ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

Annals of Oncology 28: 1015–1022, 2017
doi:10.1093/annonc/mdx035
Published online 9 February 2017

ORIGINAL ARTICLE

Genetic variants of DNA repair-related genes predict efficacy of TAS-102 in patients with refractory metastatic colorectal cancer

M. Suenaga[1], M. Schirripa[1], S. Cao[2], W. Zhang[1], D. Yang[2], S. Murgioni[3], D. Rossini[4], F. Marmorino[4], A. Mennitto[5], Y. Ning[1], S. Okazaki[1], M. D. Berger[1], Y. Miyamoto[1], R. Gopez Jr[1], A. Barzi[1], T. Yamaguchi[6], F. Loupakis[3] & H.-J. Lenz[1]*

[1]Division of Medical Oncology; [2]Department of Preventive Medicine, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, USA; [3]Medical Oncology 1 Unit, Istituto Oncologico Veneto, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Padua; [4]Polo Oncologico, Istituto Toscano Tumori, Azienda Ospedaliero-Universitaria Pisana, Pisa; [5]Medical Oncology Department, Fondazione Istituti di Ricovero e Cura a Carattere Scientifico, Istituto Nazionale dei Tumori, Milan, Italy; [6]Gastroenterology Center, Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo, Japan

Correspondence to: Dr Heinz-Josef Lenz, Division of Medical Oncology, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, 1441 Eastlake Ave, Suite 3456, Los Angeles, CA 90033, USA. Tel: +1-323-865-3967; E-mail: lenz@usc.edu

Background: Tri-phosphorylated trifluridine (FTD) incorporation into DNA is TAS-102's main anti-tumor action. We tested whether genetic polymorphisms in homologous recombination (HR) and cell cycle checkpoint pathway for DNA repair is associated with outcomes in refractory metastatic colorectal cancer (mCRC) patients treated with TAS-102.

Patients and methods: We analyzed genomic DNA extracted from 233 samples of three cohorts: an evaluation cohort of 52 patients receiving TAS-102, a validation cohort of 129 patients receiving TAS-102 and a control cohort of 52 patients receiving regorafenib. Single nucleotide polymorphisms of genes involved in HR (*ATM*, *BRCA1*, *BRCA2*, *XRCC3*, *FANCD2*, *H2AX*, *RAD51*) and cell cycle checkpoint (*ATR*, *CHEK1*, *CHEK2*, *CDKN1A*, *TP53*, *CHE1*, *PIN1*, *PCNA*) were analyzed by PCR-based direct sequencing.

Results: In univariate analysis for the evaluation cohort, patients with any G allele in *ATM* rs609429 had longer overall survival (OS) than those with the C/C variant (8.7 vs. 4.4 months, HR 0.37, 95% CI: 0.14–0.99, $P = 0.022$). Patients carrying any A allele in *XRCC3* rs861539 had significantly longer progression-free survival (PFS) (3.8 vs. 2.3 months, HR 0.44, 95% CI: 0.21–0.92, $P = 0.024$) and OS (15.6 vs. 6.3 months, HR 0.25, 95% CI: 0.08–0.79, $P = 0.012$) than those with the G/G variant. In multivariable analysis, *ATM* rs609429 remained significant for OS ($P = 0.020$). In the validation cohort, patients having *ATM* rs609429 with any G allele showed longer OS and PFS; the G/A variant in *XRCC3* rs861539 showed longer OS, though without statistical significance.

Conclusion: Genetic variants in the HR pathway may predict clinical outcome in mCRC patients receiving TAS-102.

Key words: TAS-102, ATM, DNA repair, cell cycle checkpoint, metastatic colorectal cancer

Introduction

TAS-102 is an orally administered drug combining the thymidine-based nucleoside analog, trifluridine (FTD), and tipiracil hydrochloride, a thymidine phosphorylase inhibitor (TPI) [1]. Based on results from the phase III RECOURSE trial, the FDA approved TAS-102 in 2015 for patients with refractory metastatic colorectal cancer (mCRC) who have previously received fluoropyrimidine, oxaliplatin, and irinotecan-based chemotherapy [2]. FTD is the active anti-tumor component of TAS-102. Incorporation of tri-phosphorylated FTD into DNA acts as the drug's main anti-tumor mechanism of action. The TPI ensures sufficient blood concentration of FTD by preventing its rapid degradation [3, 4]. However, the mechanism of DNA repair following FTD incorporation is still unclear. Previous preclinical

