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Original Research

Impact of diabetes and metformin use on recurrence and outcome in stage II–III colon cancer patients—A pooled analysis of three adjuvant trials

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Abstract ***Background:*** Diabetes mellitus (DM) has been associated with increased colorectal cancer (CRC) risk and worse prognosis in metastatic CRC patients. In this large, pooled analysis of non-metastatic colon cancer (CC) patients, we investigated the impact of DM and metformin treatment on recurrence and survival.
Patients and methods: A patient-level pooled analysis from three randomised adjuvant trials

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was performed. All patients had resection with curative intent of stage II or III CC and were treated with standard adjuvant fluoropyrimidine and oxaliplatin (±cetuximab). We investigated the impact of DM and metformin treatment on time to recurrence (TTR) and overall survival (OS).

Results: Of 5922 CC patients who had a median follow-up of 6.8 years, 621 patients (10.5%) had DM at CC diagnosis. Of those with DM, 327 patients (52.7%) were defined as metformin users and 294 patients (47.3%) as non-metformin users. CC patients with DM had a significantly shorter TTR (adjHR: 1.21; 95% CI, 1.03–1.42; $p = 0.017$) and OS (adjHR: 1.29; 95% CI, 1.09–1.52; $p = 0.003$) compared to non-diabetic CC patients. Diabetic CC patients not receiving metformin had a significantly worse TTR (adjHR: 1.28; 95% CI, 1.02–1.60; $p = 0.032$) and OS (adjHR: 1.41; 95% CI, 1.13–1.77; $p = 0.003$) as compared to non-diabetic patients. These worse outcomes were not significant in metformin users (TTR: adjHR: 1.16; 95% CI, 0.94–1.43; $p = 0.168$; OS: adjHR: 1.19; 95% CI, 0.95–1.48, $p = 0.127$).

Conclusions: CC patients with DM had not only a significantly worse survival but also TTR. Furthermore, our data suggest that metformin may attenuate the detrimental effect of DM on CC patient outcomes.

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1. Introduction

Colorectal cancer (CRC) accounts for a large portion of cancer-related mortality worldwide with around 915 000 new deaths reported in 2020 [1]. Diabetes mellitus (DM) has been recognised as a risk factor of CRC in the USA [2]. However, using Global International Diabetes Federation (IDF) 2015 and GLOBOCAN data, no linkage was observed between the prevalence of DM and the incidence of CRC. This raises the question of whether the country of residency could be associated with specific risk and protective factors.

The effects of metformin, a first-line drug for treating type 2 DM patients, on colorectal carcinogenesis are quite controversial. *In vitro* studies have demonstrated that metformin is able to inhibit the Wnt3a/β-catenin pathway, a pathway important in promoting tumour progression by facilitating tumour metastasis, angiogenesis, epithelial-mesenchymal transition and cancer stem cell formation [3]. However, in clinical studies where the impact of environmental exposures is not easily controlled and where different chemotherapeutic and targeted therapies are prescribed, the effects of metformin on specific cohorts of diabetic patients may vary. The most recent meta-analysis by Cheng *et al.*, [4] demonstrated better overall and cancer-specific survivals in diabetic metformin users in comparison with non-metformin users with colon cancer (CC). Nevertheless, several limitations were present in this meta-analysis due to the important heterogeneity between the ten included studies as well as limited adjustment for confounding variables.

Herein, we strictly limited our study to patients living in France, Italy and the USA who were diagnosed with stage II–III colon cancer and treated with an oxaliplatin-based standard adjuvant therapy after surgery. The objectives of our study were to investigate the impact of DM and metformin use on both recurrence and survival.

2. Materials and methods

2.1. Patients

Overall, 9440 patients from three randomised phase III clinical trials (N0147, PETACC8 and TOSCA) conducted between 2004 and 2013 were considered. All patients had undergone resection of stage II or III colon cancer (CC) with curative intent and subsequently received adjuvant therapy consisting of a fluoropyrimidine and oxaliplatin for either three or six months with or without the anti-epidermal growth factor receptor (*EGFR*) antibody cetuximab. At inclusion, patients completed self-reported standardised questionnaires with regards to comorbidities and concomitant medication intake. For this patient-level pooled analysis, we included all patients with available information with regard to a history of DM and anti-diabetic treatment with metformin. A total of 3518 patients had to be excluded due to incomplete information regarding patient self-reported comorbidities and comedication. Therefore, 5922 patients were available for this analysis (Fig. 1).

