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General discussion and future research

General discussion

Over the last decades, colorectal cancer incidence has risen worldwide.[1] In the Netherlands, 9,301 patients were diagnosed with colorectal cancer in 2001, increasing to 13,408 new diagnoses in 2012.[2] Although with the initiation of population-based screening programs a reduction in both colorectal cancer incidence and mortality is anticipated, based on earlier studies a considerate number of patients is still expected to present with interval tumors[3], underscoring the importance of continuing research for optimal treatment strategies.

In the struggle to reduce colorectal cancer mortality, it is not only important to develop new therapeutic strategies, but it is equally essential to optimize the use of currently available treatment modalities. Large costs are associated with anti-cancer treatment, both in relation to quality of life and in financial terms. Patients frequently experience severe and debilitating side-effects, even without any beneficial treatment effect. On the other hand, health care costs for colorectal cancer treatment are exceedingly high, especially for patients with advanced stage disease.[4] Therefore, pretreatment predictors are required, to identify patients with the best likelihood of treatment response, as well as those who are most susceptible to toxicity.

Pharmacogenetics, studying the effect of heritable germline genetic variation on response to drug treatment, may provide such a tool. For this thesis, we studied the association of germline polymorphisms with effects of capecitabine and oxaliplatin in patients with advanced colorectal cancer (ACC).

General pharmacogenetic considerations

The term "pharmacogenetics" entered medical literature as early as 1961.[5] Since then, this research field has evolved rapidly and, at the completion of this thesis, more than 12,000 articles are indexed in Pubmed for the word "pharmacogenetics". **Chapter 2** summarizes the literature regarding pharmacogenetic studies in colorectal cancer available at the start of the research leading up to this thesis. It provides an overview of the understanding of genetic variation in pathways involved in anti-cancer drug effects, not only for capecitabine and oxaliplatin, but also for irinotecan and the newer targeted drugs bevacizumab and cetuximab. Since the writing of the article, many newer studies have been published, and our knowledge on the effects of germline genetic variation on treatment outcome in colorectal cancer is continuously growing.

Pharmacogenetic studies aim at understanding the influence of variation in germline DNA on inter-patient differences in drug effects. Preferably, DNA for these studies should be derived from healthy tissues, such as peripheral blood leucocytes or buccal swabs. However, many of the early pharmacogenetic studies in colorectal cancer have used tumor tissue as the primary source of DNA for their analyses. This approach does not account for the potential bias of somatic mutations or loss of heterozygosity (LOH) in the tumor, and differences between tumor and germline genotype could potentially explain the often contradicting results between pharmacogenetic publications. Studies for the association of *CYP2D6* genotype

with efficacy of tamoxifen in breast cancer have occasionally found significant deviation from Hardy-Weinberg equilibrium (HWE) in tumor material, possibly due to hemizygous chromosomal deletions in this gene.[6-9] This has led some authors to conclude that studies with pharmacodynamic endpoints should not use tumor material as a source of DNA.[6] In **chapter 3** we addressed this possible cause of confounding, by comparing genotyping results in DNA isolated in peripheral blood leukocytes, with results in DNA extracted from archived colorectal cancer tumor samples in the same patients. Analyses were restricted to a defined set of genetic markers that have been frequently selected for pharmacogenetic studies in colorectal cancer, and could successfully be genotyped in all samples.

We found that only 1.4 percent of all blood-tumor pairs showed discordant results. We then evaluated if these discrepancies could be the result of LOH, using heterozygous loci adjacent to the SNP of interest as a marker for chromosomal loss. In this way, we showed that only half of the mismatches could have been induced by LOH.