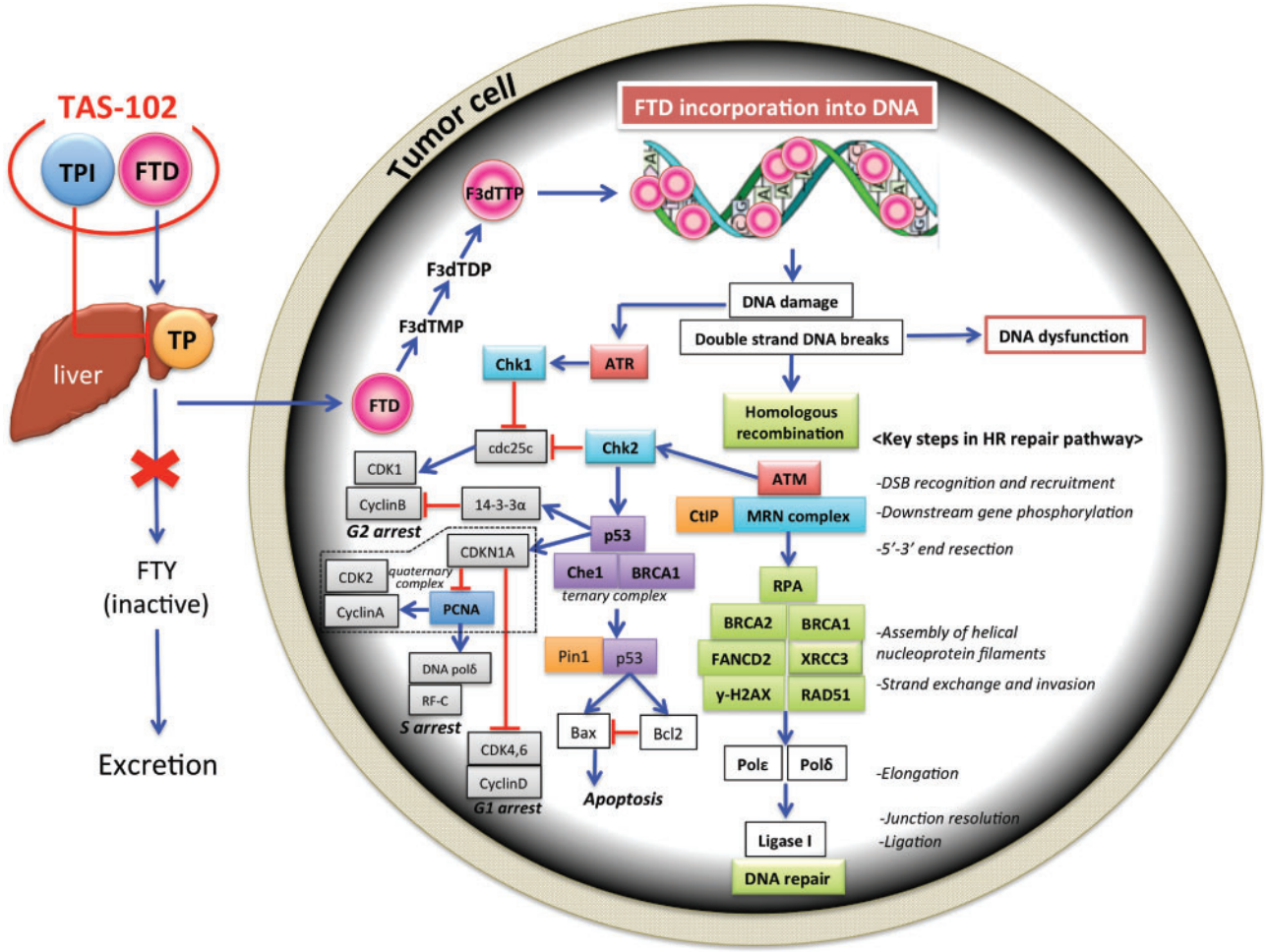

Figure 1. Mechanism of repair for FTD incorporation into DNA.

reports demonstrated that FTD incorporation into DNA induces single-strand breaks followed by double strand breaks (DSBs) during the G2/M-phase of the cell cycle. In contrast, 5-fluorouracil (5-FU) has been shown to arrest the cell cycle at the G1/S-phase in cancer cell-lines [5]. Thus, it is suggested that the antitumor activity of FTD primarily originates from DSBs that confer potent efficacy for 5-FU refractory mCRC.

Homologous recombination (HR) is the primary mechanism of DNA repair triggered by DSBs. The initial cellular DNA damage response (DDR) to DSBs is mediated by the upstream DDR kinase, ataxia telangiectasia mutated (ATM). ATM subsequently phosphorylates downstream HR-related key genes [6–9]. Cell cycle checkpoints also play an important role in DNA damage and mediate cell cycle arrest to promote DNA repair. ATM and Ataxia Telangiectasia and Rad3-Related Protein (ATR) are known as upstream proteins of checkpoints, responding to different types of DNA damage. Cell cycle arrest mediated by phosphorylation of ATR-checkpoint kinase 1 (Chk1) and the ATM-checkpoint kinase 2 (Chk2) by ATR and ATM as DDR suppresses the activity of cyclin-dependent kinase (CDK), leading to cell cycle arrest and DNA repair [6] (Figure 1).

Taken together, we hypothesized that the DDR induced by DNA DSBs will modulate TAS-102 efficacy. We therefore tested whether genetic single nucleotide polymorphisms (SNPs) in the DNA repair pathway, including HR and cell cycle checkpoints, might be associated with clinical outcome in patients with refractory mCRC treated with TAS-102.

Materials and methods

Study design and patients

This study was a retrospective exploratory study that investigated three independents cohorts consisting of patients with refractory mCRC; an evaluation cohort receiving TAS-102 ($n = 52$), a control cohort treated with regorafenib ($n = 52$) and a validation cohort receiving TAS-102 ($n = 129$). Eligible patients had a histologically confirmed diagnosis of mCRC; a history of previous standard chemotherapy consisting of 5-fluorouracil, oxaliplatin, irinotecan, bevacizumab, and cetuximab or panitumumab if *KRAS* wild-type; measurable or evaluable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1; and provided written informed consent (supplementary data, available at *Annals of Oncology* online).

Patients treated in the evaluation and validation cohort received TAS-102 (Taiho Pharmaceutical Co., Ltd., Tokyo, Japan) at 35 mg/m^2 two times daily for days 1–5 and 8–12, every 4 weeks. Patients in the control group received 160 mg regorafenib (Bayer, Leverkusen, Germany) once daily from days 1 to 21 every 4 weeks. Analyses were approved by the Institutional Review Boards of each institute and conducted at the

University of Southern California/Norris Comprehensive Cancer Center in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines.