2.2. Study design and objectives

Within this study, we aimed to investigate the impact of DM as a primary objective and metformin use as a secondary objective on recurrence and survival in early CC patients. Therefore, we compared diabetic versus non-diabetic patients, diabetic metformin users versus

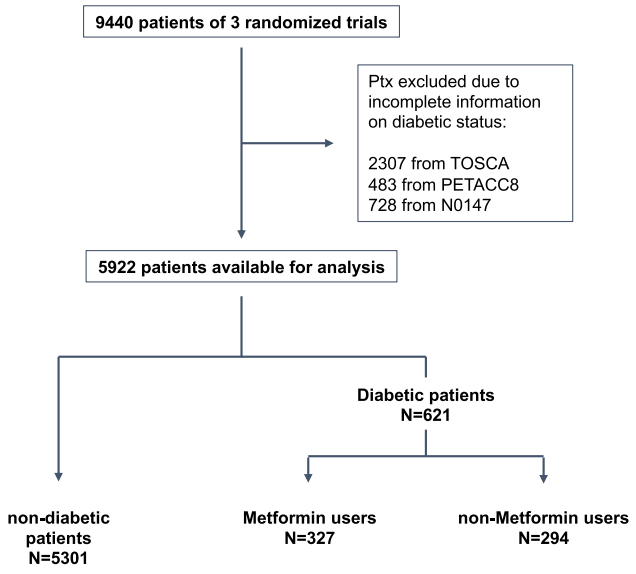

Fig. 1. Flowchart of patient inclusion (ptx, patients).

non-diabetic patients and diabetic non-metformin users versus non-diabetic patients. Patients were defined as diabetic if a DM was present at enrolment or randomization of the study or during adjuvant treatment. Metformin users were defined as diabetic patients with active metformin intake during previously described time points.

2.3. Statistical analysis

The end-points were time to recurrences (TTR), defined as time from randomization to first relapse or death linked to disease recurrence. Overall survival (OS) was defined as time from randomization to death from any cause or last date of follow-up for patients alive. For comparisons of baseline characteristics, categorical factors were compared with the chi-square test and continuous factors were compared with standard parametric or non-parametric tests. Continuous variables are presented as the mean (SD) and median interquartile range (IQR). Distributions of TTR and OS were estimated using the Kaplan–Meier method. Differences between groups of patients were compared using log-rank tests and Cox model stratified by studies. For testing the impact of DM and metformin use respectively on TTR within different molecular subgroups, interaction terms were included into the Cox models. Factors included in the multivariable analyses were baseline prognostic factors identified in univariable analyses or clinically relevant. Therefore, we adjusted for the following parameters in the multivariable analyses: age, ECOG performance status (PS), T-stage, N-stage, histological grade, location of the primary tumour and different therapy regimens. The prognostic value of the different variables was tested by Cox proportional hazard models stratified by studies. Two-tailed p-values <0.05 were considered to indicate statistical significance. All statistics were calculated using SAS version 9.4 software (SAS Institute, Cary, NC).

