British Journal of Cancer

ARTICLE

Translational Therapeutics

Germline polymorphisms in genes maintaining the replication fork predict the efficacy of oxaliplatin and irinotecan in patients with metastatic colorectal cancer

Hiroyuki Arai[1], Yi Xiao[2], Joshua Millstein[2], Jingyuan Wang[1], Francesca Battaglin[1], Natsuko Kawanishi[1], Priya Jayachandran[1], Shivani Soni[1], Wu Zhang[1], Christoph Mancao[3], Bodour Salhia[4], Shannon M. Mumenthaler[5], Aparna R. Parikh[6] and Heinz-Josef Lenz[1]✉

BACKGROUND: The TIMELESS–TIPIN complex protects the replication fork from replication stress induced by chemotherapeutic drugs. We hypothesised genetic polymorphisms of the TIMELESS–TIPIN complex may affect the response, progression-free survival (PFS), and overall survival (OS) of cytotoxic drugs in patients with metastatic colorectal cancer (mCRC).
METHODS: We analysed data from the MAVERICC trial, which compared FOLFOX/bevacizumab and FOLFIRI/bevacizumab in untreated patients with mCRC. Genomic DNA extracted from blood samples was genotyped using an OncoArray. Eight functional single nucleotide polymorphisms (SNPs) in *TIMELESS* and *TIPIN* were tested for associations with clinical outcomes.
RESULTS: In total, 324 patients were included (FOLFOX/bevacizumab arm, $n = 161$; FOLFIRI/bevacizumab arm, $n = 163$). In the FOLFOX/bevacizumab arm, no SNPs displayed confirmed associations with survival outcomes. In the FOLFIRI/bevacizumab arm, *TIMELESS* rs2291739 was significantly associated with OS in multivariate analysis (G/G vs. any A allele, hazard ratio = 3.06, 95% confidence interval = 1.49–6.25, $p = 0.004$). *TIMELESS* rs2291739 displayed significant interactions with treatment regarding both PFS and OS.
CONCLUSIONS: *TIMELESS* rs2291739 might have different effects on therapeutic efficacy between oxaliplatin- and irinotecan-based chemotherapies. Upon further validation, our findings may be useful for personalised approaches in the first-line treatment of mCRC.

British Journal of Cancer (2022) 126:72–78; https://doi.org/10.1038/s41416-021-01592-7

INTRODUCTION

Replication stress is a hallmark of cancer development [1, 2]. Endogenous and exogenous sources of replication stress promote genomic instability by leading to DNA replication fork collapse and subsequent DNA double-strand breaks (DSBs) [3]. Whereas cells that can activate an adequate DNA damage response (DDR) to DNA damage undergo apoptosis, cells with a faulty DDR system escape from apoptosis and develop mutations and chromosome aberrations that result in tumorigenesis [4, 5]. In this context, cancer cells harbour distinct molecular backgrounds adaptive to oncogene-induced replication stress, thereby shaping an environment that favours survival and encourages tumour growth [6, 7]. Recently, DDR pathway alteration has been targeted in the treatment of cancer using the concept of synthetic lethality [8]. One great success of this strategy is PARP inhibition in ovarian, breast, prostate, and pancreatic cancers with homologous recombination deficiency [9].

TIMELESS and TIMELESS-interacting protein (TIPIN), which form a complex, are components of the replication fork machinery [10]. The TIMELESS–TIPIN complex contributes to full activation of the ATR–Chk1 checkpoint signalling pathway, which plays a central role in preventing fork collapse [11]. In addition to their role in the ATR–Chk1 pathway, TIMELESS and TIPIN also interact with numerous components of the replication machinery, such as the replicative helicase components MCM2-7 and CDC45 and replicative polymerases Polε and Polδ, thereby stabilising the replication fork structure [12]. When DNA lesions transiently stall the leading-strand polymerase without impeding the movement of the rest of the replisome, uncoupling between DNA polymerase and helicase activities can be induced [13]. The TIMELESS–TIPIN complex can sense functional uncoupling of the replisome to transduce a signal to remodel and restart the fork [14]. Otherwise, in the absence of the TIMELESS–TIPIN complex, the fork collapses,

[1]Division of Medical Oncology, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA. [2]Department of Preventive Medicine, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA. [3]Oncology Biomarker Development, Genentech Inc, Basel, Switzerland. [4]Department of Translational Genomics, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA. [5]Lawrence J. Ellison Institute for Transformative Medicine, University of Southern California, Los Angeles, CA, USA. [6]Division of Hematology and Oncology, Massachusetts General Hospital, Boston, MA, USA. ✉email: lenz@usc.edu

Received: 28 July 2021 Revised: 27 September 2021 Accepted: 6 October 2021
Published online: 23 October 2021

and in turn, harmful DSBs are generated [14]. Thus, TIMELESS and TIPIN are necessary for cell survival under replication stress because of their roles in protecting the replication fork in both ATR-dependent and ATR-independent manners. Tumours, such as colorectal cancer (CRC), overexpress these molecules, and this overexpression may serve as a supportive mechanism against replication stress in cancer cells [15–17].

