Clinical Cancer Research

Pharmacogenetic Analysis of the UK MRC (Medical Research Council) MAGIC Trial: Association of Polymorphisms with Toxicity and Survival in Patients Treated with Perioperative Epirubicin, Cisplatin, and 5-fluorouracil (ECF) Chemotherapy

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Abstract

Purpose: Germline polymorphisms may affect chemotherapy efficacy and toxicity. We examined the effect of polymorphisms in drug metabolism and DNA repair genes on pathologic response rates, survival, and toxicity for patients randomized to surgery alone or perioperative ECF chemotherapy in the MRC MAGIC trial.

Experimental Design: DNA was extracted from nontumor resection formalin-fixed paraffin-embedded (FFPE) blocks. *ERCC1*, *ERCC2*, *XRCC1*, *DYPD*, and *OPRT* SNPs were evaluated using Sequenom, *GSTP1*, *GSTT1* deletion, and *TYMS* (*TS*) 5′ 2R/3R using multiplex PCR. Post PCR amplification, *TS* 2R/3R and *GSTT1* samples underwent gel electrophoresis.

Results: Polymorphism data were available for 289 of 456 (63.4%) operated patients. No polymorphism was statistically significantly associated with pathologic response to chemotherapy. Median overall survival (OS) for patients treated with surgery alone with any *TS* genotype was not different (1.76 years 2R/2R, 1.68 years 2R/3R, 2.09 years 3R/3R). Median OS for patients with a *TS* 2R/2R genotype treated with chemotherapy was not reached, whereas median OS for 2R/3R and 3R/3R patients were 1.44 and 1.60 years, respectively (log rank *P* value = 0.0053). The *P* value for the interaction between treatment arm and genotype (3R/3R and 3R/2R vs. 2R/2R) was 0.029. No polymorphism was statistically significantly associated with chemotherapy toxicity.

Conclusions: In MAGIC, patients with a *TS* 2R/2R genotype appeared to derive a larger benefit from perioperative ECF chemotherapy than patients with 3R containing genotypes. Further exploration of this potential predictive biomarker in this patient population is warranted. *Clin Cancer Res; 23(24); 7543–9. ©2017 AACR.*

Introduction

Gastric and esophageal cancers are the third and sixth most common causes of cancer death annually worldwide (1). Neoadjuvant or perioperative chemotherapy is one standard treatment for patients with operable gastric or gastroesophageal adenocarcinoma prior to surgical resection (2–5). This approach is associated with a modest (6–13%) absolute overall survival (OS) advantage in terms of OS compared to surgery alone but also with chemotherapy-related toxicities such as nausea and vomiting, and neutropenia. Furthermore, following multimodality therapy half of resected patients develop incurable, metastatic cancer, and therefore do not benefit from neoadjuvant chemotherapy (2, 4). Better selection of patients for preoperative chemotherapy might avoid needless toxicity; however, currently, gastroesophageal cancer patients who are treated with neoadjuvant chemotherapy are selected for treatment based on radiologic staging alone as there are no currently validated predictive biomarkers for chemotherapy.

Germline polymorphisms in genes associated with chemotherapy and drug metabolism have been validated as predictors of survival and toxicity outcomes across several tumor types

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Translational Relevance

Neoadjuvant or perioperative cisplatin and 5-fluorouracil–based chemotherapy plus surgery is one standard of care for patients with resectable gastroesophageal cancer. However, chemotherapy benefits only a small proportion of patients, and validated biomarkers predictive of response or toxicity have been elusive. We analyzed the effect of multiple germline polymorphisms putatively associated with response or toxicity to chemotherapy in patients treated with chemotherapy plus surgery or surgery alone in the UK Medical Research Council MAGIC Trial. One polymorphism in thymidylate synthase (*TS*), a 2R/2R tandem repeat, was significantly associated with overall survival in patients treated with chemotherapy, but not in patients treated with surgery alone. These findings suggest that neoadjuvant chemotherapy for patients with operable gastroesophageal cancer could be personalized based on germline polymorphism status.