Selection of candidate single-nucleotide polymorphism

The candidate 22 SNPs in the HR pathway and cell cycle checkpoint used in this study were *ATM*, *BRCA1*, *BRCA2*, *XRCC3*, *FANCD2*, *H2AX*, *RAD51*, *ATR*, *CHEK1*, *CHEK2*, *TP53*, *CHE1*, *PIN1*, *CDKN1A*, and *PCNA*. They were selected using one the following criteria: (i) SNP with biological significance according to published literature review, (ii) tagging SNPs selected by the HapMap genotype data with r^2 threshold = 0.8: http://snpinfo.niehs.nih.gov/snpinfo/snptag.html, or (iii) minor allele frequency ≥10% in both Caucasians and East Asian (in the Ensembl Genome Browser: http://uswest.ensembl.org/index.html). Functional significance was predicted based on functional single-nucleotide polymorphism (F-SNP) database: http://compbio.cs.queensu.ca/F-SNP/ (supplementary Table S1, available at *Annals of Oncology* online). DNA extraction and genotyping is shown in (supplementary data, available at *Annals of Oncology* online).

Statistical analysis

The primary endpoint in this study was progression-free survival (PFS), and the secondary endpoints were overall survival (OS) and disease control rate (DCR). All analyses were carried out with SAS 9.4 (SAS Institute, Cary, NC). All tests were two-sided at a significance level of 0.050. *P* values were not adjusted for multiple testing (supplementary data, available at *Annals of Oncology* online).

Results

Baseline patients and tumor characteristics

The median follow-up time, median PFS and OS were 6.4, 2.6, and 8.0 months in the evaluation cohort; 5.3, 2.0, and 5.7 months in the validation cohort; and 5.3, 1.9, and 5.3 months in the control cohort, respectively. All patients were deceased at the last follow-up time in the control cohort. The baseline characteristics of the evaluation, validation, and control cohorts are summarized in supplementary Table S2, available at *Annals of Oncology* online. Patient's gender, performance status, adjuvant treatment history and the number of prior chemotherapy were different between three cohorts. These associations between baseline characteristics and clinical outcome are summarized in supplementary Tables S3–S5, available at *Annals of Oncology* online for evaluation, validation and control cohorts, respectively. In the evaluation cohort we found that liver metastasis, *KRAS* wild-type and prior exposure to anti-EGFR antibody were significantly associated with shorter PFS, while a history of adjuvant treatment was associated with longer PFS. In addition, the number of prior chemotherapy, adjuvant treatment history and exposure of anti-EGFR anti-body were correlated with OS. In the validation cohort, age ≥61 and previous exposure of anti-EGFR antibodies were significantly associated with longer PFS and OS. However, liver metastasis and ECOG performance status ≥1 were significantly correlated with shorter PFS and OS, respectively. In the control cohort, patients with poor ECOG performance status and multiple metastatic sites had shorter PFS and OS. In addition, age <61 and liver metastasis showed correlation with significantly shorter PFS.

Association of clinical outcome and DNA repair-related genetic variants in the evaluation and validation cohort treated with TAS-102

The allelic frequencies and genotype distribution in each SNP were close to those reported for the Caucasian or Japanese population. All candidate SNPs were successfully genotyped in each cohort, except for three patients with *PCNA* rs25406 in the evaluation cohort and four patients with *XRCC3* rs861539 in the validation cohort because of poor quality extracted genomic DNA.

In univariate analysis for the evaluation cohort, patients with any G allele in *ATM* rs609429 had longer OS compared with those with the C/C variant [8.7 vs. 4.4 months, hazard ratio (HR) 0.37, 95% CI: 0.14–0.99, $P = 0.022$] (Figure 2A). Patients carrying the G/A variant in *XRCC3* rs861539 had significant longer PFS (3.8 vs. 2.3 months, HR 0.44, 95% CI: 0.21–0.92, $P = 0.024$) and OS (15.6 vs. 6.3 months, HR 0.25, 95% CI: 0.08–0.79, $P = 0.012$) than those with the G/G variant (supplementary Figure S1, available at *Annals of Oncology* online). Patients with any C allele in *CHEK2* rs2267130 had significant longer PFS (3.4 vs. 2.1 months, HR 0.48, 95% CI: 0.25–0.90, $P = 0.010$) compared with those with the T/T variant. In multivariable analysis adjusted for liver metastases and adjuvant history, *ATM* rs609429 remained significant for OS (HR 0.24, $P = 0.020$), and *XRCC3* rs861539 showed marginal significance in PFS (HR 0.52, $P = 0.091$) and OS (HR 0.31, $P = 0.056$). *CHEK2* rs2267130 did not remain as an independent factor. *PIN1* rs2233678 showed significant association with PFS and OS in both uni- and multi-variable analysis. However, the number of G/C variants was only two, which is not considered valid, proving its true impact on outcomes (Table 1; supplementary Table S6, available at *Annals of Oncology* online).

In univariate analysis for the validation cohort, patients carrying any G allele in *ATM* rs609429 showed longer PFS (2.0 vs. 1.9 months, HR 0.78, 95% CI: 0.52–1.16, $P = 0.20$) and OS (not reached vs. 4.4 months, HR 0.73, 95% CI: 0.43–1.25, $P = 0.24$) than those with the C/C variant; however, the effects were not statistically significant (Figure 2C and D). *XRCC3* rs861539 showed a different distribution of variants compared with those of the evaluation cohort having no A/A variant. However, patients with the G/A variant showed a trend of longer OS compared with the C/C variant (9.0 vs. 5.5 months, HR 0.75), which is consistent with the results in the evaluation cohort. None of the candidate SNPs remained significant in a multivariable model (Table 1; supplementary Table S7, available at *Annals of Oncology* online).