In the standard first-line treatment of metastatic CRC (mCRC), oxaliplatin or irinotecan is used as the cytotoxic agent in combination with 5-fluorouracil. The choice of these agents remains an important clinical question for optimising treatment in individual patients. Oxaliplatin and irinotecan form different structures on the DNA strands which prevent DNA replication in different manners [18, 19]. Thus, although fork protection is a global response to genotoxic treatments, it might be possible that oxaliplatin and irinotecan induce distinct responses that protect the replication fork in tumours [20]. We hypothesised that common and functional single nucleotide polymorphisms (SNPs) within *TIMELESS* and *TIPIN* are associated with the different therapeutic effects of oxaliplatin- and irinotecan-based chemotherapies in mCRC. We tested our hypothesis using genetic and clinical data from the MAVERICC trial, a randomised phase II clinical trial of patients with mCRC in the first-line setting [21].

PATIENTS AND METHODS

Patient population and study design

The subjects of this study were patients with mCRC enrolled in the MAVERICC trial (NCT01374425). Patients were randomised to treatment with either FOLFOX (oxaliplatin cohort) or FOLOFIRI (irinotecan cohort), both in combination with bevacizumab. Patients without sufficient peripheral whole blood samples, SNP data, and/or any other relevant data were excluded from this study. All patients provided informed consent for molecular research before study enrollment. The study protocol was approved by the institutional review board of each participating institution and was conducted in accordance with the tenets of the Declaration of Helsinki as well as the Good Clinical Practice and REMARK guidelines.

Genotyping and selecting polymorphisms

Genomic DNA was extracted from peripheral whole blood collected before treatment initiation using a QIAmp Kit (Qiagen, Inc., Valencia, CA, USA) in accordance with the manufacturer's protocol. The OncoArray of 530 K SNPs was used for genotyping (Illumina, Inc., San Diego, CA, USA). The candidate SNPs within *TIMELESS* and *TIPIN* were selected from dbSNP variants (http://www.ncbi.nlm.nih.gov) if the SNPs met both following criteria: (1) minor allele frequency in Caucasians (defined as 'European' in 1000 Genomes Project Phase 3 and/or 'Non-Finnish European' in gnomAD genomes r3.0) of at least 10% in the Ensemble Genome Browser (https://www.ensembl.org) and (2) missense, 3′-untranslated region (UTR), or intron/5′-UTR variants having potential biological functions based on public databases (https://snpinfo.niehs.nih.gov). SNPs exhibiting linkage disequilibrium with $R^2 > 0.8$ (https://ldlink.nci.nih.gov, among the population of 'European') were excluded. In total, eight SNPs (rs2291739, rs3759786, rs8035497, rs11071888, rs28593577, rs11637949, rs6494568, and rs12323975) met the criteria for inclusion in this study (Table S1).

Statistical analysis

The selected SNPs were evaluated for their associations with tumour response, progression-free survival (PFS), and overall survival (OS) based on the dominant and recessive genetic models. The overall response rate (ORR) was calculated as the percentage of patients with a complete or partial response using Response Evaluation Criteria in Solid Tumors version 1.1. PFS was defined as the time from randomisation to disease progression or death from any cause. OS was defined as the time from randomisation to death from any cause. Patients who did not experience any events were censored at the last follow-up date. The correlations of SNPs with ORR were examined using the likelihood ratio test. To test the associations of SNPs with PFS or OS, the Cox proportional hazards regression model and the log-rank test were performed. Multivariate analyses based on the Wald test were performed for tumour response, PFS, and OS. In the multivariate analyses, adjustment was performed for the following covariates: ethnicity, sex, age, Eastern Cooperative Oncology Group performance status, primary tumour site, primary tumour resected, number of metastases, and *KRAS* status. To formally assess the predictive value of SNPs, the treatment-by-SNP interaction was tested based on the multivariate analysis. All analyses were two-sided at a significance level of 0.05 and were performed using SAS ver. 9.4 software (SAS Institute, Cary, NC, USA).

RESULTS

Patient characteristics

In total, 324 patients were included in this study, including 161 patients in the oxaliplatin cohort and 163 patients in the irinotecan cohort (Fig. S1). Patient characteristics were balanced between the cohorts, excluding the higher proportion of patients aged ≤65 in the oxaliplatin cohort (Table 1).

Associations of SNPs in *TIMELESS* and *TIPIN* with clinical outcomes in the oxaliplatin cohort

In the oxaliplatin cohort, univariate analysis did not show significant associations between the tested SNPs and tumour response, whereas *TIPIN* rs11637949 was significantly associated with tumour response in multivariate analysis (Table 2, Tables S2, S3). Conversely, two SNPs exhibited significant associations with survival outcomes in univariate analysis (Tables 3, 4, Tables S2, S3). Specifically, the G/G genotype of *TIMELESS* rs2291739 was associated with better PFS than any A allele (median PFS, 13.9 months vs. 9.5 months, hazard ratio [HR] = 0.51, 95% confidence interval [CI] = 0.32–0.83, $p = 0.006$), and the A/A genotype of *TIPIN* rs8035497 was linked to worse OS than any G allele (median OS, 19.2 months vs. 25.5 months, HR = 1.96, 95% CI = 1.02–3.75, $p = 0.04$). However, these associated were not confirmed in multivariate analysis (Tables 3, 4, Table S3).