including colorectal and breast cancer (6–8). Although similar studies have examined the effects of polymorphisms in germline genes relating to chemotherapy metabolism in gastroesophageal cancer, most of these have been retrospective, and all lack an untreated control group (9–11). The UK MRC MAGIC trial was an open label, multicentre, phase III randomized trial comparing six cycles of perioperative ECF (epirubicin, cisplatin, and infused 5-fluorouracil) chemotherapy (three cycles pre- and three cycles postresection) plus surgery to surgery alone in patients with resectable gastroesophageal cancer (2). Patients treated with perioperative chemotherapy had improved OS compared to patients treated with surgery alone [5-year OS 36% vs. 23%; HR 0.75; 95% confidence interval (CI), 0.60–0.93; $P = 0.009$]. As a result, perioperative ECF chemotherapy became one standard treatment regimen for patients with resectable gastroesophageal adenocarcinoma. We hypothesized that selected germline polymorphisms would be associated with pathologic response to chemotherapy, OS, or chemotherapy-related toxicity in the MAGIC trial, and herein present the results of this analysis.

Methods

Hematoxylin and eosin (H&E)-stained tissue sections from resection specimens were reviewed by a histopathologist (AW). DNA was extracted from nonmalignant tissue. Five sections (10 mm thick) were cut and deparaffinized using a standard protocol, and the area of interest was dissected using a sterile scalpel blade. Genomic DNA was extracted using the QIAmp DNA Micro Kit and QIAamp DNA FFPE Tissue Kit (Qiagen), following the manufacturer's instructions. After dewaxing and rehydrating the slides, tissue was microdissected and placed into a 1.5-mL Eppendorf tube with buffer ATL and proteinase K for digestion (Qiagen). DNA was eluted in buffer ATE (Qiagen) with an elution volume of 60 mL. Quality control of the DNA was performed on the basis of 260:230 and 260:280 ratio values and visual inspection of the wavelength spectral pattern provided by the NanoDrop spectrophotometer (Thermo Scientific). A 260:230 ratio of approximately 2.0, together with a 260:280 ratio of approximately 1.8 and the presence of a peak at 260 nm with a steep decrease toward 280 nm in the wavelength spectrum was considered sufficiently good quality DNA.

Slides from all cases were reviewed and pathologic response in tumor graded using the Mandard tumor regression grading (TRG) system (12).

Genotype analysis

Ten polymorphisms were selected based on a review of the literature and expected interaction with epirubicin, cisplatin, and 5-fluorouracil (5-FU) chemotherapy. These are listed in Table 1. For detailed description of genotype analysis methodology, please see Supplementary Methods.

Statistical analysis

OS was calculated from surgery to death from any cause or last date of follow up. Date of surgery was selected as the baseline for biomarker analysis to reduce potential bias as only patients with a surgical specimen were available for inclusion. Analyses were performed within treatment arms due to the differences in timing of surgery, to further reduce potential bias in the estimates of effects. Date of surgery could not be confirmed for nine patients in the chemotherapy plus surgery arm and these patients were excluded from the survival analyses. Differences in OS by polymorphism status were assessed using the Kaplan–Meier method and compared using Cox regression. To mitigate multiplicity a P value of <0.01 was considered significant when testing for associations of genotypes with survival and toxicity, and <0.05 when testing interactions. Multiple imputation was performed to account for missing polymorphism data. OS results were adjusted for possible confounders of age, subtype, gender, site of primary, WHO, nodal status).

Proportions of patients with good pathologic response (TRG 1-2) compared with poor pathologic response (TRG 3-5) were compared for each genotype using the Fisher exact test. Proportions of patients with toxicities according to genotype were compared using Pearson chi-squared test or Fisher exact test where appropriate.

As TS 2R/2R genotype is the polymorphism of interest and is present in approximately 30% of patients, with median OS of 18 months in control (2R/3R + 3R/3R), power of 80%, 5% two-sided significance level, to detect an HR of 0.5 would require 85 events. Alternatively, as with the same assumptions as above with 70% power, 67 events would be required. With respect to pathologic response rate in TS genotyped patients, in order to detect a doubling in response rates from 15% to 30%, 206 patients would be required to achieve 70% power. This is based on a pathologic response rate of 15% in the 3R group, which accounts for 70% of patients, and 30% in the 2R/2R group (which contains 30% of patients). Because of the trial design and retrospective nature of these analyses, all results can only be seem as hypothesis

Table 1. Germline polymorphisms analyzed

Gene	Polymorphism	rsID
OPRT	G638C (Gly213Ala)	1801019
DPYD	IVS14+1G>A	DPYD2A
DPYD	A1627G	1801159
ERCC1	C118T	11615
ERCC1	C8092A	3212986
ERCC2	Lys751Gln	13181
XRCC1	A399G	25487
GSTP1	I105V	1695
TS 2R/3R 5′UTR	2R/3R repeat	
GSTT1 deletion		

generating and suggestive of future work, with significance levels set to limit the possibility of a type II error.

