# Pharmacogenetic analyses of 2,183 patients with advanced colorectal cancer; Potential role for common dihydropyrimidine dehydrogenase variants in toxicity to chemotherapy.

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**ABSTRACT**

**Background**

Inherited genetic variants may influence response to, and side effects from, chemotherapy. We sought to generate a comprehensive inherited pharmacogenetic profile for oxaliplatin and 5FU/capecitabine therapy in advanced colorectal cancer (aCRC).

**Methods**

We analysed over 200 potentially functional, common, inherited variants in genes within the 5-FU, capecitabine, oxaliplatin, and DNA repair pathways, together with 4 rare dihydropyrimidine dehydrogenase (*DPYD*) variants, in 2183 aCRC patients treated with oxaliplatin-fluoropyrimidine chemotherapy with, or without, cetuximab (from MRC COIN and COIN-B trials). Primary endpoints were response, any toxicity and peripheral neuropathy. We had >85% power to detect ORs=1.3 for variants with minor allele frequencies >20%.

**Results**

Variants in DNA repair genes (Asn279Ser in *EXO1* and Arg399Gln in *XRCC1*) were most associated with response (OR 1.9, 95% CI 1.2-2.9, *P*=0.004, and OR 0.7, 95% CI 0.5-0.9, *P*=0.003, respectively). Common variants in *DPYD* (Cys29Arg and Val732Ile) were most associated with toxicity (OR 0.8, 95% CI 0.7-1.0, *P*=0.008, and OR 1.6, 95% CI 1.1-2.1, *P*=0.006, respectively). Two rare *DPYD* variants were associated with increased toxicity (Asp949Val with neutropenia, nausea and vomiting, diarrhoea and infection; IVS14+1G>A with lethargy, diarrhoea, stomatitis, Hand-Foot Syndrome and infection; all ORs>3). Asp317His in *DCLRE1A* was most

associated with peripheral neuropathy (OR 1.3, 95% CI 1.1-1.6, *P*=0.003). No common variant associations remained significant after Bonferroni correction.

**Conclusions**

DNA repair genes may play a significant role in the pharmacogenetics of aCRC. Our data suggest that both common and rare *DPYD* variants may be associated with toxicity to fluoropyrimidine-based chemotherapy.

## INTRODUCTION

Genetic factors affect response to, and side effects from, chemotherapy and biological therapies used in the treatment of advanced colorectal cancer (aCRC). For example, somatic mutations in *KRAS* and *NRAS* in the epidermal growth factor receptor (EGFR) signalling pathway predict a lack of response to anti-EGFR mononclonal antibodies [1,2]. Germline changes in drug metabolism, transport and target genes have also been implicated in altering response [3,4]. Although several large studies have attempted to identify inherited predictive biomarkers, including the analysis of ten variants in 1188 CRC patients [5,6], 1456 5-FU pathway tagging variants in 968 patients [7], and 34 variants in 520 patients [8], none have comprehensively analysed all of the pharmacological pathways. Indeed, the vast majority of studies performed to-date have used small cohorts of patients and most findings have not been validated in independent analyses.

We have previously sought predictive biomarkers for cetuximab response and side effects by analysing 54 common, inherited EGFR pathway variants in 815 aCRC patients from the COIN [9,10] and COIN-B [11] trials that received cetuximab together with oxaliplatin-fluoropyrimidine chemotherapy [12]. Although we identified five potential biomarkers for response and four for skin rash, none remained significant after correction for multiple testing [12]. Here, we sought predictive biomarkers for oxaliplatin-fluoropyrimidine chemotherapy by analysing over 200 potentially functional common inherited variants in 2183 COIN and COIN-B patients treated with oxaliplatin-fluoropyrimidine chemotherapy with, or without, cetuximab.

## METHODS

**Patients and treatments**

All patients had metastatic or locally advanced colorectal adenocarcinoma and received no previous chemotherapy for advanced disease. All patients gave fully informed consent for this study (approved by REC [04/MRE06/60]). COIN patients were randomised 1:1:1 to receive continuous oxaliplatin and fluoropyrimidine chemotherapy (Arm A), continuous chemotherapy +cetuximab (Arm B), or intermittent chemotherapy (Arm C) (ISRCTN27286448) [9,10]. COIN-B patients were randomised 1:1 to receive intermittent chemotherapy and cetuximab (Arm D) or intermittent chemotherapy and continuous cetuximab (Arm E) (ISRCTN3837568) [11]. For the first 12-weeks, at which point the primary pharmacogenetic analyses were carried out, treatments were identical in all patients apart from the choice of fluoropyrimidine (n=834, 38% received infusional 5FU with oxaliplatin [OxMdG] and n=1349, 62% received capecitabine with oxaliplatin [Xelox]) together with the randomisation of ±cetuximab (n=815, 37% received cetuximab) (Supplementary Table 1).

**Selection of potential pharmacogenetic variants**

Potentially functional variants were sought in 62 genes identified from literature reviews as likely to play a role in the metabolic pathways associated with the agents used in COIN and COIN-B - 5FU and capecitabine (28 genes) and oxaliplatin (34 genes). Variants were considered potentially functional if there was previously reported clinical or biological evidence for an effect on response or side effects, if they were nonsynonymous, or if they occurred in the promoter region. We also sought similar variants in 155 DNA repair genes that were likely to play a role in repairing the damage caused by these agents. Variants were mined from dbSNP

(v.129, http://www.ncbi.nlm.nih.gov/SNP/) and from exome re-sequencing germline data [13], and those with a minor allele frequency (MAF) >5% (Caucasian population) were considered for genotyping.

**Genotyping**

Most variants were single nucleotide polymorphisms (SNPs) genotyped using a custom Illumina GoldenGate assay. The 'Assay Design Tool' software (Illumina) was used to anticipate genotyping success. This was based on the designability rank and validation class for a given SNP. When two or more SNPs occurred within 60bp of one another, the SNP selected for submission was chosen based on its designability score, MAF and likelihood of being functional using *in silico* analyses (PolyPhen, http://genetics.bwh.harvard.edu/pph2/ or align-GVGD, http://agvgd.iarc.fr/).