Associations of SNPs in *TIMELESS* and *TIPIN* with clinical outcomes in the irinotecan cohort

In the irinotecan cohort, univariate and multivariate analyses did not show significant associations between the tested SNPs and tumour response or PFS (Tables 2, 3, Table S4, S5). Conversely, two SNPs exhibited significant associations with OS in univariate analysis univariate (Table 4, Table S4, S5). Specifically, any G allele of *TIPIN* rs11637949 was linked to worse OS than the A/A genotype using the dominant genetic model (median OS, 23.8 months vs. 32.3 months, HR = 1.95, 95% CI = 1.17–3.24, $p = 0.009$), and the G/G genotype of *TIMELESS* rs2291739 was linked to worse OS than any A allele using the recessive genetic model (median OS, 21.3 months vs. 31.3 months, HR = 2.01, 95% CI = 1.19–3.41, $p = 0.008$, Fig. 1). Multivariate analysis confirmed the significant association between *TIMELESS* rs2291739 and OS (G/G vs. any A allele, HR = 3.06, 95% CI = 1.49–6.25, $p = 0.004$, Table 4, Table S5).

Comparing treatment efficacy between FOLFOX/bevacizumab and FOLFIRI/bevacizumab by *TIMELESS* rs2291739 genotype

In the patients having any A allele of *TIMELESS* rs2291739, FOLFIRI/bevacizumab showed significantly better OS (median OS, 31.3 months vs. 22.8 months, HR = 0.56, 95% CI = 0.38–0.83, $p = 0.004$) and PFS (median PFS, 14.0 months vs. 9.5 months, HR = 0.57, 95% CI = 0.42–0.78, $p = <0.001$) than FOLFOX/bevacizumab (Fig. S2A, B). In contrast, in the patients harbouring G/G genotype of *TIMELESS* rs2291739, FOLFIRI/bevacizumab showed worse OS (median OS, 21.3 months vs. 28.8 months, HR = 1.80, 95% CI = 0.90–3.59, $p = 0.09$) and PFS (median PFS, 9.5 months vs. 13.9 months, HR = 1.62, 95% CI = 0.92–2.85, $p = 0.10$) than FOLFOX/bevacizumab (Fig. S2C, D).

Table 1. Patient characteristics.

Characteristics	Total $N = 324$	MAVERICC		*P*-value
		FOLFOX + BEV (Oxaliplatin cohort) $N = 161$	FOLFIRI + BEV (Irinotecan cohort) $N = 163$	
Sex				0.93
Male	204	101 (62.7%)	103 (63.2%)	
Female	120	60 (37.3%)	60 (36.8%)	
Age				0.04
≤65	218	117 (72.7%)	101 (62.0%)	
>65	106	44 (27.3%)	62 (38.0%)	
Performance status				0.11
ECOG 0	178	81 (50.3%)	97 (59.5%)	
ECOG 1	145	79 (49.1%)	66 (40.5%)	
Unknown*	1	1 (0.6%)	0 (0%)	
Primary tumour site				0.80
Right-sided	131	64 (39.8%)	67 (41.1%)	
Left-sided	193	97 (60.2%)	96 (58.9%)	
Number of metastases				0.67
≤2	207	101 (62.7%)	106 (65.0%)	
>2	117	60 (37.3%)	57 (35.0%)	
Primary tumour resected				0.50
No	301	148 (91.9%)	153 (93.9%)	
Yes	23	13 (8.1%)	10 (6.1%)	
Adjuvant chemotherapy				0.39
No	289	146 (90.7%)	143 (87.7%)	
Yes	35	15 (9.3%)	20 (12.3%)	

P-values was estimated by Chi-square test.
BEV bevacizumab, *ECOG* Eastern Cooperative Oncology Group.
*Unknown group was not included in the analysis.

Table 2. Univariate and multivariate analyses for the association between SNPs and tumour response.