All analyses were conducted using Stata version 14. The MAGIC protocol was approved by the relevant ethics committees, and patients gave written informed consent for participation in the trial. The translational MAGIC protocol (TransMAGIC) received separate national ethics approval (11/LO/0566).

Results

Polymorphism data were available for 289/456 (63.4%) patients who underwent surgery in the MAGIC trial. There was no difference in distribution of sex, performance status, site of tumor, age, or treatment arm between patients with and without polymorphism data; however, patients without polymorphism data were more likely to undergo a palliative resection in the view of the operating surgeon (Supplementary Table S1). This resulted in a borderline difference in survival between patients who had polymorphism data available and those who did not, which was more pronounced in the surgery only arm (Supplementary Fig. S1).

Discordance in size-based polymorphism assessment

We found that on duplicate runs that size-based polymorphism assessment that discordance occurred at a rate of 32.7% and 4.2% for *GSTT1* and *TYMS* (*TS*), respectively (13). Because of the high rate of discordance in *GSTT1* results for this polymorphism was not analyzed further.

Genotype frequency

The frequency of each polymorphism genotype is described in Table 2. Genotype frequency was consistent with previously published data and all were in Hardy–Weinberg equilibrium with the exception of *DPYD* rs1801159.

Table 2. Frequency of each polymorphism

		Chemo+Surgery	Surgery alone	Total
TS 2R/3R 5′UTR	2R/2R	38 (31%)	53 (36%)	91 (34%)
	2R/3R	51 (41%)	61 (42%)	112 (41%)
	3R/3R	35 (28%)	32 (22%)	67 (25%)
	Total	124	146	270
GSTP1 rs1695	A	74 (56%)	73 (47%)	147 (51%)
	AG	53 (40%)	67 (43%)	120 (41%)
	G	6 (4%)	16 (10%)	22 (8%)
	Total	133	156	289
OPRT rs1801019	C	5 (4%)	3 (2%)	8 (3%)
	G	92 (69%)	92 (59%)	184 (64%)
	CG	36 (27%)	60 (39%)	96 (33%)
	Total	133	155	288
DPYD2A IVS14+1G>A	G	131 (99%)	152 (100%)	283 (>99%)
	GA	1 (1%)	0 (0%)	1 (<1%)
	Total	132	152	284
DPYD rs1801159	A	96 (73%)	100 (65%)	196 (68%)
	AG	27 (20%)	48 (31%)	75 (26%)
	G	9 (7%)	7 (4%)	16 (6%)
	Total	132	155	287
ERCC1 rs11615	C	19 (14%)	29 (19%)	48 (17%)
	CT	70 (53%)	68 (44%)	138 (48%)
	T	44 (33%)	57 (37%)	101 (35%)
	Total	133	154	287
ERCC1 rs3212986	G	61 (46%)	84 (55%)	145 (51%)
	GT	64 (49%)	60 (39%)	124 (43%)
	T	7 (5%)	10 (6%)	17 (6%)
	Total	132	154	286
ERCC2 rs13181	G	23 (17%)	20 (13%)	43 (15%)
	GT	62 (47%)	63 (41%)	125 (44%)
	T	48 (36%)	71 (46%)	119 (41%)
	Total	133	154	287
XRCC1 rs25487	A	19 (15%)	18 (12%)	37 (13%)
	AG	56 (42%)	67 (43%)	123 (43%)
	G	57 (43%)	69 (45%)	126 (44%)
	Total	132	154	286

Genotype and pathologic response to chemotherapy

The association between each polymorphism and pathologic response to chemotherapy in chemotherapy-treated patients is described in Table 3. No polymorphism was statistically significantly associated with pathologic response to chemotherapy.

Genotype and OS

Median OS for patients treated with surgery alone who had *TS* 2R/2R genotype was 1.76 years, compared to 1.68 years for 2R/3R and 2.09 years for 3R/3R (Table 4 and Fig. 1). These differences were not statistically significant. In contrast, median OS for patients with a *TS* 2R/2R genotype treated with chemotherapy was not reached, whereas survival for 2R/3R and 3R/3R were 1.44 and 1.60 years, respectively (log rank *P* value 0.0053). When all 3R genotypes were combined, median OS was 1.44 years for chemotherapy-treated patients versus not reached for 2R/2R genotype (HR = 2.4; $P = 0.003$). The effect of TS genotype on OS in chemotherapy-treated patients remained statistically significant when adjusted for the potential confounders of age, subtype, gender, site of primary, WHO, nodal status (Table 4). The *P* value for the interaction between treatment arm and TS genotype (3R/3R and 3R/2R vs. 2R/2R) was 0.029 (with an HR = 0.46).

