **G3:** *1/*1 *P*	**Severe toxicity** **G1:** 4/7 **G2:** 8/17 **G3:** 15/95 *P* < 0.001 aOR = 6.84 1.86 – 25.2	Not reported

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Marceullo, 2004[16] **Setting** Spain Hospital-based **Study design** Retrospective case-series **Inclusion criteria** • Metastatic colorectal cancer • Treated with irinotecan • Adequate bone marrow and organ function for treatment **Exclusion criteria** • ECOG > 3 • Apparent jaundice **Groups** **G1:** *28/*28 (n = 10) **G2:** *28/*1 (n = 45) **G3:** *1/*1 (n = 40) **Quality** Poor	**Objective of the study** Examine influence of UGT1A1 on toxicity profile, response rate, and overall survival in patients with metastattic colorectal cancer treated with irinotecan chemotherapy	**Pretreatment bilirubin** **G1:** 15 (6-28) **G2:** 8.7 (4-18) **G3:** 8.3 (4-22) *P* < 0.001 **Highest post treatment Bilirubin** **G1:** 22 (6-65) **G2:** 13 (5 -37) **G3:** 9.9 (4 – 25) *P* = 0.001	**Diarrhea** **G1:** 7/10 **G2:** 15/45 **G3:** 7/40 *P* = 0.005 **Asthenia** **G1:** 10/40 **G2:** 17/45 **G3:** 7/10 *P* = 0.03 **Hemotological** **G1:** 4/40 **G2:** 12/45 **G3:** 6/10 *P* = 0.2 **Nausea** **G1:** 5/40 **G2:** 10/45 **G3:** 5/10 *P* = 0.4 **Mucositis** **G1:** 0/40 **G2:** 2/45 **G3:** 1/10 *P* = 0.4 **Infection** **G1:** 0/40 **G2:** 2/45 **G3:** 7/10 *P* = 0.13	**Median survival** **G1 + G2:** 21 months **G3:** 33 months *P* = 0.09 Regression *P* = 0.8 **Clinical response** **G1:** Not stated **G2:** Not stated **G3:** *P* = 0.09 *P* = 0.3 Regression *P* = 0.1

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Massacesi 2006[19] **Setting** Italy Hospital based **Study design** Prospective cohort **Inclusion criteria** • Confirmed colorectal carcinoma • ≥ 1 bibdimensionally measureable lesion • Age 18-75 • ≥ 1 previous course of chemotherapy • Disease progression occurring during adjuvant chemo or disease free interval of 6m, • ECOG PS 0-2 • Life expectancy > 3m • Neutrophil count ≥ 1500 µg/L and platelet count ≥ 100,000 µg/L • Bilirubin < 2x upper limit of normal (ULN) • Other factors dependent on site of metastases • Normal cardiac function	**Objective of the study** Assess value of polymorphisms in UGT1A1, MTHFR, and TS genes as predictive factors of toxicity in patients with advanced colorectal cancer undergoing irinotecan and raltitrexed as at least 2nd line therapy	Not reported	**Toxicity-related mortality** **G1:** 0/7 **G2:** 1/22 **G3:** 0/27 *P* = Not stated *Numbers below are cycles, not patients **Diarrhea 2-4** **G1:** 4/19 **G2:** 23/60 **G3:** 6/90 *P* < 0.0001 Adj: UGT1A1 (*1/*1 versus *1*28/ versus *28/*28) *P* < 0.00005 **Diarrhea 3-4** **G1:** 1/19 **G2:** 8/60 **G3:** 4/90 *P* = 0.009 Adj: UGT1A1 (*1/*1 versus *1*28/ versus *28/*28) *P* = 0.012 **Neutrophil Grade 3/4** **G1:** 1/19 **G2:** 2/60 **G3:** 1/90 *P* = NS	Not reported