SNP/genetic model	Oxaliplatin cohort		Irinotecan cohort	
	Univariate analysis P^a	Multivariate analysis P^b	Univariate analysis P^a	Multivariate analysis P^b
TIMELESS rs2291739				
Dominant	0.64	0.85	0.90	0.89
Recessive	0.39	0.84	0.79	0.29
TIPIN rs3759786				
Dominant	0.97	0.90	0.80	0.28
Recessive	NA	NA	NA	NA
TIPIN rs8035497				
Dominant	0.63	0.63	0.87	0.61
Recessive	0.52	0.55	0.43	0.21
TIPIN rs11071888				
Dominant	0.47	0.77	0.22	0.56
Recessive	0.63	0.92	0.56	0.96
TIPIN rs28593577				
Dominant	0.71	0.91	0.96	0.83
Recessive	1.00	0.65	0.70	0.30
TIPIN rs11637949				
Dominant	0.61	0.23	0.73	0.68
Recessive	0.06	**0.04**	0.83	0.56
TIPIN rs6494568				
Dominant	0.72	0.25	0.74	0.50
Recessive	NA	NA	NA	NA
TIPIN rs12323975				
Dominant	0.79	0.79	0.56	0.58
Recessive	NA	NA	NA	NA

Significant values are indicated in bold characters. *TIPIN* rs3759786, *TIPIN* rs6494568, and *TIPIN* rs12323975 were not assessed with recessive genetic model because there were no patients having homozygous genotype of the recessive allele in these SNPs.
NA not assessed, *SNP* single nucleotide polymorphism.
[a]*P*-values were based on likelihood ratio test.
[b]*P*-values were based on Wald test in the multivariate model.

Table 3. Univariate and multivariate analyses for the association between SNPs and PFS.

SNP/genetic model	Oxaliplatin cohort				Irinotecan cohort			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	P^a	HR (95% CI)	P^b	HR (95% CI)	P^a	HR (95% CI)	P^b
TIMELESS rs2291739								
Dominant	1.03 (0.69–1.55)	0.87	1.25 (0.73–2.15)	0.41	1.44 (0.90–2.30)	0.13	1.26 (0.73–2.16)	0.40
Recessive	0.51 (0.32–0.83)	**0.006**	0.60 (0.31–1.17)	0.12	1.48 (0.97–2.26)	0.07	1.49 (0.77–2.87)	0.25
TIPIN rs3759786								
Dominant	0.78 (0.47–1.29)	0.33	0.88 (0.48–1.64)	0.69	0.90 (0.57–1.42)	0.65	1.06 (0.59–1.90)	0.85
Recessive	NA	NA	NA	NA	NA	NA	NA	NA
TIPIN rs8035497								
Dominant	1.22 (0.86–1.75)	0.27	1.27 (0.81–1.99)	0.30	0.87 (0.59–1.27)	0.46	0.80 (0.47–1.35)	0.40
Recessive	1.20 (0.68–2.15)	0.53	1.31 (0.66–2.60)	0.44	1.20 (0.58–2.49)	0.62	0.93 (0.37–2.38)	0.89
TIPIN rs11071888								
Dominant	0.84 (0.57–1.24)	0.37	0.90 (0.52–1.56)	0.70	1.12 (0.77–1.65)	0.55	1.04 (0.61–1.76)	0.90
Recessive	0.18 (0.03–1.29)	0.05	0.23 (0.03–1.86)	0.09	1.59 (0.65–3.93)	0.31	0.84 (0.24–2.91)	0.79
TIPIN rs28593577								
Dominant	1.02 (0.71–1.46)	0.92	1.06 (0.67–1.68)	0.80	0.92 (0.62–1.38)	0.70	0.85 (0.50–1.45)	0.55
Recessive	1.24 (0.63–2.45)	0.54	1.62 (0.76–3.46)	0.24	1.22 (0.56–2.64)	0.61	0.99 (0.39–2.52)	0.99
TIPIN rs11637949								
Dominant	1.31 (0.91–1.89)	0.15	1.27 (0.80–1.99)	0.31	1.23 (0.84–1.80)	0.28	1.17 (0.71–1.91)	0.54
Recessive	1.41 (0.68–2.92)	0.35	1.61 (0.69–3.78)	0.29	1.04 (0.53–2.08)	0.90	0.91 (0.41–2.02)	0.81
TIPIN rs6494568								
Dominant	1.26 (0.81–1.95)	0.31	1.11 (0.60–2.06)	0.74	0.84 (0.51–1.37)	0.48	0.88 (0.42–1.81)	0.72
Recessive	NA	NA	NA	NA	NA	NA	NA	NA
TIPIN rs12323975								
Dominant	0.86 (0.53–1.40)	0.55	0.83 (0.44–1.56)	0.56	1.16 (0.69–1.95)	0.58	0.74 (0.37–1.51)	0.40
Recessive	NA	NA	NA	NA	NA	NA	NA	NA

Significant values are indicated in bold characters. *TIPIN* rs3759786, *TIPIN* rs6494568, and *TIPIN* rs12323975 were not assessed with recessive genetic model because there were no patients having homozygous genotype of the recessive allele in these SNPs.
CI confidence interval, *HR* hazard ratio, *NA* not assessed, *PFS* progression-free survival, *SNP* single nucleotide polymorphism.
[a]*P*-values were based on log-rank test.
[b]*P*-values were based on Wald test in the multivariate Cox proportional hazards regression model.

Treatment-by SNP interaction

In the dominant genetic model, *TIPIN* rs8035497 had a significant interaction with treatment in terms of OS. In the recessive genetic model, three SNPs exhibited significant interactions with treatment: *TIPIN* rs8035497 and *TIPIN* rs28593577 in terms of OS and *TIMELESS* rs2291739 in terms of both OS and PFS (Table 5).