Of note, we used macro-dissection for the collection of DNA from colorectal tumor tissue, which may have unintentionally led to the inclusion of significant amounts of germline DNA from healthy stromal tissue in samples designated as tumor DNA. This may alternatively explain the high level of agreement between both sample types. Although this mixture of DNA types could have been prevented by the use of micro-dissection for DNA collection from tumor samples, this is not the method used by most previous studies. Furthermore, it was not our goal to rule out any somatic variation in tumor DNA for these polymorphisms. Regardless if the concordance is a reflection of a large stromal component or of actual agreement between germline and tumor genotype, our analyses showed that results from previous pharmacogenetic studies using DNA from macro-dissected tumor tissue, can reliably be compared with those from newer studies using blood-derived DNA. However, the inferior DNA quality in tumor-derived DNA often leads to lower call-rates. Therefore, for future pharmacogenetic research, peripheral blood should be the preferred source of DNA for genotyping.

Pathway-based approach

Capecitabine

Cytotoxicity for fluoropyrimidines is exerted, at least in part, by binding of the active metabolite, fluorodeoxyuridine monophosphate (FdUMP), to thymidylate synthase (TS). This prevents the formation of 20-deoxythymidine-50-monophosphate, an essential precursor for DNA synthesis. For the binding of FdUMP to TS, 5,10-methylenetetrahydrofolate (5,10-MTHF) is required as an essential cofactor. The amount of 5,10-MTHF available for binding to TS is under the direct influence of methylene hydrofolate reductase (MTHFR).

In **chapter 4**, we hypothesized that polymorphisms in the gene encoding for MTHFR may affect capecitabine cytotoxicity in colorectal cancer patients, by increasing the availability of 5,10-MTHF and thereby the complex formation with TS. Two common, functional polymorphisms in *MTHFR* (*MTHFR* 677C>T and *MTHFR* 1298A>C) were evaluated for their association with capecitabine-induced toxicity. We found no effect of these polymorphisms on

the incidence of severe adverse events in our analyses. Whether this absence of association is due to the overshadowing effect of other genetic and non-genetic influences, or whether there is indeed no effect of these polymorphisms on capecitabine cytotoxicity, cannot conclusively be answered by our research. However, correction for common polymorphisms in the gene encoding for TS, which have been suggested to influence fluoropyrimidine cytotoxicity, did not alter our results. It should be noted, patients who experienced severe toxicity during adjuvant treatment with 5-FU or capecitabine, may not have entered the CAIRO study and this selection could also confound our pharmacogenetic analyses. Nevertheless, if this selection had been due to a specific *MTHFR* allele, the genotype distribution at baseline would not have adhered to Hardy-Weinberg equilibrium. Therefore, we advocate that *MTHFR* SNPs are not useful in the pretreatment prediction of capecitabine-induced adverse effects (or treatment efficacy) in ACC patients.

In addition to *MTHFR* genotype, other polymorphisms haven been suggested to explain the variation in individual response to capecitabine. Recently, a genome wide association study (GWAS) in lymfoblastoid cell lines identified new possible markers for capecitabine cytotoxicity.[10] The most noticeable result was for a SNP, rs4702484, located near the gene encoding for 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*), which has been studied previously for its effect on fluoropyrimidine-induced cytotoxicity, and for its potential role in colorectal carcinogenesis.[11;12] In **chapter 5** we attempted to confirm the influence of the most common *MTRR* polymorphisms, as well as four other promising SNPs from the aforementioned GWAS, on efficacy of capecitabine in our population of ACC patients from the Dutch CAIRO trial.[13] Although rs4702484 showed a borderline significant association with increased progression free survival (PFS) for carriers of the variant allele, this effect was only present in univariate analysis in patients treated with capecitabine monotherapy. Thus, even if this marker affects capecitabine cytotoxicity, its effect is overshadowed by the influence of clinical patient characteristics and it is lost in combination therapy. This also illustrates that results from GWAS, especially *in vitro* studies, are difficult to replicate in clinical practice. Our relatively small cohort size may have compromised the power to detect genotype effects. However, for almost all analyses, no trend could be discovered for a genotype effect on treatment efficacy and it is unlikely that increasing sample size would have led to significant results. We therefore conclude that none of the tested genetic markers are helpful in the pre-treatment prediction of efficacy of capecitabine in ACC patients.