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Massacesi 2006 (continued) **Exclusion criteria** • Symptomatic central nervous system metastases • Second primary malignancy • System disorders • Chronic diarrhea • Inflammatory diseases • Bowel subocclusion • Did not receive at least 1 cycle of chemotherapy **Groups** **G1:** UGT1A1*28/*28 (n = 7) **G2:** UGT1A1*1/*28 (n = 22) **G3:** UGT1A1*1/*1 (n = 27) **Quality** Poor			**Nausea 2-4** **G1:** 5/19 **G2:** 20/60 **G3:** 7/90 *P* < 0.0001 Adj: UGT1A1 (*1/*1 versus *1*28/ versus *28/*28) *P* = .001 **Nausea 3-4** **G1:** 1/19 **G2:** 3/60 **G3:** 1/90 *P* = 0.009 Adj: UGT1A1 (*1/*1 versus *1*28/ versus *28/*28) *P* = NS **Asthenia Grade 2-3** **G1:** 4/19 **G2:** 13/60 **G3:** 2/90 *P* = < 0.0002 Adj: UGT1A1 (*1/*1 versus *1*28/ versus *28/*28) *P* = .0065 **Hepatic toxicity 3-4** **G1:** 1/19 **G2:** 3/60 **G3:** 8/90 *P* = NS Adj: UGT1A1 (*1/*1 versus *1*28/ versus *28/*28) *P* = NS	

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Mathijssen 2003[10] **Setting** The Netherlands Hospital-based **Study design** Prospective cohort **Inclusion criteria** • Histologically confirmed malignant solid tumor • No effective regime exists • Adequate hematopoietic function • Normal renal and hepatic function **Exclusion criteria** • Other drugs, dietary supplements or herbal preparations that interfere with irinotecan **Groups** **G1:** *28/*28 (n = 2) **G2:** *28/*1 (n = 19) **G3:** *1/*1 (n = 32) **Quality** Poor	**Objective of the study** Link genetic polymorphisms in transporters and enzymes in irinotecan elimination to interindividual differences in measures of drug exposure Provide stronger scientific bases for optimizing irinotecahn therapy on basis of genotype	**SN-38G/SN 38 AUC ratio** **G1:** 2.2, 5.2 (n = 2) **G2:** 7.1 +/- 3.6 (n = 19) **G3:** 7.6 +/- 4.1 (n = 32) *P* = 0.221	Not reported	Not reported

Study characteristics

Author
Mathijssen 2004[11]

Setting
The Netherlands
Hospital-based

Study design
Prospective cohort

Inclusion criteria
- Solid tumor
- Irinotecan treatment of choice
- Acceptable liver functions
- WHO PS < 1

Exclusion criteria
No use of agents that interfere with irinotecan

Groups
G1: *28/*28 (n = 3)
G2: *28/*1 (n = 15)
G3: *1/*1 (n = 12)

Quality
Poor

Objective

Objective of the study
Explore association of CYP3A with irinotecan and SN-38

Intermediate outcomes

AUC or Biliary index
G1: 1343
G2: 631
G3: 435 ng*h/mL
P = 0.006

Relative Extent of Conversion
G1: 0.042
G2: 0.030
G3: 0.018
P < 0.001

Extent of Glcuronidatiaon
G1: 3.48
G2: 5.79
G3: 9.27
P < 0.010

Toxicity

Summary:
G1 + G2 to G3 (not clear)
NCI neutropenia (*P* = 0.02)

Absolute neutrophil count (*P* = 0.26)

Percent decrease in absolute neutrophil count (.024)