To assess the effect of a 4.2% discrepancy in *TS* genotype status assessment, we performed 10,000 simulations, randomly changing 4.2% of results. From these 10,000 simulations, the 2.5 to 97.5 percentiles of the HR for the interaction between treatment arm and TS genotype (3R/3R and 3R/2R vs. 2R/2R) was 0.37–0.60, compared to the estimate from the original data of 0.46.

Patients with the (AG) genotype of *DPYD* rs1801159 Ile543Val had numerically shorter survival compared to the AA genotype in the surgery alone arm of the trial, this difference was statistically significant (HR = 1.75; $P = 0.008$). There was no evidence of an interaction between treatment arm and DPYD status. Results were similar when multiple imputation was performed for missing data.

No other genotype was statistically significantly associated with OS (see Supplementary Tables S2A–S2F).

Genotype and chemotherapy-related toxicity

The presence of grade 3 or greater toxicity and association with polymorphism status are detailed in Supplementary Table S1. *DPYD2A* IVS14+1G>A GA variant was associated with a nonstatistically significant trend towards increased rates of ≥grade 3 diarrhea ($P = 0.039$); however, only one patient was detected with this variant. No other polymorphism demonstrated a statistically significant relationship with chemotherapy related toxicity.

The mean number of cycles of chemotherapy received for most polymorphisms was five (Supplementary Table S3), with the exception of *ERCC1* rs3212986 (GT+TT) variant who had a mean of four cycles (Kruskal–Wallis equality-of-populations rank test, $P = 0.0425$).

Table 3. Genotype and pathologic response to chemotherapy

Genotype	TRG 1-2	TRG 3-5	*P*	OR for TRG 3-5	95% CI
TS					
2R/2R	9 (24.3)	28 (75.7)	(0.536)	1.0	
2R/3R	8 (16.7)	40 (83.3)	0.384	1.61	0.55–4.68
3R/3R	5 (14.7)	29 (85.3)	0.313	1.86	0.56–6.25
2R/2R	9 (24.3)	28 (75.7)		1.0	
2R/3R + 3R/3R	13 (15.9)	69 (84.2)	0.274	1.71	0.66–4.44
GSTP1 rs1695					
A	11 (15.5)	60 (84.5)	(0.812)	1.0	
AG	10 (20.0)	40 (80.0)	0.520	0.73	0.29–1.89
G	1 (16.7)	5 (83.3)	0.939	0.92	0.10–8.62
OPRT					
C	2 (40.0)	3 (60.0)	(0.308)	1.0	
GC	4 (11.8)	30 (88.2)	0.128	5.0	0.63–39.7
G	16 (18.2)	72 (81.8)	0.249	3.0	0.46–19.5
DPYD rs1801159					
A	14 (15.4)	77 (84.6)	(0.413)	1.0	
AG	5 (18.5)	22 (81.5)	0.698	0.80	0.26–2.46
G	3 (33.3)	6 (66.7)	0.186	0.36	0.08–1.62
ERCC1 rs11615					
C	3 (16.7)	15 (83.3)	(0.211)	1.0	
CT	15 (22.7)	51 (77.3)	0.580	0.68	0.17–2.67
T	4 (9.3)	39 (90.7)	0.417	1.95	0.39–9.77
ERCC1 rs3212986					
G	8 (13.6)	51 (86.4)	(0.496)	1.0	
GT	12 (19.7)	49 (80.3)	0.371	0.64	0.24–1.70
T	2 (28.6)	5 (71.4)	0.308	0.39	0.06–2.38
ERCC2 rs13181					
G	3 (14.3)	18 (85.7)	(0.879)	1.0	
GT	11 (19.0)	47 (81.0)	0.631	0.71	0.18–2.85
T	8 (16.7)	40 (83.3)	0.804	0.83	0.20–3.51
XRCC1 rs25487					
A	2 (11.1)	16 (88.9)	(0.752)	1.0	
AG	10 (18.9)	43 (81.1)	0.453	0.54	0.11–2.72
G	10 (17.9)	46 (82.1)	0.503	0.58	0.11–2.91

Abbreviation: CI, confidence interval.