Oxaliplatin

The addition of oxaliplatin to fluoropyrimidine treatment has considerably increased survival for patients with metastatic colorectal cancer.[14] However, treatment with oxaliplatin is often hindered by the occurrence of neurotoxicity, leading to dose reductions or treatment discontinuation. Preclinical data suggest that there is a relationship between the presence of specific organic cation transporters (OCTs) in dorsal ganglia, responsible for cellular uptake of oxaliplatin, and oxaliplatin-induced peripheral neuropathy. For instance, the competitive

OCT2 inhibitor cimetidine not only impairs cellular uptake of oxaliplatin *in vitro*[15], but also reduces neuropathic symptoms in oxaliplatin-exposed mice.[16] However, results from *in vitro* and *in vivo* studies are not in complete agreement on which cation transporter is most likely to be involved in oxaliplatin uptake in dorsal ganglia.[15;16] We theorized that SNPs in the genes encoding for three cellular transporters (*SLC22A1*, *SLC22A2* and *SLC47A1*) could explain the divergent expression of neurotoxicity between patients treated with oxaliplatin. This hypothesis was tested in **chapter 6**. A functional, non-synonymous SNP in OCT1, *SLC22A1* Arg61Cys, was associated with a low risk of severe oxaliplatin-induced neurotoxicity for patients carrying two variant alleles, even after correction for cumulative oxaliplatin dose. Unfortunately, the low population frequency of only 2 percent homozygote variant carriers, in combination with a small effect size, impairs the implications of this marker for clinical practice. Moreover, because our study was hindered by a low genotyping call rate, as well as a suboptimal clinical scoring system for neurotoxicity, this result needs to be validated in independent treatment cohorts and mechanistic and functional studies before implementation into clinical practice.

The anti-tumor effect of oxaliplatin stems from binding of diaminocyclohexane (DACH)-platinum (Pt) to the DNA helix, causing Pt-DNA cross-links, and ultimately leading to programmed cell death. This process is inhibited by the action of cellular DNA repair mechanisms, such as the Excision Repair Cross Complementation type I (ERCC1). **Chapter 7** focuses on the hypothesis that a common synonymous SNP in *ERCC1 (ERCC1* C118T) alters oxaliplatin cytotoxicity depending on genotype, through an effect on DNA repair capacity. Transfection experiments were performed, assessing the effect of *ERCC1* C118T on DNA repair capacity *in vitro.* In addition, we performed a clinical association study for the effect of this SNP on survival upon oxaliplatin-based chemotherapy in ACC patients from the CAIRO study.[13] We showed that both *in vitro* cell survival after oxaliplatin-administration, and DNA repair-capacity of the transfected cells, depend on the presence of a functional *ERCC1* gene. However, we found that *ERCC1* C118T neither changes the *in vitro* capacity for DNA repair, nor affects survival of ACC patients receiving treatment with oxaliplatin. Strictly speaking, the population size for the clinical association study was not sufficiently powered to definitely rule out any effect of *ERCC1* genotype on patient survival. However, our results concur with a meta-analysis published in 2011, showing that PFS following oxaliplatin-based chemotherapy for colorectal cancer patients was not influenced by *ERCC1* C118T genotype in Caucasian populations.[17] Although transfection experiments do not account for the normal cellular regulation of gene expression, *in vivo* ERCC1 functionality depends on the co-expression of ERCC4. Any super-natural ERCC1 protein levels due to unregulated plasmid derived transcription would therefore not have resulted in more effective DNA repair in our experiments. We therefore believe both the clinical and the transfection model provide valid evidence that *ERCC1* C118T does not alter cellular or clinical response to oxaliplatin.