Eight variants were assayed 'in house' because they were not suitable for (n=7), or failed (n=1), GoldenGate genotyping. The c.1-99 28bp repeat in the *TYMS* promoter (rs34743033) and the c.939+450 6bp deletion in the *TYMS* 3' untranslated region (UTR) (rs34489327) were assayed using the primers 5'-GGGTTTCCTAAGACTCTCAG-3' and 5'- CCGAGCCGGCCACAGGCATA-3', and 5'-CATCCAAACCAGAATACAGCAC-3' and 5'-CTTTGAGTTAACTCACTGAGGG-3', respectively, and the c.1-1671 insertion A in the *MMP3* promoter (rs35068180) was assayed using the primers 5'-AGCTGCCACAGCTTCTACAC-3' and 5'-GTATTCTATGGTTCTCCATTC-3'. One of the primers for each pair was fluorescently labelled and PCR products were analysed on an ABI3100 using the GeneScan Analysis Software (ABI). The *GSTT1* and *GSTM1* copy number deletions (Accession numbers CG962889 and CN973733, respectively [HGMD,

www.hgmd.cf.ac.uk]) and the variants Phe212Val in *FCGR3A* (rs396991) and His46 (synonymous) in *ERCC5* (rs1047768) were assayed using Taqman real time quantitative PCR assays (ABI). The G>C variant in the 28bp repeat within the *TYMS* promoter (rs2853542) was assayed by direct sequencing without success.

We assayed for four rare *DPYD* variants (IVS14+1G>A [rs3918290], Asp949Val [rs67376798], Lys259Glu [rs45589337] and Ser534Asn [rs1801158]) using KASPar assays (LGC).

**Clinical parameters assessed**

The primary efficacy endpoint was 12-week response, defined as complete response or partial response versus stable disease or progressive disease at 12-weeks, and secondary efficacy endpoints were overall survival (OS) and overall response rate (ORR). The primary endpoints for toxicity were: (i) a dose reduction or delay in chemotherapy in the first 12-weeks of treatment due to any toxicity except peripheral neuropathy (PN), and, (ii) grade ≥2 PN or dose reduction or delay due to PN *versus* grade <2 PN despite no oxaliplatin dose modification in the first 12-weeks. Secondary toxicity endpoints were grade ≥2 at any point versus grade <2 for neutropenia, lethargy, nausea or vomiting, diarrhoea, stomatitis, Hand-Foot Syndrome (HFS), infection (infection with grade ≥3 neutropenia *versus* infection with normal absolute neutrophil count or no infection) and PN (COIN Arms A and B) at 24-weeks.

**Power considerations**

Patients from all arms of COIN and COIN-B had similar efficacy and toxicity outcomes at 12-weeks [9-11], so were combined to increase power (n=2183). Based on 2183 patients, we had >85% power ($P$<0.05) to detect an OR of 1.3, corresponding to a 7% difference in response or toxicity (45% responded and 35% had toxicity) [9-11], for a variant with a MAF>20%, and an OR of 1.6, corresponding to an 11% difference in response, for a variant with a MAF>5%.

**Statistical analyses**

Genotypes were tested for deviation from the Hardy Weinberg Equilibrium (HWE) using a chi-squared test and those with $P<2.5\times10^{-4}$ (multiple testing for 202 common variants) were excluded. Pharmacogenetic analyses were carried out using Stata 12.1 with a co-dominant model, and tested using the likelihood-ratio chi-squared statistic. For significant associations ($P$<0.05), subsequent analyses were carried out using logistic regression under the best-fitting allele model, adjusted for cetuximab use and type of fluoropyrimidine. Correction for multiple testing was by Bonferroni. Our methods and reporting conform to REMARK criteria [14].

**RESULTS**

Two hundred and thirty-six potentially functional, common, coding and promoter-region variants were identified in either 39 genes likely to play a role in the metabolic pathways associated with the chemotherapeutic agents used in COIN and COIN-B, or, in 97 genes that were likely to play a role in repairing the damage caused by these agents (Fig.1). Of these, 226 passed *in silico* locus conversion on the GoldenGate platform and 195 were successfully assayed. Eight variants were assayed 'in house' of which 7 were successfully genotyped. Only genotypes for the

c.1-99 28bp repeat in *TYMS* deviated from the HWE and were excluded. Therefore, in total, 201 common variants were considered in the pharmacogenetic analyses of 2183 unrelated patients with aCRC from the UK national trials COIN (2070 of the 2445 randomised) and COIN-B (113 of the 226 randomised) in which all patients received oxaliplatin and fluoropyrimidine chemotherapy with, or without, cetuximab (Supplementary Table 2).

**Primary analyses**

Eight variants were associated ($P$<0.05) with response, eight with toxicity and five with PN, prior to correction for multiple testing (Table 1, Supplementary Table 3); none were significant after Bonferroni correction.

For response, the most significant associations were with variants in DNA repair genes. Seventy-one percent of patients with at least one allele encoding serine at residue 279 in *EXO1* responded, as compared to 57% of patients homozygous for alleles encoding asparagine (OR 1.9, 95% CI 1.2-2.9, $P$=0.004). Also, 48% of patients homozygous for alleles encoding glutamine at residue 399 in *XRCC1* responded, as compared to 59% of patients with at least one allele encoding arginine (OR 0.7, 95% CI 0.5-0.9, $P$=0.003).

For toxicity, the most significant associations were Cys29Arg and Val732Ile in *DPYD*. These variants were in low linkage disequilibrium (LD) ($r^2$=0.0, D'=0.5) suggesting that they may represent independent associations. Arginine at residue 29 reduced toxicity, particularly diarrhoea and stomatitis (34% of patients with at least one allele encoding arginine had severe toxicity as compared to 38% of patients

homozygous for alleles encoding cysteine, OR 0.8, 95% CI 0.7-1.0, *P*=0.008). Although reduced toxicity was observed with both fluoropyrimidine regimens, it was only statistically significant with Xelox (OR 0.4, 95% CI 0.2-0.8, *P*=0.004) (Supplementary Table 4).

Forty-five percent of patients with at least one allele encoding isoleucine at residue 732 had severe toxicity as compared to 36% of patients homozygous for alleles encoding valine (OR 1.6, 95% CI 1.1-2.1, *P*=0.006). Increased toxicity was observed with both fluoropyrimidine regimens, but was statistically significant with OxMdG (OR 2.0, 95% CI 1.1-3.5, *P*=0.014) (Supplementary Table 4). The association was primarily caused by neutropenia (20% *versus* 14% of patients, OR 1.9, 95% CI 1.2-3.1, *P*=0.005) (Table 2).