Discussion

Our study is the first to evaluate the association between germline polymorphisms and pathologic response, OS, and chemotherapy-related toxicity for patients with operable gastroesophageal cancer in a randomized trial with a control group. We found that patients who have a 2R/2R thymidylate synthase (*TS*) genotype who were treated with perioperative ECF chemotherapy had statistically superior OS compared to those who had a 2R/3R or 3R/3R genotype. This difference was not apparent in patients who were treated with surgery alone, and a significant interaction between *TS* genotype status and treatment arm was noted. In addition, in our study, patients with a *TS* 2R2R genotype had a nonstatistically significant higher rate of good pathologic response (TRG 1–2) at 24% compared to 3R allele containing patients. These findings are important as if validated pharmacogenomic genotyping could be used in future to select patients who are more likely to benefit from perioperative chemotherapy.

TS acts to produce thymidylate which is an essential precursor for DNA synthesis. The activity of TS is blocked by 5-fluorodeoxyuridylate (5FdUMP), the active metabolite of 5-FU and it is via this mechanism that 5-FU exerts cytotoxicity. The human thymidylate synthase gene is polymorphic through the presence of either double (2R) or triplet (3R) 28 base tandem repeats which are sited upstream of the *TS* translational start site (14). These repeats control the transcription and translation of the *TS* gene; individuals with 3R tandem repeats have higher levels of TS expression in tissue and consequently lower rates of response to fluoropyrimidine chemotherapy (15). Our findings are in keeping with this biology. Several other series have reported comparable improvements in response rates OS similar results in gastric cancer patients with the favorable 2R genotype treated with fluoropyrimidine 5-FU-based chemotherapy, however none of these were a randomized trial with an untreated control group (14–16). However, opposing results have also been demonstrated (17, 18). Potential reasons for this include small, heterogeneous, ethnically diverse populations treated with variable chemotherapy regimens in both advanced and resectable disease settings, and the addition of other related polymorphisms such as the *TS* 3′UTR 6 base pair polymorphism to analyses (19). We caveat our discussion with an awareness that length based polymorphism assessment resulted in a discordance rate of 4.2% for TS polymorphism status. However, as our findings for the 2R/2R genotype are quite striking, even when a stringent *P* value is applied to

Table 4. Association between TS genotype and OS (second HR and *P* value are adjusted for age, subtype, gender, site of primary, WHO, nodal status)

	Chemotherapy			Surgery alone			Overall		
	2R/2R	2R/3R	3R/3R	2R/2R	2R/3R	3R/3R	2R/2R	2R/3R	3R/3R
Patients	38 (31%)	51 (41%)	35 (28%)	51 (36%)	59 (41%)	32 (23%)	89 (34%)	110 (41%)	67 (25%)
Events	15	36	20	31	38	25	46	74	45
Median survival	Not reached	1.44	1.60	1.76	1.68	2.09	3.31	1.62	1.84
Log-rank *P* value	0.0053			0.7212			0.0326		
HR	1 (REF)	2.66	2.10	1 (REF)	1.03	1.23	1 (REF)	1.59	1.53
		3.06	2.64		1.07	1.45		1.73	1.79
HR *P* value		0.002	0.032		0.896	0.448		0.013	0.043
		0.001	0.009		0.778	0.190		0.005	0.008

	Combined analysis					
	2R/2R	2R/3R + 3R/3R	2R/2R	2R/3R + 3R/3R	2R/2R	2R/3R + 3R/3R
Patients	38 (31%)	86	51 (36%)	91	89 (34%)	177
Events	15	56	31	63	46	119
Median survival	Not reached	1.44	1.76	1.84	3.31	1/71
Log-rank *P* value	0.002		0.6519		0.009	
HR	1 (REF)	2.43	1 (REF)	1.10	1 (REF)	1.57
		2.89		1.15		1.75
HR *P* value		0.003		0.652		0.010
		0.001		0.531		0.002