DISCUSSION

Our findings revealed for the first time that genetic variations in the TIMELESS–TIPIN complex are associated with survival outcomes in patients with mCRC treated with irinotecan-based first-line chemotherapy. Our findings highlight the significance of this pathway and its impact on therapeutic efficacy based on the difference in outcomes between irinotecan- and oxaliplatin-based chemotherapies. These data suggest that genes regulating the replication fork in tumour cells are related to the distinct response to cytotoxic agents with different DNA-damaging activities.

We identified a significant association of *TIMELESS* rs2291739 with OS in patients treated with FOLFIRI plus bevacizumab using the recessive genetic model, and this finding was confirmed in multivariate analyses. The association with PFS was consistent with the OS data, but statistical significance was not reached. Meanwhile, no confirmed associations with clinical outcomes were observed in patients treated with FOLFOX plus bevacizumab. Of note, *TIMELESS* rs2291739 displayed significant interactions with treatment in terms of both PFS and OS.

Our findings are supported by the consistent mechanisms of action and tumour biology. First, *TIMELESS* rs2291739 is a missense variant that causes a protein function-altering amino acid change. Second, the molecular mechanisms protecting the replication fork are differently affected by irinotecan and oxaliplatin even though both drugs are DNA-damaging agents. Irinotecan is a topoisomerase I (TOP1) inhibitor that inhibits the dissociation of the TOP1 cleavage complex (TOP1-cc), a transient structure formed in front of the replication fork that permits TOP1 to resolve topological stress [22]. In the presence of irinotecan, stabilised TOP1-cc collides with the replication fork during DNA replication and transcription, resulting in DSBs [19]. TIMELESS was identified as a TOP1-binding factor [23]. Interestingly, a previous study revealed that the TIMELESS–TIPIN complex destabilises TOP1-cc by interacting with TOP1, which prevents the generation of irinotecan-induced DSBs [24]. These data support our findings of an association between a functional SNP of *TIMELESS* and OS of irinotecan-treated patients. Specifically, patients with the *TIMELESS* rs2291739 G/G genotype had worse OS than those with any A allele, suggesting that the G/G genotype is associated with increased function of the TIMELESS–TIPIN complex, thereby protecting cancer cells against irinotecan-induced cytotoxicity. Meanwhile, oxaliplatin is a

Table 4. Univariate and multivariate analyses for the association between SNPs and OS.

SNP/ genetic model	Oxaliplatin cohort				Irinotecan cohort			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	P^a	HR (95% CI)	P^b	HR (95% CI)	P^a	HR (95% CI)	P^b
TIMELESS rs2291739								
Dominant	1.03 (0.64–1.66)	0.90	1.41 (0.76–2.60)	0.27	1.32 (0.70–2.48)	0.39	1.47 (0.73–2.96)	0.27
Recessive	0.63 (0.34–1.14)	0.12	0.83 (0.36–1.94)	0.66	2.01 (1.19–3.41)	**0.008**	3.06 (1.49–6.25)	**0.004**
TIPIN rs3759786								
Dominant	0.78 (0.42–1.45)	0.44	0.68 (0.32–1.42)	0.28	0.80 (0.42–1.50)	0.48	1.03 (0.47–2.27)	0.94
Recessive	NA	NA	NA	NA	NA	NA	NA	NA
TIPIN rs8035497								
Dominant	1.13 (0.73–1.75)	0.59	1.41 (0.81–2.43)	0.22	0.59 (0.35–1.01)	0.05	0.53 (0.26–1.10)	0.08
Recessive	1.96 (1.02–3.75)	**0.04**	2.02 (0.93–4.42)	0.09	0.21 (0.03–1.52)	0.09	0.20 (0.02–1.57)	0.06
TIPIN rs11071888								
Dominant	0.64 (0.39–1.06)	0.08	0.63 (0.32–1.24)	0.17	1.32 (0.80–2.20)	0.28	0.85 (0.41–1.76)	0.66
Recessive	0.46 (0.06–3.30)	0.43	0.42 (0.05–3.57)	0.38	2.01 (0.73–5.58)	0.17	0.62 (0.15–2.54)	0.50
TIPIN rs28593577								
Dominant	0.87 (0.56–1.36)	0.54	1.00 (0.58–1.75)	0.99	0.78 (0.45–1.35)	0.37	0.64 (0.30–1.35)	0.23
Recessive	1.52 (0.69–3.33)	0.29	1.42 (0.59–3.40)	0.45	0.24 (0.03–1.74)	0.13	0.21 (0.03–1.67)	0.07
TIPIN rs11637949								
Dominant	1.04 (0.66–1.64)	0.86	1.01 (0.58–1.76)	0.96	1.95 (1.17–3.24)	**0.009**	1.81 (0.93–3.51)	0.08
Recessive	1.87 (0.81–4.32)	0.14	2.16 (0.84–5.55)	0.14	1.38 (0.59–3.21)	0.45	1.10 (0.43–2.82)	0.84
TIPIN rs6494568								
Dominant	1.39 (0.83–2.33)	0.21	0.99 (0.47–2.07)	0.98	0.92 (0.48–1.76)	0.79	2.18 (0.91–5.19)	0.09
Recessive	NA	NA	NA	NA	NA	NA	NA	NA
TIPIN rs12323975								
Dominant	0.70 (0.38–1.29)	0.25	0.67 (0.30–1.46)	0.29	1.21 (0.61–2.39)	0.58	0.54 (0.19–1.49)	0.21
Recessive	NA	NA	NA	NA	NA	NA	NA	NA