Genome wide approach

In chapters 4 to 7, a pathway-based, candidate gene approach was used to identify predictive markers for efficacy and toxicity of capecitabine and oxaliplatin. However, this approach is restricted by the limited knowledge of the pathways and genes involved in individual drug response. Therefore, as described in **chapter 8**, we performed a GWAS to identify novel markers for the prediction of PFS on treatment with a multidrug schedule for ACC. For this study, patients were accrued from the CAIRO2 trial, which compared treatment with capecitabine, oxaliplatin and bevacizumab (CAPOX-B) with CAPOX-B plus cetuximab.[18] One marker on chromosome 2 showed a significant effect on PFS, that was opposite in patients treated with CAPOX-B, compared to those treated with cetuximab in addition to CAPOX-B. This marker could therefore be a potential marker for cetuximab efficacy in ACC. This SNP is located in *GnT-IVa*, in an intronic region that is a predicted binding site for microRNA-34A. We presented a pathophysiological hypothesis based on this remarkable finding, which will be further evaluated in pre-clinical studies. Even if a functional effect of this marker is validated in laboratory experiments, these results should also be replicated in clinical patient cohorts. However, since cetuximab is no longer prescribed in combination with bevacizumab, and it is often included in second- or third-line treatment only, finding a patient cohort similar to ours is extremely difficult.

Future research

Colorectal cancer survival is highly dependent on tumor stage. For patients with stage I-III disease, treatment is aimed at curation by surgical removal of the tumor, mostly followed by adjuvant systemic treatment for patients with stage III or high risk stage II disease.[19;20] Over time, the boundaries of what is considered to be curable disease have broadened. In current practice, not only liver metastases, but also solitary lung metastases are often treated with localized therapies in hope of curation.[19] However, for most patients with metastatic disease, treatment still consists of systemic therapy with palliative intent.

Unfortunately, there is great disparity in individual response to chemotherapy, both in terms of efficacy and toxic events. Because of the poor prognosis of ACC, and the increasing emphasis on quality (rather than prolongation) of life, it can be questioned whether this disease should be treated by a one-size-fits-all regime. If the goal is to offer the most effective, least toxic therapy to each individual upfront, we need a form of personalized medicine.

Ideally, in the near future, the choice for a specific treatment will become tailor-made, taking into account a multitude of biomarkers, as well as clinical factors, such as age, renal function, co-medication and patient preference. Pharmacogenetics provides valuable pre-treatment markers of efficacy and toxicity, and is slowly beginning to enter clinical practice. It is now generally accepted that carriers of the rare *DPYD**2A allele are prone to severe fluoropyrimidine-induced toxicity, and should be treated with reduced dose or alternative

treatment.[21;22] Patients carrying the variant *UGT1A1*28* allele are at increased risk of febrile neutropenia when treated with irinotecan, and dose reductions are advised.[21-23] A genotype-based dosing system has recently been proposed according to *UGT1A1*28* genotype, which is a further step toward genotype-guided, personalized cancer-care.[24]

In addition to its merits for clinical practice, pharmacogenetic research could also aid in the development of new drugs. Currently, new agents are still tested in genetically heterogeneous populations, without recognition of genotype-phenotype interactions. In case of insufficient survival benefit or intolerable toxicity for the total study population, the drug will not enter clinical practice. However, the treatment under investigation could be a safe and effective option for a genetically distinct subset of patients, and its development could be continued for this specific group. In fact, it has been shown that such stratification markers improve the success rate of drug development programs.[25]

Our GWAS results, described in **chapter 8**, also provide support for this assumption. Although the addition of cetuximab to CAPOX-B in first-line treatment of ACC has a negative effect on survival in unselected, genetically heterogeneous populations[18;26], we found that carriers of the variant allele of the common SNP rs885036, conversely, may benefit from the addition of cetuximab. Although these data are preliminary and need to be further validated, they illustrate that analysis of germline genetic variation could indeed identify groups of patients who differ significantly in their response to a specific drug regimen.