Association of clinical outcome and DNA repair-related genetic variants in the control cohort treated with regorafenib

In the control cohort receiving regorafenib without previous treatment with TAS-102, genotyping for the all candidate SNPs was available in all patients. Uni- and multivariate analyses showed no significant association between *ATM* rs609429, *XRCC3* rs861539, and *CHEK2* rs2267130 genotypes with PFS or OS (Figure 2B and Table 1).

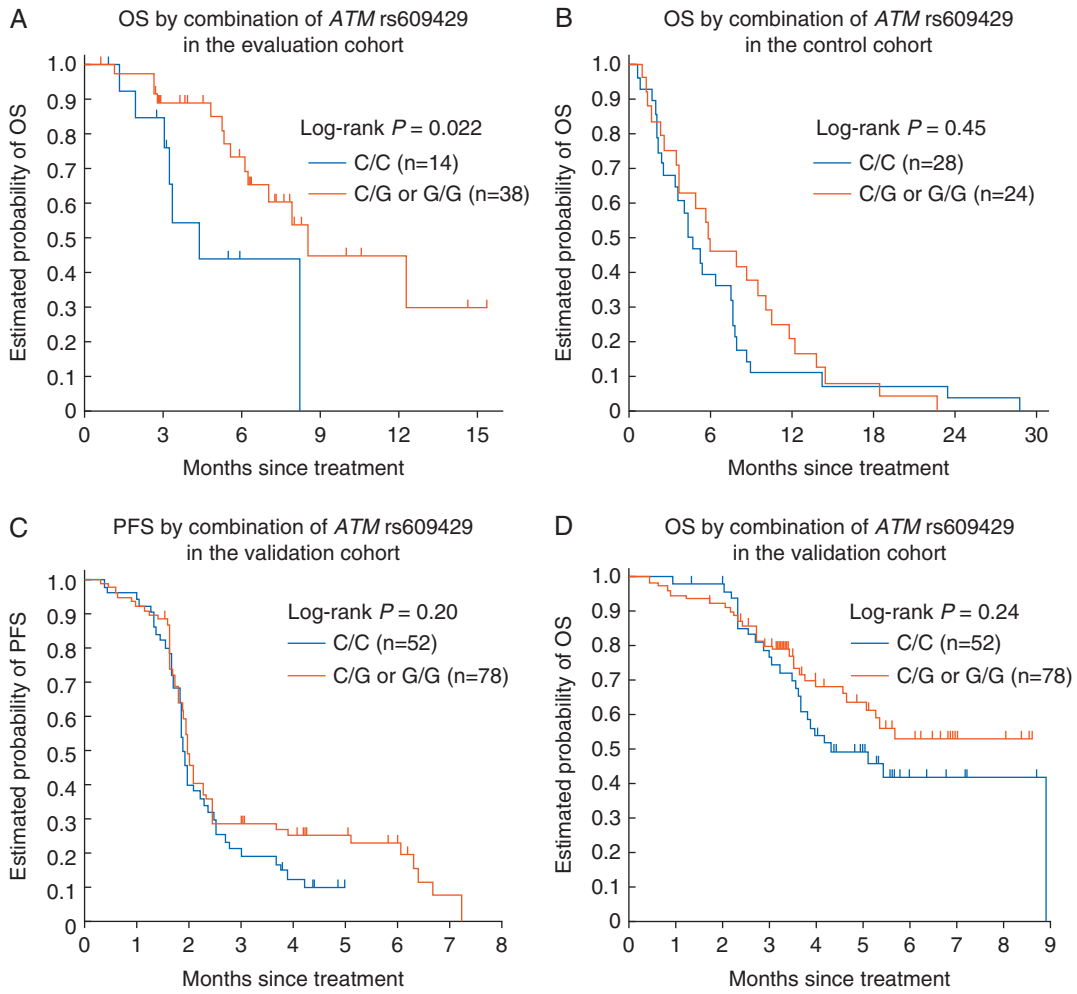

Figure 2. Overall survival (OS) by *ATM* rs609429 variants, C/C or any G (C/G or G/G) in the evaluation cohort treated with TAS-102 (A) and the control cohort (B) treated with regorafenib. Progression-free survival (PFS) (C) and OS (D) by *ATM* rs609429 variants, C/C or any G (C/G or G/G) in the validation cohort treated with TAS-102.

SNPs and TAS-102-related toxicity with clinical outcome

Well-known TAS-102-related grade 3≤ toxicities including neutropenia, leukopenia, anemia, anorexia, nausea and diarrhea were analyzed for association with clinical outcomes. In univariate analysis for the evaluation cohort, grade 3≤ neutropenia ($n = 20$) showed longer PFS and OS compared with that less than grade 3 ($n = 32$), but it was not statistically significant (PFS, 3.4 vs. 2.1 months, HR 0.68, 95% CI: 0.37–1.28, $P = 0.21$; OS, 8.3 vs. 6.3 months, HR 0.59, 95% CI: 0.24–1.43, $P = 0.22$). These findings were confirmed in the validation cohort for PFS (2.3 vs. 1.9 months, HR 0.50, 95% CI: 0.32–0.77, $P < 0.001$) and OS (8.8 vs. 3.7 months, HR 0.21, 95% CI: 0.10–0.44, $P < 0.001$) (supplementary Table S8, available at *Annals of Oncology* online).