Significant values are indicated in bold characters. *TIPIN* rs3759786, *TIPIN* rs6494568, and *TIPIN* rs12323975 were not assessed with recessive genetic model because there were no patients having homozygous genotype of the recessive allele in these SNPs.
CI confidence interval, *HR* hazard ratio, *NA* not assessed, *OS* overall survival, *SNP* single nucleotide polymorphism.
[a]*P*-values were based on log-rank test.
[b]*P*-values were based on Wald test in the multivariate Cox proportional hazards regression model.

platinum compound forming two types of DNA crosslinks: intra-strand crosslinks on the same strand of DNA and inter-strand crosslinks between the two complementary strands of DNA [18, 25]. Platinum drugs produce a high proportion of intra-strand crosslinks, which contribute to their cytotoxic activity [26]. This type of crosslinks can avoid the development of DSBs if uncoupling activities between DNA polymerase and helicase are properly sensed by the TIMELESS–TIPIN complex in associated with checkpoint mechanisms [13]. Conversely, inter-strand crosslinks are more toxic because they physically block DNA replication and induce DSBs by covalently linking both DNA strands [27]. The repair of inter-strand crosslinks is highly dependent on homologous recombination and Fanconi anaemia pathways [28, 29]. We speculate that these mechanisms of inter-strand crosslinks, which are independent of TIMELESS–TIPIN complex function, attenuated the impact of *TIMELESS* rs2291739 on clinical outcomes in oxaliplatin-treated patients. Based on these molecular bases, we supposed that the function of the TIMELESS–TIPIN complex is more relevant to the efficacy of irinotecan than to that of oxaliplatin.

Our findings suggest a novel approach of clinical decision making based on the *TIMELESS* rs2291739 genotype. Specifically, FOLFIRI plus bevacizumab may be a favourable treatment option for the patients with any A allele because our results showed it led to better survival outcomes compared to FOLFOX plus bevacizumab in this patient subset. In contrast, FOLFOX plus bevacizumab may be favourable for the patients with G/G genotype because this patient subset was more likely to benefit from FOLFOX plus bevacizumab than from FOLFIRI plus bevacizumab. This personalised strategy in the choice of backbone chemotherapy should be assessed in further clinical studies.

This study had several limitations. Because of the retrospective design, the results require validation in prospective clinical trials. Furthermore, we tested the association between SNPs and the efficacy of oxaliplatin and irinotecan in one study cohort, however, these data are preliminary and the predictive value of SNPs need to be confirmed. Thus, further validation studies are needed. However, the significant results demonstrated by the formal interaction test in this study support the predictive potential of *TIMELESS* rs2291739 concerning the selection of cytotoxic agents in the first-line setting.

In conclusion, our study provided the first evidence that germline polymorphisms in replication fork-protecting genes were associated with the efficacy of FOLFIRI plus bevacizumab, but not with that of FOLFOX plus bevacizumab, in patients with mCRC. Our findings may be useful for personalised approaches in selecting cytotoxic drugs in the first-line treatment of mCRC, and validation in prospective studies is warranted.

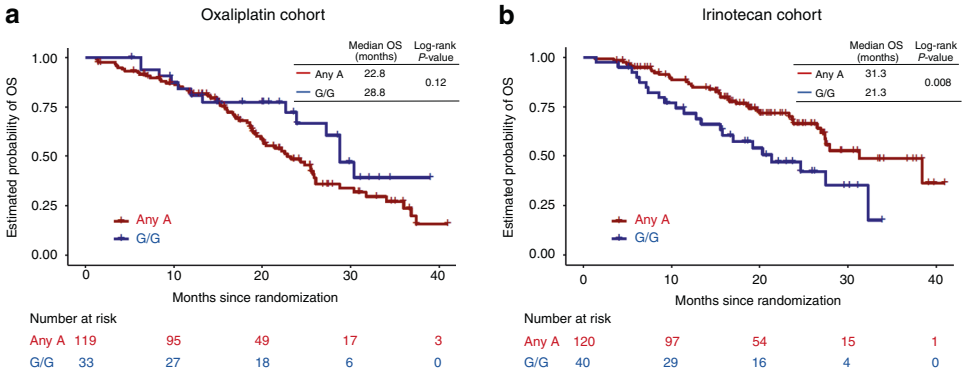

Fig. 1 Overall survival of patients *TIMELESS* rs2291739 variants. a Oxaliplatin cohort (FOLFOX plus bevacizumab arm). **b** Irinotecan cohort (FOLFIRI plus bevacizumab arm). Abbreviations: OS, overall survival.