As our knowledge of the genome is increasing, so are the technological possibilities for genotyping. Whereas the original dogma was that our genome was made up of protein-encoding genes surrounded by non-functional DNA[27], it was later discovered that it harbors a vast amount of non-coding RNA isoforms, involved in regulation of transcription.[28] New technologies for genotyping have been developed, allowing us to include these former "gene deserts" into our analyses. GWAS address between 500,000 and 1,000,000 SNPs across the genome, although analyses are usually restricted to polymorphisms with a population frequency of >0.05. In addition, next generation sequencing (NGS) offers the potential of genotyping all coding regions, or even the whole genome.[29]

Even if the proof of principle has been delivered, cost-effectiveness and clinical utility of pharmacogenetics continue to be questioned.[30] Cost-effectiveness is determined by many different aspects, including drug price and cost of genotyping. Costs for whole genome genotyping with NGS have decreased from $95.000.000 in 2001, to $4.000 in 2014.[31] For various clinical purposes, a multitude of SNPs are tested at the same time, with complete arrays at less than €500 per patient.[32] Both these arrays and NGS yield information on a myriad of polymorphisms, important for drugs currently prescribed to the patient, but also for potential future prescriptions. This reduces cost per genotyped SNP to only a few cents. Independent of financial cost, clinical utility is dependent on prevalence and penetrance of the allele in question, test specificity and sensitivity, cost of an alternative drug, and on spendings saved by increased survival or better quality of life.[33] Therefore, clinical applicability is not the same for all markers, but dependent on the characteristics of the SNP, the population in which it is

tested, the drug and the disease under investigation. With reducing genotyping cost per SNP associated with NGS, and with increasing drug cost, the break-even point will be met more easily in future.

Despite pharmacogenetics finding its way into clinical practice, we do not know yet which amount of inter-individual variation in drug response can ultimately be explained by genetic variation, and how many different loci influence each drug effect. Although past studies showed that heritability explained around 97-99 percent of the variation in elimination of number of non-cancer drugs[34-36], an *in vitro* study using lymphoblastoid cell lines found that heritability of 5-FU cytotoxicity is only 26-65 percent, depending on the administered dose.[37] In contrast, *DPYD**2A alone predicts 50 percent of all cases of grade IV febrile neutropenia in patients treated with standard dose 5-FU.[38] The degree to which germline pharmacogenetics explains drug behavior is likely to depend not only on the drug and gene at hand, but also on the administered dose, the method of administration and whether the endpoint is efficacy or toxicity. Furthermore, epigenetic regulation may even lead to day-to-day variations in genetic influences on drug behavior.

The influence of genetic variation on drug behavior is best analyzed in extreme phenotype populations, because of the large effect size. Patients experiencing severe toxicity on chemotherapeutic treatment form such a population. On the one hand, extreme phenotypes could be explained by a small number of rare polymorphisms, each individually evoking the phenotype in a proportion of patients, through various mechanisms. Although rare variants often embody protein-changing mutations, and are therefore predisposed to causing extreme phenotypes, half of all variants at a minor allele frequency of 0.5 percent are found in only one single ethnic population, restricting the world-wide implementations in pharmacogenetic guidelines.[39] The effect of *DPYD**2A, with a population frequency of only 1.8 percent heterozygotes in Western populations and a large effect on 5-FU induced toxicity, fits this rare variant hypothesis.[40] Also consistent with the hypothesis, this SNP is not found in Asian populations and therefore cannot explain fluoropyrimidine-induced toxicity in Asian cancer patients.[41]