For PN, the most significant association was with Asp317His in *DCLRE1A.* Twenty-one percent of patients homozygous for alleles encoding histidine had PN, in comparison to 17% of those with a single allele encoding histidine and 13% of those homozygous for alleles encoding aspartic acid (OR 1.3, 95% CI 1.1-1.6, *P*=0.003) (Table 2).

**Extended profiling of *DPYD***

Since two common *DPYD* variants influenced toxicity and previous observations that rare *DPYD* variants also affect toxicity [15], we assayed an extended panel of rare (MAFs <5%) nonsynonymous and splicing *DPYD* variants in all patients using KASPar.

Asp949Val, in 1.4% of patients (30/2116), was associated with increased toxicity (OR 2.2, 95% CI 1.1-4.5, *P*=0.038), specifically neutropenia (OR 3.2, 95% CI 1.2-8.2, *P*=0.019), nausea and vomiting (OR 3.4, 95% CI 1.5-7.3, *P*=0.002), diarrhoea (OR 4.6, 95% CI 2.1-10.1, *P*<0.001) and infection (OR 5.5, 95% CI 1.3-24.2, *P*=0.024) (Table 2). We found significantly increased infection with Xelox (OR 31.9, 95% CI 5.7-178) as compared to OxMdG (OR 1.2, 95% CI 0.1-13.0, $P_{interaction}$=0.026; Supplementary Table 4).

IVS14+1G>A, in 1.1% of patients (23/2105), was associated with increased lethargy (OR 5.3, 95% CI 1.9-14.9, *P*=0.002), diarrhoea (OR 4.4, 95% CI 1.7-11.0, *P*=0.002), stomatitis (OR 4.6, 95% CI 1.7-12.6, *P*=0.003), HFS (OR 3.8, 95% CI 1.2-11.8, *P*=0.021) and infection (OR 19.2, 95% CI 5.0-73.8, *P*<0.001) (Table 2). These were consistent across fluoropyrimidine regimens (Supplementary Table 4).

**Secondary analyses**

Thirteen variants were associated with ORR (n=7) or OS (n=6) (Supplementary Table 5). In addition, 11 variants were associated with lethargy, 17 with nausea/vomiting, 13 with diarrhoea, 3 with stomatitis, 11 with HFS, 8 with infection and 8 with PN at 24-weeks (Supplementary Table 6). Upon rigorous correction for multiple testing, none of these associations remained statistically significant.

## DISCUSSION

Fluoropyrimidines have several mechanisms of cytotoxicity including disruption of the dioxynucleotide pools from thymidylate synthase inhibition and the direct incorporation of fluoropyrimidines into DNA [16]. Platinums cause bulky adducts to

be introduced into DNA. The consequences of these agents are the mutagenic effects of base analogues or mispairs in DNA, the inhibition of replication and the fragmentation of DNA created in the cell's attempts to repair these lesions. The base excision repair (BER), nucleotide excision repair, mismatch repair (MMR) and double strand break repair systems have all been suggested to modify response [16]. In our study, the most significant associations for response to therapy were with variants in DNA repair genes. EXO1 has exonuclease activity and plays a role in MMR and homologous recombination, and XRCC1 is involved in the repair of single-strand breaks following BER. Interestingly, others have also shown a predictive role for Arg399Gln in *XRCC1* in response to oxaliplatin/5-FU treatment for aCRC [17] and in platinum based therapy of oesophageal cancers [18]. We also found that Asp317His in *DCLRE1A* was associated with PN, an oxaliplatin-associated toxicity of chronic peripheral nerve damage causing sensory ataxia and functional impairment [19]. *DCLRE1A* is involved in the repair of interstrand cross-links [20]. Together, these data support a key role for DNA repair in the pharmacogenetics of cancer therapy.

Given that our study was an exploratory analysis, we provided uncorrected *P*-values; however, we also adjusted these for multiple testing by Bonferroni. Although no associations with common variants remained statistically significant after correction, it is noteworthy that the two common variants most significantly associated with toxicity, were both in *DPYD*. *DPYD* encodes DPD, the key enzyme for the catabolism of 5-FU, and reduced DPD activity is thought to cause severe 5-FU induced toxicities. Previous studies have clearly shown that two rare *DPYD* variants are associated with severe toxicity in patients receiving 5-FU [15,21,22] and our data support these observations. Interestingly, we noted that Asp949Val was associated

with increased infection with Xelox as compared to OxMdG. This difference warrants further investigation and may potentially relate to variants within the folinic acid metabolism pathway not studied *herein*.

As yet, there is no consensus on the role of common *DPYD* variants in contributing to toxicity to therapy, but our data provide supportive evidence for their role. Cys29Arg (MAF=21%) has previously been associated with reduced toxicity (OR 0.5 for gastrointestinal toxicity, 95% CI 0.2-1.0 [23], and, *P*=0.041 [24]) and our data support a protective role for this variant (OR 0.8, 95% CI 0.7-1.0). Interestingly, this variant shows significantly higher enzymatic activity as compared to wild type DPD when expressed in mammalian cells [25], supporting a model in which hyperactive forms of DPD reduce mean circulating levels of 5-FU by increased drug catabolism [25].

Val732Ile (MAF=4%) has previously been associated with increased fluorouracil-related adverse events (OR 1.7, 95% CI 1.3-2.4) including hematologic adverse events (OR 1.9, 95% CI 1.4-2.6), and neutropenia (OR 1.8, 95% CI 1.3-2.4) in CRC patients who received standard adjuvant FOLFOX4 or FOLFOX4 in combination with cetuximab, and these findings were validated in aCRC patients receiving FOLFOX4 [26]. Furthermore, others have associated Val732Ile with leucopenia (OR 8.2, 95% CI 2.4-27.3) and neutropenia (OR 2.8, 95% CI 1.0-7.5) [23]. Our data also support this variant having an association with toxicity.

In addition to these common coding region variants, a recent study has shown that common tagging variants outside of the *DPYD* coding sequence also affect capecitabine toxicity [7].