Grade 3≤ neutropenia was more frequent in patients with any G allele ($n = 16/38$) in *ATM* rs609429 compared with those with the C/C variant ($n = 4/14$) (42% vs. 29%, $P = 0.52$), though no significance was observed in the evaluation cohort. In the validation cohort, patients with any G allele ($n = 34/78$) in *ATM* rs609429 had low statistical significance for association with high incidence of grade 3≤ neutropenia compared with those with the C/C variant ($n = 13/51$) (44% vs. 26%, $P = 0.060$). There was no significant association between other SNPs and toxicity (supplementary Table S9, available at *Annals of Oncology* online).

Discussion

To our knowledge, this study is the first to report that the HR pathway-related gene polymorphisms, *ATM* rs609429, and *XRCC3* rs861539, are potentially associated with clinical outcomes in mCRC patients receiving TAS-102. Our results suggested that the incorporation of FTD into DNA induces DSBs followed by DNA repair mainly through the HR pathway.

The role of HR-related gene polymorphisms in the risk of developing colorectal cancer and the chemosensitivity of mCRC still remains unclear. Landi et al. hypothesized that genetic variation of DNA repair and cell cycle control genes play an important role in determining susceptibility to lung cancer. They tested 102 SNPs in 34 key genes in lung cancer patients and determined that the *ATM* rs609429 G/G variant was associated with a decreased risk of lung cancer [10]. Angeles et al. reported that *ATM* rs609429 GG and *ATM* rs664677 CC variants were associated with increased breast cancer risk, and mentioned the

Table 1. Association between gene polymorphism and clinical outcome

	N	Disease control			Progression-free survival					Overall survival				
		CR/PR/SD[a]	PD	*P* value	Median, months (95% CI)	HR (95% CI)[a]	*P* value	HR (95% CI)[b]	*P* value	Median, months (95% CI)	HR (95% CI)[a]	*P* value	HR (95% CI)[b]	*P* value
Evaluation cohort														
ATM rs609429				0.49			0.52		0.70			0.055		0.036
C/C	14	5 (45%)	6 (55%)		3.3 (1.6, 6.0[c])	1 (Reference)		1 (Reference)		4.4 (3.1, 8.3[c])	1 (Reference)		1 (Reference)	
G/C	27	8 (32%)	17 (68%)		2.9 (1.8, 3.4)	1.53 (0.68, 3.43)		1.30 (0.53, 3.19)		12.4 (6.3, 15.6[c])	0.32 (0.11, 0.91)		0.19 (0.05, 0.68)	
G/G	11	2 (20%)	8 (80%)		2.6 (1.2, 4.1)	1.55 (0.59, 4.04)		1.56 (0.56, 4.37)		8.7 (1.2, 14.8[c])	0.52 (0.16, 1.73)		0.39 (0.10, 1.54)	
				0.46			0.25		0.48			0.022		0.020
C/C	14	5 (45%)	6 (55%)		3.3 (1.6, 6.0[c])	1 (Reference)		1 (Reference)		4.4 (3.1, 8.3[c])	1 (Reference)		1 (Reference)	
Any G	38	10 (29%)	25 (71%)		2.7 (2.1, 3.4)	1.53 (0.70, 3.35)		1.37 (0.57, 3.26)		8.7 (6.3, 15.6[c])	0.37 (0.14, 0.99)		0.24 (0.07, 0.80)	
XRCC3 rs861539				0.33			0.024		0.091			0.012		0.056