On the other hand, extreme phenotypes may be explained by a multitude of different polymorphisms with a higher population frequency, all causing a very small fraction of the variation in drug response in each affected individual. Chemotherapy-induced toxicity is not an ordinal endpoint, but rather a continuous scale, ranging from minor complaints to severe and life-threatening events. Although the array of possible outcomes could be explained by the effect of many different rare variants with an equal number of different effect sizes, it is better explained by this common variant hypothesis. Because we are far from understanding all processes and gene products involved in individual drug behavior, we need a hypothesis-free approach to unravel all of these contributing variants. It is only because of modern technologies such as GWAS or NGS, that we are now able to perform such broad searches. However, because of the relatively small effect sizes, very large patient populations are needed to identify and validate these markers, before incorporation into clinical practice. This necessitates the inclusion of pharmacogenetic research into all clinical trials involving systemic anti-cancer

treatment. For definite conclusions on these small effect genetic markers, the formation of consortia and the conduction of meta-analyses from observational studies is indispensable. A dedicated, randomized controlled trial, withholding genotyping in half of the patients, may be regarded unethical if retrospective evidence for the genotype-phenotype interaction is overwhelming. Consequently, focus should also be shifted from clinical validation to gathering functional proof by laboratory studies.

In conclusion, results of pharmacogenetic research are already being incorporated into clinical practice of anti-cancer therapy. In a survey in 2012, more than two thirds of oncologists in the United States reported using a pharmacogenetic test in the previous six months.[42] In future, new technological possibilities, increasing availability and decreasing financial costs of genotyping will further increase the applicability of pre-emptive pharmacogenetic testing for clinical practice. Current knowledge on genes, and molecular and clinical effects is now integrated into pathways and registered online, in the Pharmacogenomics Knowledge Base (PharmGKB).[43] Implications for clinical practice are being formulated by consortia[21;44], and the applicability for clinical practice is under current investigation.[32]