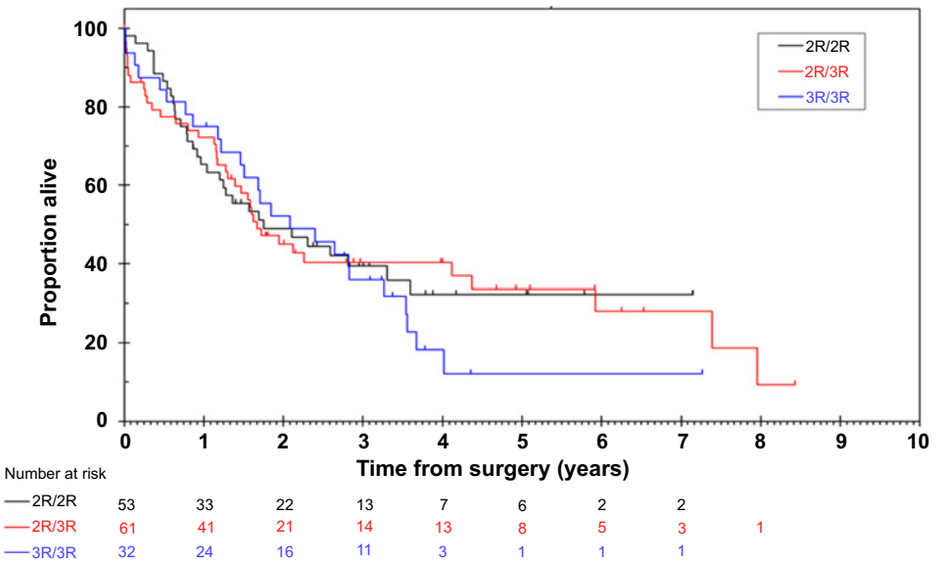

Figure 1.
Overall survival from surgery by TS genotype (surgery-alone arm).

correct for multiplicity, and were confirmed with repeated simulation testing to account for any discrepancy in *TS* genotype assessment, we do not think that this is likely to have unduly affected these results.

Dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme in 5-fluorouracil catabolism and variation in DPD levels and activity have profound effects of fluoropyrimidine metabolism and toxicity. The most well described of these is a *DPYD* 2* splice variant polymorphism, which results in a nonfunctional enzyme and is associated with fluoropyrimidine-related toxicity in many studies (6, 20–22). Although low patient deleterious allele frequency and lack of statistical significance due to correction for multiplicity means that we cannot be definitive in our conclusions, our results are consistent with these data. However, we think that these results are of secondary importance to the survival outcomes presented.

We asked two questions from our dataset: first, can genotyping be used to differentiate between those who derive a survival benefit from perioperative chemotherapy and those who do not; and second, if these genotypes were assessed preoperatively, would it possible to predict excessive toxicity prior to commencing chemotherapy? Regarding survival benefit, our findings relating the favorable effects of the *TS* 2R/2R genotype are shared with several other large studies (23). Therefore, is further validation with a clinical trial required? One small genotype directed clinical trial clinical trial

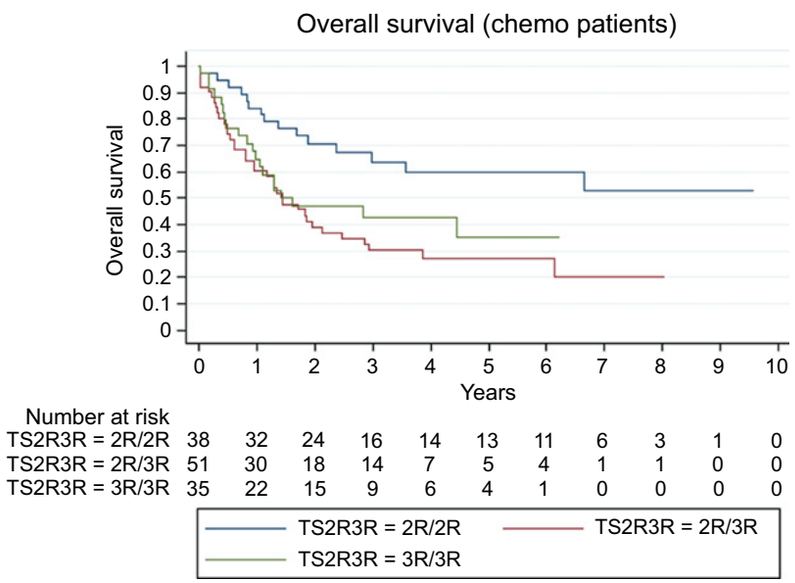

Figure 2.
Overall survival in chemotherapy patients.