Table 5. Treatment-by-SNP interaction test.

SNP	Dominant genetic model			Recessive genetic model		
	TR	PFS	OS	TR	PFS	OS
	Interaction *P*	Interaction *P*	Interaction *P*	Interaction *P*	Interaction *P*	Interaction *P*
TIMELESS rs2291739	0.73	0.86	0.90	0.44	**0.04**	**0.007**
TIPIN rs3759786	0.54	0.73	0.53	NA	NA	NA
TIPIN rs8035497	0.34	0.07	**0.02**	0.87	0.55	**0.01**
TIPIN rs11071888	0.90	0.28	0.49	0.91	0.08	0.67
TIPIN rs28593577	0.66	0.28	0.24	0.55	0.45	**0.04**
TIPIN rs11637949	0.61	0.99	0.15	0.12	0.73	0.49
TIPIN rs6494568	0.65	0.42	0.27	NA	NA	NA
TIPIN rs12323975	0.65	0.40	1.00	NA	NA	NA

Significant values are indicated in bold characters. *TIPIN* rs3759786, *TIPIN* rs6494568, and *TIPIN* rs12323975 were not assessed with recessive genetic model because there were no patients having homozygous genotype of the recessive allele in these SNPs.
NA not assessed, *OS* overall survival, *PFS* progression-free survival, *SNP* single nucleotide polymorphism, *TR* tumour response.

DATA AVAILABILITY

The data sets used and analysed during this study are available from the corresponding author on reasonable request.

REFERENCES

1. Negrini S, Gorgoulis VG, Halazonetis TD. Genomic instability-an evolving hallmark of cancer. Nat Rev Mol Cell Biol. 2010;11:220–8. https://doi.org/10.1038/nrm2858.
2. Macheret M, Halazonetis TD. DNA replication stress as a hallmark of cancer. Annu Rev Pathol. 2015;10:425–48. https://doi.org/10.1146/annurev-pathol-012414-040424.
3. Berti M, Cortez D, Lopes M. The plasticity of DNA replication forks in response to clinically relevant genotoxic stress. Nat Rev Mol Cell Biol. 2020;21:633–51. https://doi.org/10.1038/s41580-020-0257-5.
4. Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, Luise C, et al. Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. Nature. 2006;444:638–42. https://doi.org/10.1038/nature05327.
5. Bartkova J, Horejsi Z, Koed K, Kramer A, Tort F, Zieger K, et al. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature. 2005;434:864–70. https://doi.org/10.1038/nature03482.
6. Lecona E, Fernandez-Capetillo O. Replication stress and cancer: it takes two to tango. Exp cell Res. 2014;329:26–34. https://doi.org/10.1016/j.yexcr.2014.09.019.
7. Bartek J, Mistrik M, Bartkova J. Thresholds of replication stress signaling in cancer development and treatment. Nat Struct Mol Biol. 2012;19:5–7. https://doi.org/10.1038/nsmb.2220.
8. Cleary JM, Aguirre AJ, Shapiro GI, D'Andrea AD. Biomarker-guided development of DNA repair inhibitors. Mol Cell. 2020;78:1070–85. https://doi.org/10.1016/j.molcel.2020.04.035.
9. Mateo J, Lord CJ, Serra V, Tutt A, Balmana J, Castroviejo-Bermejo M, et al. A decade of clinical development of PARP inhibitors in perspective. Ann Oncol. 2019;30:1437–47. https://doi.org/10.1093/annonc/mdz192.
10. Leman AR, Noguchi E. Local and global functions of Timeless and Tipin in replication fork protection. Cell cycle. 2012;11:3945–55. https://doi.org/10.4161/cc.21989.
11. Kemp MG, Akan Z, Yilmaz S, Grillo M, Smith-Roe SL, Kang TH, et al. Tipin-replication protein A interaction mediates Chk1 phosphorylation by ATR in response to genotoxic stress. J Biol Chem. 2010;285:16562–71. https://doi.org/10.1074/jbc.M110.110304.
12. Cho WH, Kang YH, An YY, Tappin I, Hurwitz J, Lee JK. Human Tim-Tipin complex affects the biochemical properties of the replicative DNA helicase and DNA polymerases. Proc Natl Acad Sci USA. 2013;110:2523–7. https://doi.org/10.1073/pnas.1222494110.
13. Byun TS, Pacek M, Yee MC, Walter JC, Cimprich KA. Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint. Genes Dev. 2005;19:1040–52. https://doi.org/10.1101/gad.1301205.
14. Errico A, Costanzo V. Mechanisms of replication fork protection: a safeguard for genome stability. Crit Rev Biochem Mol Biol. 2012;47:222–35. https://doi.org/10.3109/10409238.2012.655374.
15. Mazzoccoli G, Panza A, Valvano MR, Palumbo O, Carella M, Pazienza V, et al. Clock gene expression levels and relationship with clinical and pathological features in colorectal cancer patients. Chronobiol Int. 2011;28:841–51. https://doi.org/10.3109/07420528.2011.615182.
16. Pasero P, Tourriere H. Overexpression of the Fork Protection Complex: a strategy to tolerate oncogene-induced replication stress in cancer cells. Mol Cell Oncol. 2019;6:1607455 https://doi.org/10.1080/23723556.2019.1607455.
17. Bianco JN, Bergoglio V, Lin YL, Pillaire MJ, Schmitz AL, Gilhodes J, et al. Overexpression of Claspin and Timeless protects cancer cells from replication stress in a checkpoint-independent manner. Nat Commun. 2019;10:910 https://doi.org/10.1038/s41467-019-08886-8.
18. Perego P, Robert J. Oxaliplatin in the era of personalized medicine: from mechanistic studies to clinical efficacy. Cancer Chemother Pharmacol. 2016;77:5–18. https://doi.org/10.1007/s00280-015-2901-x.