For patients with advanced colorectal cancer, tailoring therapy is of great importance, because of the small window of opportunity for effective treatment, and the burden of adverse effects associated with anti-cancer drugs. Through its incorporation into drug development programs and clinical trials, and through collaborating efforts for the introduction into clinical practice, pharmacogenetics will help maximize the chances of efficacy and minimize the risks of adverse reactions for all patients.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. GLOBOCAN 2008 v2.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]. Lyon, France: International Agency for Research on Cancer; 2010. Available from: http://globocan.iarc.fr, accessed on 01/07/2014.
2. Integraal Kankercentrum Nederland. Available from: http://www.cijfersoverkanker.nl/selecties/Incidentie_darmkanker/img534e81c492d09; Accessed 01/07/2014.
3. Paimela H, Malila N, Palva T, Hakulinen T, Vertio H, Jarvinen H. Early detection of colorectal cancer with faecal occult blood test screening. Br J Surg 2010 Oct;97(10):1567-71.
4. Kriza C, Emmert M, Wahlster P, Niederlander C, Kolominsky-Rabas P. Cost of illness in colorectal cancer: an international review. Pharmacoeconomics 2013 Jul;31(7):577-88.
5. Evans DA, Clarke CA. Pharmacogenetics. Br Med Bull 1961 Sep;17:234-40.
6. Nakamura Y, Ratain MJ, Cox NJ, McLeod HL, Kroetz DL, Flockhart DA. Re: CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the Breast International Group 1-98 trial. J Natl Cancer Inst 2012 Aug 22;104(16):1264-8.
7. Pharoah PD, Abraham J, Caldas C. Re: CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the Breast International Group 1-98 trial and Re: CYP2D6 and UGT2B7 genotype and risk of recurrence in tamoxifen-treated breast cancer patients. J Natl Cancer Inst 2012 Aug 22;104(16):1263-4.
8. Regan MM, Leyland-Jones B, Bouzyk M, Pagani O, Tang W, Kammler R, et al. CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the breast international group 1-98 trial. J Natl Cancer Inst 2012 Mar 21;104(6):441-51.
9. Stanton V, Jr. Re: CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the Breast International Group 1-98 trial. J Natl Cancer Inst 2012 Aug 22;104(16):1265-6.
10. O'Donnell PH, Stark AL, Gamazon ER, Wheeler HE, McIlwee BE, Gorsic L, et al. Identification of novel germline polymorphisms governing capecitabine sensitivity. Cancer 2012 Aug 15;118(16):4063-73.
11. Pardini B, Kumar R, Naccarati A, Novotny J, Prasad RB, Forsti A, et al. 5-Fluorouracil-based chemotherapy for colorectal cancer and MTHFR/MTRR genotypes. Br J Clin Pharmacol 2011 Jul;72(1):162-3.
12. Zhou D, Mei Q, Luo H, Tang B, Yu P. The polymorphisms in methylenetetrahydrofolate reductase, methionine synthase, methionine synthase reductase, and the risk of colorectal cancer. Int J Biol Sci 2012;8(6):819-30.
13. Koopman M, Antonini NF, Douma J, Wals J, Honkoop AH, Erdkamp FL, et al. Sequential versus combination chemotherapy with capecitabine, irinotecan, and oxaliplatin in advanced colorectal cancer (CAIRO): a phase III randomised controlled trial. Lancet 2007 Jul 14;370(9582):135-42.
14. de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 2000 Aug;18(16):2938-47.
15. Zhang S, Lovejoy KS, Shima JE, Lagpacan LL, Shu Y, Lapuk A, et al. Organic cation transporters are determinants of oxaliplatin cytotoxicity. Cancer Res 2006 Sep 1;66(17):8847-57.
16. Sprowl JA, Ciarimboli G, Lancaster CS, Giovinazzo H, Gibson AA, Du G, et al. Oxaliplatin-induced neurotoxicity is dependent on the organic cation transporter OCT2. Proc Natl Acad Sci U S A 2013 Jul 2;110(27):11199-204.
17. Yin M, Yan J, Martinez-Balibrea E, Graziano F, Lenz HJ, Kim HJ, et al. ERCC1 and ERCC2 polymorphisms predict clinical outcomes of oxaliplatin-based chemotherapies in gastric and colorectal cancer: a systemic review and meta-analysis. Clin Cancer Res 2011 Mar 15;17(6):1632-40.
18. Tol J, Koopman M, Cats A, Rodenburg CJ, Creemers GJ, Schrama JG, et al. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. N Engl J Med 2009 Feb 5;360(6):563-72.
19. Benson AB, III, Bekaii-Saab T, Chan E, Chen YJ, Cooper HS, Engstrom PF, et al. NCCN Guidelines: colon cancer. [2], 141-152. 27-1-2014. 2-7-2014.
20. Nielsen LB, Wille-Jorgensen P. National and international guidelines for rectal cancer. Colorectal Dis 2014 May 28.
21. Caudle KE, Thorn CF, Klein TE, Swen JJ, McLeod HL, Diasio RB, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clin Pharmacol Ther 2013 Dec;94(6):640-5.
22. Swen JJ, Nijenhuis M, de BA, Grandia L, Maitland-van der Zee AH, Mulder H, et al. Pharmacogenetics: from bench to byte--an update of guidelines. Clin Pharmacol Ther 2011 May;89(5):662-73.
23. Kweekel DM, Gelderblom H, van der Straaten T, Antonini NF, Punt CJ, Guchelaar HJ. UGT1A1*28 genotype and irinotecan dosage in patients with metastatic colorectal cancer: a Dutch Colorectal Cancer Group study. Br J Cancer 2008 Jul 22;99(2):275-82.
24. Innocenti F, Schilsky RL, Ramirez J, Janisch L, Undevia S, House LK, et al. Dose-Finding and