evaluated FOLFOX chemotherapy in 25 patients with *TS* 2R containing genotypes (2R/2R and 2R/3R) and found that radiologic response rates did not differ compared to historical control (24). However, based on our results only the 2R/2R genotype would benefit from this approach; this was also suggested in subgroup analysis of that study. As patients with 3R containing genotypes did not appear to benefit from fluoropyrimidine-based chemotherapy in MAGIC, we suggest that alternative treatment options should be evaluated for these patients. Omitting perioperative chemotherapy completely is unlikely to be acceptable as many patients (especially those with proximal tumors) require downstaging prior to surgical resection. Alternatively, patients with 3R containing genotypes could be treated with higher doses of fluoropyrimidines, although this could be result in increased toxicity. This approach in UGT1A1 genotyped patients has demonstrated that patients who are wild type or heterozygous for the deleterious *28 allele can tolerate increased doses of irinotecan compared to UGT1A1 *28 homozygotes (25, 26). Finally, a non-fluoropyrimidine containing regimen could be considered; for patients with tumors of the gastroesophageal junction or esophagus chemoradiotherapy with carboplatin and paclitaxel would seem a reasonable alternative.

With respect to avoiding toxicity, the relative rarity of alleles which predict for significant toxicity such as *DPYD 2A** is associated with significant screening costs even when toxicity is reduced by the use of pre-emptive dose reductions (27). As such, neither the European Medicines Agency nor the U.S. Food Drug Administration require testing for *DPYD* variants prior to treatment with fluoropyrimidines despite the availability of advice from expert groups such Clinical Pharmacogenetics Implementation Consortium and the Dutch Pharmacogenetics Working Group, which provide clinical practice guidelines on genotype-based drug dosing (28, 29). In the MAGIC trial, the most common grade 3 or greater chemotherapy-associated toxicity was neutropenia, which is likely to be due to epirubicin and which is not predicted by any of the polymorphisms which we examined. Therefore, routine testing for *DPYD 2A** polymorphisms is unlikely to significantly decrease toxicity in patients treated with MAGIC type chemotherapy.

The interaction between chemotherapy and genotype is complex, and colored by many other clinical variables such as age, ethnicity, gender, hepatorenal function, and the interaction between individual components of each chemotherapy regimen. This has profound implications for the accuracy of toxicity or outcome prediction using genotyping. One potential flaw relating specifically to this work is that not all MAGIC trial participants were included in this study as not all provided tissue for analysis, therefore we caution that the analysis could be underpowered to detect small effect sizes. On one hand, if a patient did not undergo surgery due to failure to respond to treatment then no tissue was available for analysis. Alternatively, patients with excessive toxicity due to chemotherapy may also have stopped chemotherapy prior to surgery. These potential biases may be reflected in the borderline improved OS demonstrated for patients with polymorphism data available. Thus, although germline genotype will not be altered by treatment, any true assessment of the predictive power of genotype would preferentially be performed in pretreatment samples for these reasons. A second issue relates to the technical challenges associated with length-based polymorphism assessment; moving forward advances in high throughput next generation sequencing technologies should ensure improved accuracy and speed of results with decreased DNA requirements.

In summary, ours is the first study to examine the effect of germline polymorphisms on pathologic response and survival outcomes for patients treated with perioperative chemotherapy for operable esophagogastric cancer, with a randomized control group. We found that patients with a *TS* 2R/2R genotype (representing 34% of the population) had excellent survival when treated with perioperative ECF chemotherapy. In contrast, patients with a 3R containing genotype did not appear to derive a similar benefit from standard dose fluoropyrimidine-based chemotherapy when compared to patients treated with surgery alone. It is salutary to note that despite recent progress in our understanding of the molecular biology underpinning gastroesophageal cancer that only one targeted drug, trastuzumab, is licensed in this disease, and that almost all patients will receive platinum and fluoropyrimidine-based chemotherapy as a component of their treatment (30, 31). Therefore, use of available data relating to patient selection for standard of care chemotherapy to design a prospective would appear to be a sound choice.

Disclosure of Potential Conflicts of Interest

E. Smyth is a consultant/advisory board member for Bristol Myers Squibb, Five Prime Therapeutics, Gritstone Oncology, and Servier. D. Cunningham reports receiving commercial research grants from Amgen, AstraZeneca, Bayer, Celgene, Medimmune, Merck Serono, Merrimack, and Sanofi. W. Allum reports receiving speakers bureau honoraria from Lilly and Nestle. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

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