G/G	35	8 (27%)	22 (73%)		2.3 (1.9, 3.3)	1 (Reference)		1 (Reference)		6.3 (4.9, 12.4[c])	1 (Reference)		1 (Reference)	
G/A	17	7 (44%)	9 (56%)		3.8 (1.6, 6.6)	0.44 (0.21, 0.92)		0.52 (0.24, 1.11)		15.6[c] (8.1, 15.6[c])	0.25 (0.08, 0.79)		0.31 (0.09, 1.03)	
CHEK2 rs2267130				0.075			0.028		0.089			0.38		0.36
T/T	22	3 (15%)	17 (85%)		2.1 (1.6, 2.9)	1 (Reference)		1 (Reference)		8.1 (4.4, 8.7[c])	1 (Reference)		1 (Reference)	
T/C	22	9 (47%)	10 (53%)		3.7 (2.1, 5.2)	0.44 (0.22, 0.89)		0.46 (0.22, 0.94)		12.4 (6.3, 15.6[c])	0.59 (0.22, 1.60)		0.56 (0.20, 1.55)	
C/C	8	3 (43%)	4 (57%)		2.7 (0.9, 4.6[c])	0.62 (0.25, 1.58)		0.59 (0.23, 1.51)		5.7 (1.4, 10.7[c])	1.21 (0.37, 3.99)		1.31 (0.39, 4.42)	
				0.031			0.010		0.031			0.43		0.47
T/T	22	3 (15%)	17 (85%)		2.1 (1.6, 2.9)	1 (Reference)		1 (Reference)		8.1 (4.4, 8.7[c])	1 (Reference)		1 (Reference)	
Any C	30	12 (46%)	14 (54%)		3.4 (2.1, 4.6)	0.48 (0.25, 0.90)		0.49 (0.26, 0.94)		8.3 (5.7, 15.6[c])	0.71 (0.29, 1.74)		0.71 (0.29, 1.77)	
Validation cohort														
ATM rs609429				0.29			0.41		0.23			0.48		0.27
C/C	51	11 (22%)	39 (78%)		1.9 (1.8, 2.3)	1 (Reference)		1 (Reference)		4.4 (3.7, 9.0[c])	1 (Reference)		1 (Reference)	
C/G	63	20 (32%)	43 (68%)		2.0 (1.9, 2.3)	0.76 (0.50, 1.16)		0.77 (0.50, 1.20)		8.7[c] (4.7, 8.7[c])	0.71 (0.40, 1.26)		0.71 (0.39, 1.30)	
G/G	15	6 (40%)	9 (60%)		2.0 (1.2, 6.1)	0.85 (0.45, 1.63)		1.32 (0.67, 2.59)		5.8 (2.7, 7.1[c])	0.82 (0.34, 2.00)		1.43 (0.56, 3.65)	
				0.23			0.20		0.44			0.24		0.44
C/C	51	11 (22%)	39 (78%)		1.9 (1.8, 2.3)	1 (Reference)		1 (Reference)		4.4 (3.7, 9.0[c])	1 (Reference)		1 (Reference)	
Any G	78	26 (33%)	52 (67%)		2.0 (1.9, 2.3)	0.78 (0.52, 1.16)		0.85 (0.56, 1.29)		8.7[c] (5.1, 8.7[c])	0.73 (0.43, 1.25)		0.80 (0.45, 1.41)	
XRCC3 rs861539				0.13			0.40		0.54			0.48		0.76
G/G	50	16 (32%)	34 (68%)		2.1 (1.7, 2.5)	1 (Reference)		1 (Reference)		5.5 (3.6, 8.7[c])	1 (Reference)		1 (Reference)	
G/A	50	16 (33%)	33 (67%)		2.0 (1.9, 2.3)	0.96 (0.62, 1.50)		0.95 (0.59, 1.51)		9.0 (4.6, 9.0[c])	0.75 (0.40, 1.43)		0.79 (0.41, 1.51)	
A/A	25	3 (12%)	22 (88%)		1.9 (1.8, 2.0)	1.34 (0.80, 2.25)		1.26 (0.74, 2.14)		4.2 (3.6, 8.8[c])	1.13 (0.57, 2.22)		0.93 (0.46, 1.88)	
				0.54			0.72		0.82			0.65		0.55
G/G	50	16 (32%)	34 (68%)		2.1 (1.7, 2.5)	1 (Reference)		1 (Reference)		5.5 (3.6, 8.7[c])	1 (Reference)		1 (Reference)	
Any A	75	19 (26%)	55 (74%)		2.0 (1.9, 2.1)	1.07 (0.72, 1.61)		1.05 (0.69, 1.60)		5.8 (4.1, 9.0[c])	0.88 (0.51, 1.54)		0.84 (0.47, 1.49)	
				0.049			0.18		0.28			0.40		0.89
Any G	100	32 (32%)	67 (68%)		2.0 (1.9, 2.3)	1 (Reference)		1 (Reference)		5.8 (4.7, 9.0[c])	1 (Reference)		1 (Reference)	
A/A	25	3 (12%)	22 (88%)		1.9 (1.8, 2.0)	1.37 (0.86, 2.18)		1.30 (0.81, 2.09)		4.2 (3.6, 8.8[c])	1.29 (0.70, 2.39)		1.05 (0.56, 1.97)	
CHEK2 rs2267130				0.76			0.88		0.50			0.97		0.50