3. Results

3.1. Patients' characteristics

Overall, 5922 patients with stage II–III CC treated by surgery and adjuvant therapy combining a fluoropyrimidine with oxaliplatin were included into this analysis. Globally, six months of FOLFOX was given to 2647 (44.7%) patients, six months of FOLFOX + cetuximab to 2240 (37.8%) patients, three months of FOLFOX to 404 (6.8%) patients, six months of CAPOX to 307 (5.2%) patients and three months of CAPOX to 324 (5.5%) patients. Of these 5922 patients, 621 patients (10.5%) presented with a history of diabetes (diabetics), whereas 5301 patients (89.5%) had no diabetes (non-diabetics) reported. Among diabetic patients, 327/621 patients (52.7%) were metformin users (metformin diabetics) and 294/621 patients (47.3%) did not report use of metformin (non-metformin diabetics). The following patient characteristics were significantly more frequently observed in diabetic patients: older age ($p < 0.001$), male gender ($p < 0.001$), ECOG performance status over 0 ($p < 0.001$), higher body mass index (BMI) ($p < 0.001$), treatment discontinuations ($p < 0.001$), presence of cardiovascular disease ($p < 0.001$) and hypertension ($p < 0.001$). Tumour-associated patient characteristics were generally well balanced between groups. Patient characteristics are listed in detail in Table 1. The median follow-up time was 6.75 years (95% CI: 6.65–6.82).

3.2. Association of DM with survival and recurrence

OS was significantly shorter in diabetic patients compared to non-diabetic patients (adjHR: 1.29; 95% CI, 1.09–1.52; $p = 0.003$). Patients with DM had a significantly shorter TTR as compared to patients without DM (adjHR: 1.21; 95% CI, 1.03–1.42; $p = 0.017$). Corresponding Kaplan–Meier curves are displayed within Fig. 2A, B. Univariable and multivariable analyses are summarised within Table 2.

3.3. Association of metformin use with survival and recurrence

Non-metformin diabetic patients had a significantly worse OS compared to non-diabetic patients (adjHR: 1.41; 95% CI, 1.13–1.77; $p = 0.003$), but this difference was not significant for diabetic patients treated with metformin (adjHR: 1.19; 95% CI, 0.95–1.48; $p = 0.127$). Similarly, TTR was observed to be worse in non-metformin diabetic patients (adjHR: 1.28; 95% CI, 1.02–1.60; $p = 0.032$) compared to non-diabetic

Table 1
Patient characteristics according to non-diabetic patients, metformin diabetics, and non-metformin diabetics (BMI, body mass index; CVD, cardiovascular disease; dMMR, deficient mismatch repair; ECOG PS, ECOG performance status; HR, hazard ratio; MMR, mismatch repair; pMMR, proficient mismatch repair; W, Wilcoxon test; X^2, chi-squared test).