## CONCLUSIONS

It is now standard practice in many European cancer centres to test for a small number of rare genetic variants in *DPYD* before starting patients on 5FU or capecitabine [27,28]. Upfront genotyping, and dose adjustment, has been shown to be feasible and cost effective by reducing the financial burden of managing preventable toxicities [29]. Whilst this strategy is specific, it is far from being sensitive for predicting excessive toxicities [30]. Partial DPD deficiency which is not picked up by current genetic testing may be caused by the presence of other, more common, genetic variants. Our study provides supportive evidence for two such variants in a very large cohort of patients and adds weight to the body of published data suggesting the genetic profiling of both common and rare *DPYD* variants could now be used to guide accurate dosing of 5FU and capecitabine. This would require validation in a prospective trial and might need to be combined with tests assessing DPD function pre-therapeutically [31], or 5FU pharmacokinetics post-therapeutically [32].

## APPENDICIES

**Appendix A.** Supplementary data

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## AUTHOR CONTRIBUTIONS

JPCheadle and TSM obtained funding for this study. The study was designed by JPCheadle, AM, TSM, DF and RSK, and was carried out under the direction of JPCheadle. AM carried out the literature searches and, with JPColley, identified the variants for genotyping. TSM was CI of COIN, HW was CI of COIN-B and, RAA and AM were COIN trial fellows; all provided clinical advice and assistance, and supported the translational research. AMM and RSK managed the COIN and COIN-B trials and facilitated access to the clinical data. DF undertook all of the statistical analyses. AM and JPCheadle interpreted the data with input from DF, RAA and TSM. SI extracted the blood DNA samples and, with RH, prepared them for genotyping at Illumina. VH and JM undertook the in-house genotyping under the direction of JPColley. JPCheadle and AM wrote the paper with input from DF, and all authors provided comments.

## CONFLICTS OF INTEREST

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## REFERENCES

[1] De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, Kalogeras KT, Kotoula V, Papamichael D, Laurent-Puig P, Penault-Llorca F, Rougier P, Vincenzi B, Santini D, Tonini G, Cappuzzo F, Frattini M, Molinari F, Saletti P, De Dosso S, Martini M, Bardelli A, Siena S, Sartore-Bianchi A, Tabernero J, Macarulla T, Di Fiore F, Gangloff AO, Ciardiello F, Pfeiffer P, Qvortrup C, Hansen TP, Van Cutsem E, Piessevaux H, Lambrechts D, Delorenzi M, Tejpar S. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 2010;**11**:753-62.

[2] Douillard JY, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Williams R, Rong A, Wiezorek J, Sidhu R, Patterson SD. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med* 2013;**369**:1023-34.

[3] Marcuello E, Altés A, del Rio E, César A, Menoyo A, Baiget M. Single nucleotide polymorphism in the 5' tandem repeat sequences of thymidylate synthase gene predicts for response to fluorouracil-based chemotherapy in advanced colorectal cancer patients. *Int J Cancer* 2004;**112**:733-37.

[4] Braun MS, Quirke P, Seymour MT. Molecular markers of chemotherapeutic response and toxicity in colorectal cancer. *Expert Rev Anticancer Ther* 2007;**7**:489-501.

[5] Braun MS, Richman SD, Quirke P, Daly C, Adlard JW, Elliott F, Barrett JH, Selby P, Meade AM, Stephens RJ, Parmar MK, Seymour MT. Predictive biomarkers

of chemotherapy efficacy in colorectal cancer: results from the UK MRC FOCUS trial. *J Clin Oncol* 2008;**26**:2690-8.

[6] Braun MS, Richman SD, Thompson L, Daly CL, Meade AM, Adlard JW, Allan JM, Parmar MK, Quirke P, Seymour MT. Association of molecular markers with toxicity outcomes in a randomized trial of chemotherapy for advanced colorectal cancer: the FOCUS trial. *J Clin Oncol* 2009;**27**:5519-28.

[7] Rosmarin D, Palles C, Pagnamenta A, Kaur K, Pita G, Martin M, Domingo E, Jones A, Howarth K, Freeman-Mills L, Johnstone E, Wang H, Love S, Scudder C, Julier P, Fernández-Rozadilla C, Ruiz-Ponte C, Carracedo A, Castellvi-Bel S, Castells A, Gonzalez-Neira A, Taylor J, Kerr R, Kerr D, Tomlinson I. A candidate gene study of capecitabine-related toxicity in colorectal cancer identifies new toxicity variants at DPYD and a putative role for ENOSF1 rather than TYMS. *Gut* 2015;**64**:111-20.

[8] McLeod HL, Sargent DJ, Marsh S, Green EM, King CR, Fuchs CS, Ramanathan RK, Williamson SK, Findlay BP, Thibodeau SN, Grothey A, Morton RF, Goldberg RM. Pharmacogenetic predictors of adverse events and response to chemotherapy in metastatic colorectal cancer: results from North American Gastrointestinal Intergroup Trial N9741. *J Clin Oncol* 2010;**28**:3227-33.

[9] Maughan TS, Adams RA, Smith CG, Meade AM, Seymour MT, Wilson RH, Idziaszczyk S, Harris R, Fisher D, Kenny SL, Kay E, Mitchell JK, Madi A, Jasani B, James MD, Bridgewater J, Kennedy MJ, Claes B, Lambrechts D, Kaplan R, Cheadle JP; MRC COIN Trial Investigators. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet* 2011;**377**:2103-14.

[10] Adams RA, Meade AM, Seymour MT, Wilson RH, Madi A, Fisher D, Kenny SL, Kay E, Hodgkinson E, Pope M, Rogers P, Wasan H, Falk S, Gollins S, Hickish T, Bessell EM, Propper D, Kennedy MJ, Kaplan R, Maughan TS; MRC COIN Trial Investigators. Intermittent versus continuous oxaliplatin and fluoropyrimidine combination chemotherapy for first-line treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet Oncol* 2011;**12**:642-53.

[11] Wasan H, Meade AM, Adams R, Wilson R, Pugh C, Fisher D, Sydes B, Madi A, Sizer B, Lowdell C, Middleton G, Butler R, Kaplan R, Maughan T; COIN-B investigators. Intermittent chemotherapy plus either intermittent or continuous cetuximab for first-line treatment of patients with KRAS wild-type advanced colorectal cancer (COIN-B): a randomised phase 2 trial. *Lancet Oncol* 2014;**15**:631-9.