Continued

doi:10.1093/annonc/mdx035 | 1019

Table 1. *Continued*

	N	Disease control			Progression-free survival					Overall survival				
		CR/PR/SD[a]	PD	*P* value	Median, months (95% CI)	HR (95% CI)[a]	*P* value	HR (95% CI)[b]	*P* value	Median, months (95% CI)	HR (95% CI)[a]	*P* value	HR (95% CI)[b]	*P* value
T/T	35	8 (24%)	26 (76%)		1.9 (1.9, 2.0)	1 (Reference)		1 (Reference)		5.8 (3.7, 9.0[C])	1 (Reference)		1 (Reference)	
T/C	63	20 (32%)	43 (68%)		2.0 (1.7, 2.3)	1.12 (0.69, 1.79)		1.17 (0.72, 1.91)		8.7[C] (3.8, 8.7[C])	0.93 (0.50, 1.75)		0.83 (0.44, 1.58)	
C/C	31	9 (29%)	22 (71%)		2.0 (1.8, 2.4)	1.08 (0.62, 1.86)		0.87 (0.49, 1.54)		5.4 (3.9, 7.1[C])	0.95 (0.45, 2.00)		0.48 (0.21, 1.09)	
				0.51			0.64		0.83			0.82		0.26
T/T	35	8 (24%)	26 (76%)		1.9 (1.9, 2.0)	1 (Reference)		1 (Reference)		5.8 (3.7, 9.0[C])	1 (Reference)		1 (Reference)	
Any C	94	29 (31%)	65 (69%)		2.0 (1.8, 2.3)	1.10 (0.71, 1.72)		1.05 (0.66, 1.67)		5.5 (4.4, 8.7[C])	0.94 (0.52, 1.69)		0.70 (0.38, 1.30)	
Control cohort														
ATM rs609429				0.89										
C/C	28	8 (32%)	17 (68%)											
C/G	21	8 (38%)	13 (62%)											
G/G	3	1 (33%)	2 (67%)											
				0.77			0.86		0.84			0.45		0.54
C/C	28	8 (32%)	17 (68%)		1.9 (1.6, 2.8)	1 (Reference)		1 (Reference)		4.6 (2.6, 7.6)	1 (Reference)		1 (Reference)	
Any G	24	9 (38%)	15 (63%)		2.0 (1.7, 3.1)	0.95 (0.55, 1.66)		1.06 (0.60, 1.87)		5.9 (3.5, 10.2)	1.91 (0.44, 8.31)		0.84 (0.47, 1.48)	
XRCC3 rs861539				0.77			0.83		0.61			0.56		0.43
G/G	15	4 (27%)	11 (73%)		1.7 (1.0, 2.2)	1 (Reference)		1 (Reference)		4.1 (1.7, 6.0)	1 (Reference)		1 (Reference)	
G/A	30	10 (37%)	17 (63%)		1.9 (1.8, 3.1)	0.83 (0.44, 1.56)		0.92 (0.47, 1.79)		6.8 (3.6, 8.7)	0.86 (0.25, 2.96)		0.76 (0.40, 1.44)	
A/A	7	3 (43%)	4 (57%)		2.7 (1.7, 4.3)	0.83 (0.34, 2.06)		0.62 (0.23, 1.65)		5.4 (2.3, 9.1)	0.34 (0.04, 2.95)		0.54 (0.21, 1.40)	
				0.53			0.54		0.63			0.29		0.27
G/G	15	4 (27%)	11 (73%)		1.7 (1.0, 2.2)	1 (Reference)		1 (Reference)		4.1 (1.7, 6.0)	1 (Reference)		1 (Reference)	
Any A	37	13 (38%)	21 (62%)		2.0 (1.8, 3.1)	0.83 (0.45, 1.53)		0.85 (0.44, 1.63)		5.9 (3.8, 8.0)	0.72 (0.22, 2.39)		0.71 (0.38, 1.31)	
CHEK2 rs2267130				0.79			0.90		0.76			0.70		0.73
T/T	15	4 (31%)	9 (69%)		1.9 (1.4, 3.4)	1 (Reference)		1 (Reference)		3.8 (2.2, 8.0)	1 (Reference)		1 (Reference)	
T/C	26	10 (40%)	15 (60%)		1.8 (1.6, 3.1)	1.02 (0.54, 1.95)		1.15 (0.58, 2.27)		6.2 (2.4, 8.9)	0.77 (0.40, 1.48)		0.76 (0.38, 1.53)	
C/C	11	3 (27%)	8 (73%)		2.0 (1.5, 2.7)	1.18 (0.54, 2.59)		1.35 (0.61, 2.99)		5.7 (2.0, 7.8)	0.89 (0.41, 1.97)		0.90 (0.41, 2.00)	
				1.00			0.83		0.54			0.46		0.52
T/T	15	4 (31%)	9 (69%)		1.9 (1.4, 3.4)	1 (Reference)		1 (Reference)		3.8 (2.2, 8.0)	1 (Reference)		1 (Reference)	
Any C	37	13 (36%)	23 (64%)		1.8 (1.7, 2.8)	1.07 (0.58, 1.95)		1.22 (0.65, 2.28)		5.9 (4.4, 7.9)	0.80 (0.44, 1.48)		0.81 (0.42, 1.54)	

[a]*P* value was based on Fisher's exact test for response, log-rank test in the univariate analysis.

[b]Wald test in the multivariable analysis within Cox regression model adjusted for liver metastasis and adjuvant history in the evaluation cohort; age group (<61 vs. ≥61), liver metastasis, ECOG performance status, previous anti-EGFR in the validation cohort; and ECOG performance status and number of metastases in the control cohort.

[C]Estimates not reached yet.

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

biological function of individual genetic variants. The study referred to *in vitro* data measuring the *ATM* mRNA transcript in a cell line carrying the variants, and demonstrated that the minor allele (G) of *ATM* rs609429 generates a weak 5′ splice site and decreases gene expression [11]. An *in vitro* study examined the mode of action of FTD-induced DNA damage after its incorporation into DNA. The results demonstrated *ATM* phosphorylation in FTD-treated cells, indicating the presence of double strand DNA breaks [12]. The results of two preclinical studies suggest the dominant C allele activates *ATM* phosphorylation corresponding to DSBs induced by FTD incorporation into DNA [11, 12]. Our study revealed that patients carrying the C/C variant had poor outcomes in both the evaluation and validation cohorts treated with TAS-102 though different frequency of the C/C variant in *ATM* rs609429 (26.9% vs. 39.5%) was observed.

XRCC3 is involved in the maintenance of genome stability in HR repair for DNA double-strand breaks [13]. Some studies for gastrointestinal cancer risk have been reported including colorectal cancer [14, 15] and gastric cancer [16]. Jiang et al. carried out a meta-analysis to evaluate the role of *XRCC1* and *XRCC3* genotypes in CRC susceptibility, and identified no association between *XRCC3* T241M (rs861539) and *XRCC1* with colorectal cancer risk [14]. Wang et al. derived discordant results from a meta-analysis, where a possible correlation between *XRCC3* T241M (rs861539) Met/Met (A/A) variant and an elevated risk of CRC was reported in a subgroup of Asian population [15]. No evidence of association of the SNPs with response to chemotherapy or clinical outcomes was observed in the studies.

In our study, we observed an ethnic difference in the allele frequency in *XRCC3* rs861539 that showed the lack of A/A variant (0% vs. 20.0%) and high frequency of the G/G variant (67.3% vs. 40.0%) in the evaluation cohort compared with those of the validation cohort. However, a trend toward shorter OS in the G/G variant compared with the G/A variant was confirmed in the validation cohort when we excluded the A/A variant from the analysis. These results are consistent with those observed in the evaluation cohort, although not statistically significant.