Health outcomes

Not reported

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Paoluzzi 2004[12] **Setting** USA Hospital based **Study design** Prospective cohort **Inclusion criteria** • Histologically confirmed solid tumor • ANC > 2.0x10*9/L • Platelet > 100x10*9/L **Exclusion criteria** • None stated **Groups** **G1:** *28/*28 (n = 5) **G2:** *28/*1 (n = 37**)** **G3:** *1/*1 (n = 44) **Quality** Poor	**Objective of the study** Explore allelic frequencies and functional consequences of UGT1A1*28m UGT1A9*3 and UGT1A9*5 variants in patients treated with irinotecan	**AUC irinotecan** **G1:** 20,071 **G2:** 17,167 **G3:** 18,957 *P* = 0.422 **AUC SN-38** **G1:** 600 **G2:** 600 **G3:** 508 *P* = 0.253 **AUC SN-38G** **G1:** 2450 **G2:** 3157 **G3:** 3363 *P* = 0.467 **AUC ratio SN-38/irinotecan** **G1:** 3.08 **G2:** 2.97 **G3:** 2.67 *P* = 0.0.055 **AUC SN-38G/SN38** **G1:** 2.51 **G2:** 6.26 **G3:** 7.00 *P* = 0.022	Diarrhea graded 2 - 4 was not significantly associated with UGT1A1 genotype (*P* = 0.74)	Not reported

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Routis 2004[20] **Setting** France Hospital or population base etc. **Study design** Retrospective cross sectional **Inclusion criteria** • Irinotecan-based chemotherapy • Metastatic colorectal cancer **Exclusion criteria** • None stated **Groups** **G1:** *28/*28 (n = 7) **G2:** *28/*1 (n = 35) **G3:** *1/*1 (n = 31) **Quality** Poor	**Objective of the study** Test and evaluate sequencing technique and correlation of genotype to irinotecan tolerance	Not reported	**Toxicity-related mortality** **G1:** 1/7 **G2:** 0/35 **G3:** 0/31 *P* = not stated **Diarrhea Grade 4** **G1:** 2/7 **G2:** 2/35 **G3:** 3/31 *P* = 0.001 **Neutopenia 3-4** **G1:** 4/7 **G2:** 14/35 **G3:** 3/31 *P* = not stated **Mucositis 3-4:** **G1:** 0/7 **G2:** 0/35 **G3:** 1/31 *P* not reported **Treatment Postponement** **G1:** 5/7 **G2:** 21/35 **G3:** 10/31 *P* not reported **Toxicity Related Hospitalization** **G1:** 5/7 **G2:** 3/35 **G3:** 0/31 *P* not reported	Not reported

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Sai 2004[13] **Setting** Japan Hospital-based **Study design** Observational cross sectional **Inclusion criteria** • No previous irinotecan based chemo • Total bilirubin < 2mg/dl, AST < 105 IU/L, ALT < 120IU/L, creatinine < 1.5 mg/dl • Performance status 0-2 (not defined; possibly http://www.ecog.org/general/perf_stat.html) **Exclusion criteria** • Not reported **Groups** **G1:** *28/*28 (n = 3) **G2:** *28/*1 (n = 15) **G3:** *1/*1 (n = 23) **Quality** Poor	**Objective of the study** Conduct haplotype analysis of UGT1A1 Examine association between haplotypes and phenotypes	**SN-38G/38 AUC ratio** **G1:** 3.57 (n = 3) **G2:** 3.45 **G3:** 6.36 *P* = 0.0014 Regression coefficient = -1.666 *P* = 0.0012 **Total Bilirubin** **G1:** 1.1 **G2:** 0.7 **G3:** 0.50 *P* = 0.007 Regression coefficient = -0.213 *P* = < 0.0001	Not reported	Not reported

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Soepenberg 2005[26] **Setting** Country not stated Apparently hospital based **Study design** Prospective cohort **Inclusion criteria** • Histologically confirmed solid tumor • Refractory to conventional chemotherapy **Exclusion criteria** • Prior treatment with Irinotecan, • Concomitant treatment with CYPA4 inhibitors • Symptomatic brain metastases • Leptomeningeal involvement • Inflammatory bowel disease • Bowel sub-obstruction • Chronic diarreha • Chronic malabsorbtion or total colectomy • Other surgery that alter transit or absorption **Groups** **G1:** *28/*28 (n = 1) **G2:** *28/*1 (n = 8) **G3:** *1/*1 (n = 13**)** **G4:** *1 / 5(TA) (n = 1) **Quality** Poor	**Objective of the study** • Determine maximum tolerated dose of capsules of irinotecan • Characterize pharmokinetics of irinotecan and SN-38 • Examine correlation of genetic polymorphisms with toxicity • Analyze effect of food on bioavailability • Evaluate preliminary antitumor activity	**Dose-normalized peak SN-38 concentration** **G1:** Not stated **G2:** Not stated **G3:** Not stated *P* = .026	Stated UGT1A1 not associated with toxicity	Not reported