[12] Madi A, Fisher D, Maughan TS, Colley JP, Meade AM, Tejpar S, Van den Bosch B, Maynard J, Humphreys V, Wasan H, Adams RA, Idziaszczyk S, Harris R, Kaplan RS, Cheadle JP. Comprehensive pharmacogenetic profiling of the epidermal growth factor receptor pathway for biomarkers of response to, and toxicity from, cetuximab. *J Med Genet* 2017;**54**:567-71.

[13] Sjöblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N, Szabo S, Buckhaults P, Farrell C, Meeh P, Markowitz SD, Willis J, Dawson D, Willson JK, Gazdar AF, Hartigan J, Wu L, Liu C, Parmigiani G, Park BH, Bachman KE, Papadopoulos N, Vogelstein B, Kinzler KW, Velculescu VE. The consensus coding sequences of human breast and colorectal cancers. *Science* 2006;**314**:268-74.

[14] McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM; Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics. REporting recommendations for tumour MARKer prognostic studies (REMARK). *Br J Cancer* 2005;**93**:387-91.

[15] Schwab M, Zanger UM, Marx C, Schaeffeler E, Klein K, Dippon J, Kerb R, Blievernicht J, Fischer J, Hofmann U, Bokemeyer C, Eichelbaum M; German 5-FU Toxicity Study Group. Role of genetic and nongenetic factors for fluorouracil treatment-related severe toxicity: a prospective clinical trial by the German 5-FU Toxicity Study Group. *J Clin Oncol* 2008;**26**:2131-8.

[16] Meyers M, Hwang A, Wagner MW, Bruening AJ, Veigl ML, Sedwick WD, Boothman DA. A role for DNA mismatch repair in sensing and responding to fluoropyrimidine damage. *Oncogene* 2003;**22**:7376-88.

[17] Stoehlmacher J, Ghaderi V, Iobal S, Groshen S, Tsao-Wei D, Park D, Lenz HJ. A polymorphism of the XRCC1 gene predicts for response to platinum based treatment in advanced colorectal cancer. *Anticancer Res* 2001;**21**:3075-9.

[18] Findlay JM, Middleton MR, Tomlinson I. A systematic review and meta-analysis of somatic and germline DNA sequence biomarkers of esophageal cancer survival, therapy response and stage. *Ann Oncol* 2015;**26**:624-44.

[19] Quasthoff S, Hartung HP. Chemotherapy-induced peripheral neuropathy. *J Neurol* 2002;**249**:9-17.

[20] Dronkert ML, de Wit J, Boeve M, Vasconcelos ML, van Steeg H, Tan TL, Hoeijmakers JH, Kanaar R. Disruption of mouse SNM1 causes increased sensitivity to the DNA interstrand cross-linking agent mitomycin C. *Molec Cell Biol* 2000;**20**:4553-61.

[21] Morel A, Boisdron-Celle M, Fey L, Soulie P, Craipeau MC, Traore S, Gamelin E. Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance. *Mol Cancer Ther* 2006;**5**:2895-904.

[22] Lee AM, Shi Q, Pavey E, Alberts SR, Sargent DJ, Sinicrope FA, Berenberg JL, Goldberg RM, Diasio RB. DPYD variants as predictors of 5-fluorouracil toxicity in adjuvant colon cancer treatment (NCCTG N0147). *J Natl Cancer Inst* 2014;**106**:pii: dju298.

[23] Kleibl Z, Fidlerova J, Kleiblova P, Kormunda S, Bilek M, Bouskova K, Sevcik J, Novotny J. Influence of dihydropyrimidine dehydrogenase gene (DPYD) coding sequence variants on the development of fluoropyrimidine-related toxicity in patients with high-grade toxicity and patients with excellent tolerance of fluoropyrimidine-based chemotherapy. *Neoplasma* 2009;**56**:303-16.

[24] Etienne-Grimaldi MC, Boyer JC, Beroud C, Mbatchi L, van Kuilenburg A, Bobin-Dubigeon C, Thomas F, Chatelut E, Merlin JL, Pinguet F, Ferrand C, Meijer J, Evrard A, Llorca L, Romieu G, Follana P, Bachelot T, Chaigneau L, Pivot X, Dieras V, Largillier R, Mousseau M, Goncalves A, Roché H, Bonneterre J, Servent V, Dohollou N, Château Y, Chamorey E, Desvignes JP, Salgado D, Ferrero JM, Milano G. New advances in DPYD genotype and risk of severe toxicity under capecitabine. *PLoS One* 2017;**12**:e0175998.

[25] Offer SM, Wegner NJ, Fossum C, Wang K, Diasio RB. Phenotypic profiling of DPYD variations relevant to 5-fluorouracil sensitivity using real-time cellular analysis and in vitro measurement of enzyme activity. *Cancer Res* 2013;**73**:1958-68.

[26] Boige V, Vincent M, Alexandre P, Tejpar S, Landolfi S, Le Malicot K, Greil R, Cuyle PJ, Yilmaz M, Faroux R, Matzdorff A, Salazar R, Lepage C, Taieb J, Laurent-Puig P. DPYD Genotyping to Predict Adverse Events Following Treatment With Fluorouracil-Based Adjuvant Chemotherapy in Patients With Stage III Colon Cancer: A Secondary Analysis of the PETACC-8 Randomized Clinical Trial. *JAMA Oncol* 2016;doi:10.1001/jamaoncol.2015.5392.

[27] Henricks LM, Kienhuis E, de Man FM, van der Veldt AAM, Hamberg P, van Kuilenburg ABP. Treatment algorithm for homozygous or compound heterozygous DPYD variant allele carriers with low-dose capecitabine. *JCO Precision Oncology* 2017; DOI:10.1200/PO.17.00118

[28] Boisdron-Celle M, Capitain O, Faroux R, Borg C, Metges JP, Galais MP, Kaassis M, Bennouna J, Bouhier-Leporrier K, Francois E, Baumgaertner I, Guerin-Meyer V, Cojocarasu O, Roemer-Becuwe C, Stampfli C, Rosenfeld L, Lecompte T, Berger V, Morel A, Gamelin E. Prevention of 5-fluorouracil-induced early severe toxicity by pre therapeutic dihydropyrimidine dehydrogenase deficiency screening: Assessment of a multiparametric approach. *Seminars in Oncology* 2017;**44**:13–23.