Taken together, our data and previously reported studies demonstrate the dominant wild-type variants, C/C in *ATM* rs609429 and G/G in *XRCC3* rs861539, affect chemosensitivity to TAS-102 treatment leading to shorter PFS or OS. This is likely because of insufficient DNA repair for DSBs by FTD incorporation into DNA. In addition, we found an association between grade 3≤ neutropenia and *ATM* rs609429. This could be explained by the maintenance of increased FTD concentration in tumor cells of patients carrying the G allele compared with the concentration level in those with the C/C variant. These results require further testing in a clinical study to compare the effect of the combination of TAS-102 with biologic agents targeting HR-related genes (such as ATM-inhibitor) with TAS-102 alone with respect to survival [17, 18].

Our study is limited by a retrospective study design with limited sample size especially in evaluation cohort and insufficient evidence that explains the function of the SNPs. However, we further demonstrated the DNA repair mechanism and identified candidate markers for clinical outcomes in mCRC patients treated with TAS-102. This was achieved by comparing three independent cohorts including an evaluation cohort for discovery, a control cohort with comparable clinical characteristics and disease stage, and a larger validation cohort with comparable clinical characteristics that received the same treatment.

In conclusion, genetic variants in the HR pathway, *ATM* rs609429 and *XRCC3* rs861539, may serve as potential predictive and prognostic markers in refractory mCRC patients receiving TAS-102. These results also provide insight for novel formulations for combination treatment with TAS-102.

Acknowledgements

MS is the recipient of Takashi Tsuruo Memorial Fund. MDB received grants from the Swiss Cancer League (BIL KLS-3334-02-2014) and the Werner and Hedy Berger-Janser Foundation for cancer research. YM received a grant from the Japan Society for the Promotion of Science (S2606).

Funding

This work was partially supported by the National Institutes of Health (P30CA014089-27S1), the Gloria Borges Wunderglo Project, the Dhont Family Foundation, the Dave Butler Research Fund, and the Call to Cure Research Fund.

Disclosure

The authors have declared no conflicts of interest.

References

1. Hong DS, Abbruzzese JL, Bogaard K et al. Phase I study to determine the safety and pharmacokinetics of oral administration of TAS-102 in patients with solid tumors. Cancer 2006; 107: 1383–1390.
2. Mayer RJ, Van Cutsem E, Falcone A et al. Randomized trial of TAS-102 for refractory metastatic colorectal cancer. N Engl J Med 2015; 372: 1909–1919.
3. Temmink OH, Emura T, de Bruin M et al. Therapeutic potential of the dual-targeted TAS-102 formulation in the treatment of gastrointestinal malignancies. Cancer Sci 2007; 98: 779–789.
4. Bijnsdorp IV, Peters GJ, Temmink OH et al. Differential activation of cell death and autophagy results in an increased cytotoxic potential for trifluorothymidine compared to 5-fluorouracil in colon cancer cells. Int J Cancer 2010; 126: 2457–2468.
5. Suzuki N, Nakagawa F, Nukatsuka M, Fukushima M. Trifluorothymidine exhibits potent antitumor activity via the induction of DNA double-strand breaks. Exp Ther Med 2011; 2: 393–397.
6. Branzei D, Foiani M. Regulation of DNA repair throughout the cell cycle. Nat Rev Mol Cell Biol 2008; 9: 297–308.
7. Liu J, Ehmsen KT, Heyer WD, Morrical SW. Presynaptic filament dynamics in homologous recombination and DNA repair. Crit Rev Biochem Mol Biol 2011; 46: 240–270.
8. Krajewska M, Fehrmann RS, de Vries EG, van Vugt MA. Regulators of homologous recombination repair as novel targets for cancer treatment. Front Genet 2015; 6: 96.
9. Jasin M, Rothstein R. Repair of strand breaks by homologous recombination. Cold Spring Harb Perspect Biol 2013; 5: a012740.
10. Landi S, Gemignani F, Canzian F et al. DNA repair and cell cycle control genes and the risk of young-onset lung cancer. Cancer Res 2006; 66: 11062–11069.
11. Angèle S, Romestaing P, Moullan N et al. ATM haplotypes and cellular response to DNA damage: association with breast cancer risk and clinical radiosensitivity. Cancer Res 2003; 63: 8717–8725.

12. Peng Z, Liao Z, Dziegielewska B et al. Phosphorylation of serine 51 regulates the interaction of human DNA ligase I with replication factor C and its participation in DNA replication and repair. J Biol Chem 2012; 287: 36711–36719.
13. Goode EL, Ulrich CM, Potter JD. Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev 2002; 11: 1513–1530.
14. Jiang Z, Li C, Xu Y, Cai S. A meta-analysis on XRCC1 and XRCC3 polymorphisms and colorectal cancer risk. Int J Colorectal Dis 2010; 25: 169–180.
15. Wang Z, Zhang W. Association between XRCC3 Thr241Met polymorphism and colorectal cancer risk. Tumour Biol 2013; 34: 1421–1429.
16. Wang Z, Chen X, Liu B et al. Quantitative assessment of the associations between DNA repair gene XRCC3 Thr241Met polymorphism and gastric cancer. Tumour Biol 2014; 35: 1589–1598.
17. Hickson I, Zhao Y, Richardson CJ et al. Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. Cancer Res 2004; 64: 9152–9159.
18. Batey MA, Zhao Y, Kyle S et al. Preclinical evaluation of a novel ATM inhibitor, KU59403, in vitro and in vivo in p53 functional and dysfunctional models of human cancer. Mol Cancer Ther 2013; 12: 959–967.