Pharmacokinetic Study to Optimize the Dosing of Irinotecan According to the UGT1A1 Genotype of Patients With Cancer. J Clin Oncol 2014 Jun 23.
25. Hayashi K, Masuda S, Kimura H. Impact of biomarker usage on oncology drug development. J Clin Pharm Ther 2013 Feb;38(1):62-7.
26. Saltz L, Badarinath S, Dakhil S, Bienvenu B, Harker WG, Birchfield G, et al. Phase III trial of cetuximab, bevacizumab, and 5-fluorouracil/leucovorin vs. FOLFOX-bevacizumab in colorectal cancer. Clin Colorectal Cancer 2012 Jun;11(2):101-11.
27. Strausberg RL, Feingold EA, Grouse LH, Derge JG, Klausner RD, Collins FS, et al. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. Proc Natl Acad Sci U S A 2002 Dec 24;99(26):16899-903.
28. Carninci P. RNA dust: where are the genes? DNA Res 2010 Apr;17(2):51-9.
29. Ong FS, Lin JC, Das K, Grosu DS, Fan JB. Translational utility of next-generation sequencing. Genomics 2013 Sep;102(3):137-9.
30. Sorich MJ, Wiese MD, Pekarsky B. Cost-effectiveness of genotyping to guide treatment. Pharmacogenomics 2014 Apr;15(6):727-9.
31. Wetterstrand KA. DNA Sequencing Costs: Data from the NHGRI Genome Sequencing Program (GSP). 2014. 9-7-2014.
32. O'Donnell PH, Danahey K, Jacobs M, Wadhwa NR, Yuen S, Bush A, et al. Adoption of a clinical pharmacogenomics implementation program during outpatient care--initial results of the University of Chicago "1,200 Patients Project". Am J Med Genet C Semin Med Genet 2014 Mar;166C(1):68-75.
33. Flowers CR, Veenstra D. The role of cost-effectiveness analysis in the era of pharmacogenomics. Pharmacoeconomics 2004;22(8):481-93.
34. Vesell ES, Page JG. Genetic control of dicumarol levels in man. J Clin Invest 1968 Dec;47(12):2657-63.
35. Vesell ES, Page JG. Genetic control of drug levels in man: antipyrine. Science 1968 Jul 5;161(3836):72-3.
36. Vesell ES, Page JG. Genetic control of drug levels in man: phenylbutazone. Science 1968 Mar 29;159(3822):1479-80.
37. Watters JW, Kraja A, Meucci MA, Province MA, McLeod HL. Genome-wide discovery of loci influencing chemotherapy cytotoxicity. Proc Natl Acad Sci U S A 2004 Aug 10;101(32):11809-14.
38. van Kuilenburg AB, Meinsma R, Zoetekouw L, Van Gennip AH. Increased risk of grade IV neutropenia after administration of 5-fluorouracil due to a dihydropyrimidine dehydrogenase deficiency: high prevalence of the IVS14+1g>a mutation. Int J Cancer 2002 Sep 20;101(3):253-8.
39. Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, et al. An integrated map of genetic variation from 1,092 human genomes. Nature 2012 Nov 1;491(7422):56-65.
40. van Kuilenburg AB, Muller EW, Haasjes J, Meinsma R, Zoetekouw L, Waterham HR, et al. Lethal outcome of a patient with a complete dihydropyrimidine dehydrogenase (DPD) deficiency after administration of 5-fluorouracil: frequency of the common IVS14+1G>A mutation causing DPD deficiency. Clin Cancer Res 2001 May;7(5):1149-53.
41. Cho HJ, Park YS, Kang WK, Kim JW, Lee SY. Thymidylate synthase (TYMS) and dihydropyrimidine dehydrogenase (DPYD) polymorphisms in the Korean population for prediction of 5-fluorouracil-associated toxicity. Ther Drug Monit 2007 Apr;29(2):190-6.
42. Stanek EJ, Sanders CL, Taber KA, Khalid M, Patel A, Verbrugge RR, et al. Adoption of pharmacogenomic testing by US physicians: results of a nationwide survey. Clin Pharmacol Ther 2012 Mar;91(3):450-8.
43. Thorn CF, Klein TE, Altman RB. PharmGKB: the Pharmacogenomics Knowledge Base. Methods Mol Biol 2013;1015:311-20.
44. Swen JJ, Wilting I, de Goede AL, Grandia L, Mulder H, Touw DJ, et al. Pharmacogenetics: from bench to byte. Clin Pharmacol Ther 2008 May;83(5):781-7.