[29] Deenen MJ, Meulendijks D, Cats A, Sechterberger MK, Severens JL, Boot H, Smits PH, Rosing H, Mandigers CM, Soesan M, Beijnen JH, Schellens JH. Upfront Genotyping of DPYD*2A to Individualize Fluoropyrimidine Therapy: A Safety and Cost Analysis. *J Clin Oncol* 2016;**34**:227-34.

[30] Boisdron-Celle M, Remaud G, Traore S, Poirier AL, Gamelin L, Morel A, Gamelin E. 5-Fluorouracil-related severe toxicity: A comparison of different methods

for the pretherapeutic detection of dihydropyrimidine dehydrogenase deficiency. *Cancer Letters* 2007**;249**:271–82.

[31] Remaud G, Boisdron-Celle M, Hameline C, Morel A, Gamelin E. An accurate dihydrouracil/uracil determination using improved high performance liquid chromatography method for preventing fluoropyrimidines-related toxicity in clinical practice. *J Chromatogr B Analyt Technol Biomed Life Sci* 2005;**823**:98-107.

[32] Gamelin E, Delva R, Jacob J, Merrouche Y, Raoul JL, Pezet D, Dorval E, Piot G, Morel A, Boisdron-Celle M. Individual fluorouracil dose adjustment based on pharmacokinetic follow-up compared with conventional dosage: results of a multicenter randomized trial of patients with metastatic colorectal cancer. *J Clin Oncol* 2008;**26**:2099-105.

**Table 1 - Variants with *P*<0.05 for the primary endpoints**

| Endpoint | rs no. | Gene | Variant | Endpoint +/- | AA | AB | BB | $X^2$ (df), *P*-value[a] | OR (95% CI), *P*-value[b] |
|---|---|---|---|---|---|---|---|---|---|
| 12-week response | rs4149909 | *EXO1* | Asn279Ser | + | 983 | 75 | 0 | 8.7 (1), 0.003 | 1.9 (1.2-2.9), 0.004 (d) |
| | | | | - | 758 | 31 | 0 | | |
| | rs25487 | *XRCC1* | Arg399Gln | + | 119 | 490 | 450 | 9.6 (2), 0.008 | 0.7 (0.5-0.9), 0.003 (r) |
| | | | | - | 127 | 357 | 304 | | |
| | rs144848 | *BRCA2* | Asn372His | + | 572 | 409 | 78 | 7.2 (2), 0.027 | 0.8 (0.7-1.0), 0.008 (a) |
| | | | | - | 386 | 323 | 80 | | |
| | rs1047768 | *ERCC5* | His46 | + | 368 | 456 | 196 | 7.2 (2), 0.027 | 1.4 (1.1-1.8), 0.008 (r) |
| | | | | - | 282 | 362 | 108 | | |
| | rs17714854 | *EME1* | Phe63Leu | + | 1024 | 34 | 0 | 4.7 (1), 0.029 | 2.0 (1.0-3.8), 0.037 (d) |
| | | | | - | 776 | 13 | 0 | | |
| | rs2273535 | *AURKA* | Phe31Ile | + | 675 | 331 | 51 | 6.5 (2), 0.039 | 0.8 (0.7-1.0), 0.011 (d) |
| | | | | - | 458 | 281 | 47 | | |
| | rs1805388 | *LIG4* | Thr9Ile | + | 23 | 274 | 760 | 6.3 (2), 0.042 | 0.8 (0.6-1.0), 0.014 (d) |
| | | | | - | 24 | 241 | 524 | | |
| | rs2229109 | *ABCB1* | Ser400Asn | + | 0 | 97 | 961 | 3.9 (1), 0.049 | 1.4 (1.0-2.0), 0.052 (d) |
| | | | | - | 0 | 53 | 736 | | |
| Any Toxicity (except PN) | rs1801265 | *DPYD*[c] | Cys29Arg | + | 506 | 252 | 28 | 8.5 (2), 0.015 | 0.8 (0.7-1.0), 0.008 (a) |
| | | | | - | 834 | 465 | 81 | | |
| | rs1801160 | *DPYD*[c] | Val732Ile | + | 2 | 79 | 705 | 7.4 (2), 0.025 | 1.6 (1.1-2.1), 0.006 (d) |
| | | | | - | 2 | 97 | 1282 | | |
| | rs4986850 | *BRCA1*[d] | Asp397Asn | + | 2 | 113 | 671 | 7.3 (2), 0.026 | 0.2 (0.1-1.0), 0.046 (r) |
| | | | | - | 17 | 169 | 1195 | | |
| | rs1979277 | *SHMT1* | Leu474Phe | + | 79 | 380 | 327 | 6.3 (2), 0.043 | 1.2 (1.0-1.4), 0.053 (d) |
| | | | | - | 156 | 591 | 633 | | |
| | rs5745459 | *MSH4* | Tyr589Cys | + | 764 | 22 | 0 | 4.1(1), 0.044 | 1.9 (1.0-3.5), 0.043 (d) |
| | | | | - | 1360 | 21 | 0 | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | rs12022378 | *DCLRE1B* | His61Tyr | +<br>- | 31<br>29 | 229<br>391 | 526<br>961 | 6.2 (2), 0.046 | 1.9 (1.1-3.2), 0.016 (r) |
| | rs1799966 | *BRCA1*[d] | Ser430Gly | +<br>- | 343<br>619 | 374<br>602 | 69<br>160 | 6.1 (2), 0.047 | 0.7 (0.5-1.0), 0.044 (r) |
| | rs1800566 | *NQO1* | Pro187Ser | +<br>- | 27<br>38 | 213<br>449 | 546<br>894 | 6.0 (2), 0.049 | 0.8 (0.7-1.0), 0.042 (d) |
| | rs3750898 | *DCLRE1A* | Asp317His | +<br>- | 27<br>105 | 119<br>603 | 149<br>1016 | 8.6 (2), 0.014 | 1.3 (1.1-1.6), 0.003 (a) |
| | rs1800058 | *ATM* | Leu72Phe | +<br>- | 0<br>0 | 18<br>52 | 277<br>1670 | 5.5 (1), 0.019 | 2.0 (1.1-3.4), 0.015 (d) |
| **PN** | rs3093921 | *PARP2* | Asp222Gly | +<br>- | 277<br>1668 | 18<br>56 | 0<br>0 | 4.9 (1), 0.027 | 1.9 (1.1-3.3), 0.020 (d) |
| | rs13181 | *ERCC2* | Lys751Gln | +<br>- | 107<br>722 | 153<br>752 | 35<br>249 | 6.7 (2), 0.036 | 1.3 (1.0-1.6), 0.082 (d) |
| | rs9352 | *CHAF1A* | Ala923Val | +<br>- | 68<br>526 | 156<br>828 | 71<br>370 | 6.1 (2), 0.048 | 1.4 (1.1-1.9), 0.016 (d) |