19. Pommier Y. Topoisomerase I inhibitors: camptothecins and beyond. Nat Rev Cancer. 2006;6:789–802. https://doi.org/10.1038/nrc1977.
20. Zellweger R, Dalcher D, Mutreja K, Berti M, Schmid JA, Herrador R, et al. Rad51-mediated replication fork reversal is a global response to genotoxic treatments in human cells. J Cell Biol. 2015;208:563–79. https://doi.org/10.1083/jcb.201406099.
21. Parikh AR, Lee FC, Yau L, Koh H, Knost J, Mitchell EP, et al. MAVERICC, a randomized, biomarker-stratified, phase II study of mFOLFOX6-bevacizumab versus FOLFIRI-bevacizumab as first-line chemotherapy in metastatic colorectal cancer. Clin Cancer Res. 2019;25:2988–95. https://doi.org/10.1158/1078-0432.CCR-18-1221
22. Pommier Y, Barcelo JM, Rao VA, Sordet O, Jobson AG, Thibaut L, et al. Repair Topoisomerase I-Mediated DNA Damage. Prog Nucleic Acid Res Mol Biol.2006;81:179–229. https://doi.org/10.1016/s0079-6603(06)81005-6.
23. Park H, Sternglanz R. Identification and characterization of the genes for two topoisomerase I-interacting proteins from Saccharomyces cerevisiae. Yeast. 1999;15:35–41. 10.1002/(SICI)1097-0061(19990115)15:1<35::AID-YEA340>3.0.CO;2-R.
24. Hosono Y, Abe T, Higuchi M, Kajii K, Sakuraba S, Tada S, et al. Tipin functions in the protection against topoisomerase I inhibitor. J Biol Chem. 2014;289:11374–84. https://doi.org/10.1074/jbc.M113.531707.
25. McHugh PJ, Spanswick VJ, Hartley JA. Repair of DNA interstrand crosslinks: molecular mechanisms and clinical relevance. Lancet Oncol. 2001;2:483–90. https://doi.org/10.1016/s1470-2045(01)00454-5.
26. Kartalou M, Essigmann JM. Recognition of cisplatin adducts by cellular proteins. Mutat Res. 2001;478:1–21. https://doi.org/10.1016/s0027-5107(01)00142-7
27. Hashimoto S, Anai H, Hanada K. Mechanisms of interstrand DNA crosslink repair and human disorders. Genes Environ. 2016;38:9 https://doi.org/10.1186/s41021-016-0037-9.
28. Deans AJ, West SC. DNA interstrand crosslink repair and cancer. Nat Rev Cancer. 2011;11:467–80. https://doi.org/10.1038/nrc3088.
29. Ben-Yehoyada M, Wang LC, Kozekov ID, Rizzo CJ, Gottesman ME, Gautier J. Checkpoint signaling from a single DNA interstrand crosslink. Mol Cell. 2009;35:704–15. https://doi.org/10.1016/j.molcel.2009.08.014.

ACKNOWLEDGEMENTS

We thank all patients who contributed to this study.

AUTHOR CONTRIBUTIONS

HA primarily planned, designed, and drafted the manuscript. H-JL supervised and administered the project, and acquired funding. YX and JM did statistical analyses. All authors made substantial contributions to data collection and drafting the manuscript. All authors read and approved the final manuscript.

FUNDING INFORMATION

This work was supported by the National Cancer Institute [P30CA 014089 to HJL], Gloria Borges WunderGlo Foundation, Dhont Family Foundation, Daniel Butler Memorial Fund, Victoria and Philip Wilson Research Fund, and San Pedro Peninsula Cancer Guild.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All patients provided informed consent for molecular research prior to study enrollment. The study protocol was approved by the Institutional Review Boards of each participating institution and was conducted in accordance with the tenets of the Declaration of Helsinki, as well as the Good Clinical Practice and REMARK guidelines.

CONSENT TO PUBLISH

Not applicable.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41416-021-01592-7.

Correspondence and requests for materials should be addressed to Heinz-Josef Lenz.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.