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Tobin 2006[17] **Setting** Australia Hospital-based **Study design** Prospective cohort **Inclusion criteria** • Previous failure of 5-FU containing regimen. • Bidimensionally measurable • Histologically confirmed colorectal cancer • Life expectancy ≥ 12weeks • Serum creatinine < 110micro mol/l • Serum bilirubin < 18 micromol/l **Exclusion criteria** • Active infection • Pre-existing intestinal disease causing diarrhea • Inflammatory bowel • Bowel obstruction • Gilbert's syndrome • Psychiatric disorders • Heart disease • Epilepsy requiring treatment • Previous treatment with irinotecan. **Groups** **G1:** *28/*1 (n = 3) **G2:** *1/*1 (n = 17) **Quality** Poor	**Objective of the study** Examine safety of combining single agent CPT-11 with chrysin	No relationship of *28/*1 genotype with SN39G/SN-38 ratio or to total plasma bilirubin.	No relationship of *28/*1 genotype with diarrhea.	Not reported

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Wright 2005[14] **Setting** USA Multi-site hospital-based **Study design** Prospective cohort **Inclusion criteria** • Cancer patients for which irinotecan was a suitable therapy • Locally advanced but not resectable primary or recurrent solid tumors • Metastatic disease who failed other therapy • Metastatic adrenocarcinoma • PS ≥ 2 • ANC > 2,000/ mcL • Platelet count > 100,000/ mcL • Bilirubin and creatinine < 1.6 mg/dL • Aspartate aminotransferase < 4x normal **Exclusion criteria** • Central nervous system primary tumor or metastases • Active ischemic heart disease • Congestive heart failure Class III or IV • Symptomatic arrhythmia • Active infections • Other serious medical conditions that would prevent treatment **Groups** **G1:** *28/*28 or *28/*1 (n = 21) **G2:** *1/*1 (n = 9) **Quality** Poor	**Objective of the study** Evaluate sequential IV infusions of irinotecan followed by 48 hour infusions of leucovorin and fluorouracil	**Ratio of SN38 / SN38G** **G1:** 0.31 (.15 - .62) **G2:** 0.2 (.06 - .38) *P* = .03	Not reported	Not reported

Study characteristics	Objective	Intermediate outcomes	Toxicity	Health outcomes
Author Zhou 2005[27] **Setting** Singapore Hospital-based **Study design** Prospective cohort **Inclusion criteria** • Histologically confirmed Nasopharyngeal carcinoma • 18-70yrs of age • ECOG PS < 3 • Life expectancy ≥ 3ms • Wbc > 3500microliters • Adequate hepatic function • Adequate renal function **Exclusion criteria** • Infectious disease • Pre-existing cardiac disease • Uncontrolled diabetes, bleeding • Colitis • Concurrent malignancies • Brain metastatases • Lactating, pregnant, or willing to be pregnant • Other medical problems that would prevent protocol compliance **Groups** **G1:***28/*28 (n = 2) **G2:** *28/*1 (n = 10) **G3:** *1/*1 (n = 17) **Quality** Poor	**Objective of the study** Investigate associations between genetic polymorphism in genes involved in irinotecan disposition and pharmacokinetic parameters	**Relative extent of glucuronidation** **G1:** 33.8 +/1 2.5 **G2:** 36.8 +/- 1.7 **G3:** 33.4 +/- 2.5 *P* = NS	Not reported	Not reported

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