Results shown using a co-dominant model[a] and, odds ratios (ORs) and 95% confidence intervals using the best model that fitted the data[b] [models for (d) = dominant, (r) = recessive, and, (a) = additive, alleles]. *P*-values uncorrected for multiple testing; none were significant after Bonferroni correction. For endpoints, + = patients that responded, had any toxicity or PN, - = patients that did not respond or did not have any toxicity or PN. The *DPYD* variants[c] Cys29Arg and Val732Ile were in low LD ($r^2$=0.0, D'=0.5) and therefore may represent independent associations. The *BRCA1* variants[d] Asp397Asn and Ser430Gly variants were in high LD ($r^2$=0.2, D'=1) so likely to be associated with the same signal. The common allele (A/B) encodes the wild type amino acid, so for Asn279Ser the A allele encodes Asn, for Arg399Gln the B allele encodes Arg, for Cys29Arg the A allele encodes Cys and for Val732Ile the B allele encodes Val. PN – Peripheral neuropathy.

## Table 2 – Profiling of *DPYD* and associations with toxicity

| Variant(s) & rs no. | Presence (+) or absence (-) of toxicity | Any 12-week toxicity | | | Specific toxicity | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Neutropenia | | | Lethargy | | | Nausea & vomiting | | | Diarrhoea | | | Stomatitis | | | HFS | | | Infection | | |
| | | AA | AB | BB | AA | AB | BB | AA | AB | BB | AA | AB | BB | AA | AB | BB | AA | AB | BB | AA | AB | BB | AA | AB | BB |
| Cys29Arg rs1801265 | + | 506 | 252 | 28 | 178 | 92 | 12 | 435 | 218 | 31 | 251 | 127 | 19 | 331 | 164 | 20 | 142 | 63 | 9 | 120 | 58 | 8 | 45 | 20 | 2 |
| | - | 834 | 465 | 81 | 1,061 | 576 | 88 | 804 | 450 | 69 | 988 | 541 | 81 | 908 | 504 | 80 | 1,097 | 605 | 91 | 1,119 | 610 | 92 | 1,041 | 575 | 86 |
| | $\chi2$ (d.f.), *P* | 8.5 (2), 0.015 | | | 1.1 (2), 0.57 | | | 1.5 (2), 0.46 | | | 0.3 (2), 0.87 | | | 3.2 (2), 0.20 | | | 3.8 (2), 0.15 | | | 1.0 (2), 0.60 | | | 2.3 (2), 0.32 | | |
| | OR (95% CI), *P* | 0.8 (0.7-1.0), 0.008 | | | - | | | - | | | - | | | - | | | - | | | - | | | - | | |
| Met166Val rs2297595 | + | 655 | 122 | 9 | 239 | 41 | 2 | 567 | 114 | 3 | 330 | 67 | 1 | 426 | 83 | 6 | 175 | 37 | 2 | 148 | 37 | 1 | 54 | 13 | 0 |
| | - | 1,134 | 237 | 9 | 1,422 | 289 | 14 | 1,094 | 216 | 13 | 1,331 | 263 | 15 | 1,235 | 247 | 10 | 1,486 | 293 | 14 | 1,513 | 293 | 15 | 1,398 | 290 | 0 |
| | $\chi2$ (d.f.), *P* | 2.0 (2), 0.36 | | | 1.3 (2), 0.52 | | | 1.3 (2), 0.53 | | | 2.0 (2), 0.37 | | | 1.2 (2), 0.55 | | | 0.0 (2), 0.98 | | | 0.9 (2), 0.64 | | | 0.0 (1), 0.85 | | |
| | OR (95% CI), *P* | - | | | - | | | - | | | - | | | - | | | - | | | - | | | - | | |
| Lys259Glu rs45589337 | + | 753 | 14 | 0 | 256 | 7 | 0 | 646 | 13 | 0 | 382 | 5 | 0 | 484 | 11 | 0 | 196 | 1 | 0 | 178 | 2 | 0 | 60 | 1 | 0 |
| | - | 1,315 | 29 | 0 | 1,654 | 34 | 0 | 1,264 | 28 | 0 | 1,528 | 36 | 0 | 1,426 | 30 | 0 | 1,714 | 40 | 0 | 1,732 | 39 | 0 | 1,634 | 35 | 0 |
| | $\chi2$ (d.f.), *P* | 0.6 (1), 0.43 | | | 0.4 (1), 0.53 | | | 0.0 (1), 0.94 | | | 1.5 (1), 0.22 | | | 0.0 (1), 0.91 | | | 4.4 (1), 0.036 | | | 1.1 (1), 0.29 | | | 0.0 (1), 0.92 | | |
| | OR (95% CI), *P* | - | | | - | | | - | | | - | | | - | | | 0.2 (0.0-1.4), 0.11 | | | - | | | - | | |
| Ser534Asn rs1801158 | + | 725 | 39 | 0 | 255 | 9 | 0 | 639 | 19 | 0 | 375 | 12 | 0 | 473 | 20 | 0 | 191 | 7 | 0 | 173 | 8 | 0 | 61 | 1 | 0 |
| | - | 1,298 | 43 | 0 | 1,619 | 64 | 0 | 1,235 | 54 | 0 | 1,499 | 61 | 0 | 1,401 | 53 | 0 | 1,683 | 66 | 0 | 1,701 | 65 | 0 | 1,599 | 65 | 0 |
| | $\chi2$ (d.f.), *P* | 4.4 (1), 0.036 | | | 0.1 (1), 0.79 | | | 2.2 (1), 0.14 | | | 0.6 (1), 0.43 | | | 0.1 (1), 0.75 | | | 0.0 (1), 0.95 | | | 0.3 (1), 0.58 | | | 1.0 (1), 0.31 | | |
| | OR (95% CI), *P* | 1.7 (1.1-2.6), 0.026 | | | - | | | - | | | - | | | - | | | - | | | - | | | - | | |
| Ile543Val rs1801159 | + | 491 | 273 | 22 | 165 | 109 | 8 | 435 | 229 | 19 | 253 | 133 | 12 | 339 | 161 | 14 | 140 | 71 | 3 | 124 | 58 | 4 | 45 | 20 | 2 |
| | - | 902 | 434 | 44 | 1,129 | 543 | 53 | 859 | 423 | 42 | 1,041 | 519 | 49 | 955 | 491 | 47 | 1,154 | 581 | 58 | 1,170 | 594 | 57 | 1,115 | 536 | 51 |
| | $\chi2$ (d.f.), *P* | 2.6 (2), 0.27 | | | 5.1 (2), 0.08 | | | 0.9 (2), 0.63 | | | 0.4 (2), 0.81 | | | 0.3 (2), 0.85 | | | 2.0 (2), 0.37 | | | 0.4 (2), 0.83 | | | 0.1 (2), 0.97 | | |
| | OR (95% CI), *P* | - | | | - | | | - | | | - | | | - | | | - | | | - | | | - | | |
| Val732Ile rs1801160 | + | 2 | 79 | 705 | 1 | 32 | 249 | 0 | 62 | 622 | 1 | 41 | 356 | 1 | 50 | 464 | 0 | 23 | 191 | 0 | 23 | 163 | 1 | 10 | 56 |
| | - | 2 | 97 | 1,282 | 3 | 130 | 1,593 | 0 | 100 | 1,220 | 3 | 121 | 1,486 | 3 | 112 | 1,378 | 0 | 139 | 1,651 | 0 | 139 | 1,679 | 3 | 132 | 1,568 |
| | $\chi2$ (d.f.), *P* | 7.4 (2), 0.025 | | | 7.5 (2), 0.023 | | | 1.1 (1), 0.29 | | | 2.7 (2), 0.27 | | | 1.9 (2), 0.39 | | | 2.0 (1), 0.16 | | | 2.6 (1), 0.11 | | | 3.5 (2), 0.17 | | |
| | OR (95% CI), *P* | 1.6 (1.1-2.1), 0.006 | | | 1.9 (1.2-3.1), 0.005 | | | - | | | - | | | - | | | - | | | - | | | - | | |

| | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Asp949Val rs67376798 | + | 750 | 16 | 0 | 255 | 8 | 0 | 651 | 10 | 0 | 377 | 12 | 0 | 480 | 16 | 0 | 196 | 1 | 0 | 180 | 1 | 0 | 58 | 3 | 0 |
| | - | 1,336 | 14 | 0 | 1,674 | 19 | 0 | 1,278 | 17 | 0 | 1,552 | 15 | 0 | 1,449 | 11 | 0 | 1,733 | 26 | 0 | 1,749 | 26 | 0 | 1,654 | 19 | 0 |
| | χ2 (d.f.), *P* | 4.3 (1), 0.038 | | | 5.0 (1), 0.026 | | | 0.1 (1), 0.77 | | | 8.6 (1), 0.003 | | | 14.7 (1), <0.001 | | | 1.5 (1), 0.22 | | | 1.2 (1), 0.28 | | | 4.0 (1), 0.046 | | |
| | OR (95% CI), *P* | 2.2 (1.1-4.5), 0.038 | | | 3.2 (1.2-8.2), 0.019 | | | - | | | 3.4 (1.5-7.3), 0.002 | | | 4.6 (2.1-10.1), <0.001 | | | - | | | - | | | 5.5 (1.3-24.2), 0.024 | | |
| IVS14+1 G>A rs3918290 | + | 753 | 12 | 0 | 258 | 7 | 0 | 644 | 14 | 0 | 380 | 6 | 0 | 482 | 11 | 0 | 190 | 7 | 0 | 174 | 5 | 0 | 56 | 6 | 0 |
| | - | 1,329 | 11 | 0 | 1,669 | 12 | 0 | 1,283 | 5 | 0 | 1,547 | 13 | 0 | 1,445 | 8 | 0 | 1,737 | 12 | 0 | 1,753 | 14 | 0 | 1,654 | 9 | 0 |
| | χ2 (d.f.), *P* | 2.2 (1), 0.13 | | | 3.5 (1), 0.06 | | | 11.6 (1), <0.001 | | | 1.7 (1), 0.19 | | | 9.6 (1), 0.002 | | | 7.3 (1), 0.007 | | | 4.5 (1), 0.034 | | | 15.6 (1), <0.001 | | |
| | OR (95% CI), *P* | - | | | - | | | 5.3 (1.9-14.9), 0.002 | | | - | | | 4.4 (1.7-11.0), 0.002 | | | 4.6 (1.7-12.6), 0.003 | | | 3.8 (1.2- 11.8), 0.021 | | | 19.2 (5.0-73.8), <0.001 | | |

Results shown using a co-dominant model and, for those that were significant (*shaded & underneath*), odds ratios and 95% confidence intervals using the best model that fitted the data (all were dominant apart from Cys29Arg which was additive). *P*-values uncorrected for multiple testing. Neither Lys259Glu nor Ser534Asn significantly increased 5FU-related toxicities (Ser534Asn was associated with skin rash). The common allele (A/B) encodes the wild type amino acid.

## LEGEND TO FIGURE

**CONSORT diagram of the study design and analyses.** Shown are the numbers of variants analysed from genes that were likely to play a role in the metabolic or DNA damage repair pathways associated with the agents used in COIN and COIN-B, together with the numbers of patients studied, and the primary and secondary endpoints. MAF, minor allele frequency; pts, patients; PN, peripheral neuropathy; OS, overall survival; ORR, overall response rate.