		Non-diabetics	Diabetics		Total	*p*-value
			Non-metformin diabetics	Metformin diabetics		
		(N = 5301)	(N = 294)	(N = 327)	(N = 5922)	
Study	n	5301	294	327	5922	X^2: <0.0001
	N0147	1691 (31.9%)	152 (51.7%)	115 (35.2%)	1958 (33.1%)	
	PETACC8	2291 (43.2%)	74 (25.2%)	147 (45.0%)	2512 (42.4%)	
	TOSCA	1319 (24.9%)	68 (23.1%)	65 (19.9%)	1452 (24.5%)	
Treatment arm	n	5301	294	327	5922	X^2: 0.5706
	FOLFOX	2733 (51.6%)	154 (52.4%)	164 (50.2%)	3051 (51.5%)	
	FOLFOX + cetuximab	1994 (37.6%)	111 (37.8%)	135 (41.3%)	2240 (37.8%)	
	CAPOX	574 (10.8%)	29 (9.9%)	28 (8.6%)	631 (10.7%)	
Sex	n	5301	294	327	5922	X^2: 0.0001
	Male	2839 (53.6%)	185 (62.9%)	205 (62.7%)	3229 (54.5%)	
	Female	2462 (46.4%)	109 (37.1%)	122 (37.3%)	2693 (45.5%)	
Age	n	5301	294	327	5922	W: <0.0001
	median	60.00	64.00	65.00	61.00	
	range (years)	19–86	29–83	41–80	19–86	
Baseline BMI	n	4381	289	324	4994	W: <0.0001
	median	25.81	28.36	28.70	26.13	
	range (kg/m2)	14.1–57.7	15.5–49.5	18.7–56.4	14.1–57.7	
ECOG PS	n	5193	288	325	5806	X^2: <0.0001
	0	4375 (84.2%)	210 (72.9%)	250 (76.9%)	4835 (83.3%)	
	1	801 (15.4%)	75 (26.0%)	72 (22.2%)	948 (16.3%)	
	2	16 (0.3%)	3 (1.0%)	3 (0.9%)	22 (0.4%)	
	3	1 (0.0%)	0 (0.0)	0 (0.0)	1 (0.0%)	
T-stage	n	5282	294	327	5903	X^2: 0.2491
	T1/T2	607 (11.5%)	39 (13.3%)	49 (15.0%)	695 (11.8%)	
	T3	3825 (72.4%)	214 (72.8%)	232 (70.9%)	4271 (72.4%)	
	T4	850 (16.1%)	41 (13.9%)	46 (14.1%)	937 (15.9%)	
N-stage	n	5292	294	327	5913	X^2: 0.5949
	N0	450 (8.5%)	22 (7.5%)	23 (7.0%)	495 (8.4%)	
	N1	3051 (57.7%)	180 (61.2%)	198 (60.6%)	3429 (58.0%)	
	N2	1791 (33.8%)	92 (31.3%)	106 (32.4%)	1989 (33.6%)	
Histological grade	n	5242	293	322	5857	X^2: 0.6637
	G1/G2	3972 (75.8%)	221 (75.4%)	251 (78.0%)	4444 (75.9%)	
	G3/G4	1270 (24.2%)	72 (24.6%)	71 (22.0%)	1413 (24.1%)	
Location	n	5297	294	327	5918	X^2: 0.0553
	Left colon	2968 (56.0%)	148 (50.3%)	162 (49.5%)	3278 (55.4%)	
	Right colon	2232 (42.1%)	142 (48.3%)	158 (48.3%)	2532 (42.8%)	
	Both	97 (1.8%)	4 (1.4%)	7 (2.1%)	108 (1.8%)	
Duration of adjuvant treatment	n	5301	294	327	5922	X^2: 0.6994
	6 months	4643 (87.6%)	260 (88.4%)	291 (89.0%)	5194 (87.7%)	
	3 months	658 (12.4%)	34 (11.6%)	36 (11.0%)	728 (12.3%)	
Treatment discontinuations	n	5301	294	327	5922	X^2: 0.0009
	no	4031 (76.0%)	199 (67.7%)	262 (80.1%)	4492 (75.9%)	
	yes	1270 (24.0%)	95 (32.3%)	65 (19.9%)	1430 (24.1%)	
KRAS status	n	3339	203	228	3770	X^2: 0.4419
	mutant	1136 (34.0%)	64 (31.5%)	85 (37.3%)	1285 (34.1%)	
	wild type	2203 (66.0%)	139 (68.5%)	143 (62.7%)	2485 (65.9%)	
BRAF status	n	3156	199	214	3569	X^2: 0.2605
	mutant	358 (11.3%)	30 (15.1%)	23 (10.7%)	411 (11.5%)	
	wild type	2798 (88.7%)	169 (84.9%)	191 (89.3%)	3158 (88.5%)	
MMR status	n	3202	204	220	3626	X^2: 0.2912
	pMMR	2845 (88.9%)	178 (87.3%)	202 (91.8%)	3225 (88.9%)	
	dMMR	357 (11.1%)	26 (12.7%)	18 (8.2%)	401 (11.1%)	
CVD	n	3982	226	262	4470	X^2: <0.0001
	Not present	3789 (95.2%)	204 (90.3%)	234 (89.3%)	4227 (94.6%)	
	Present	193 (4.8%)	22 (9.7%)	28 (10.7%)	243 (5.4%)	

(*continued on next page*)

Table 1 (*continued*)

		Non-diabetics	Diabetics		Total	*p*-value
			Non-metformin diabetics	Metformin diabetics		
		(N = 5301)	(N = 294)	(N = 327)	(N = 5922)	
Hypertension	n	3982	226	262	4470	X^2: <0.0001
	Not present	2881 (72.4%)	121 (53.5%)	111 (42.4%)	3113 (69.6%)	
	Present	1101 (27.6%)	105 (46.5%)	151 (57.6%)	1357 (30.4%)	

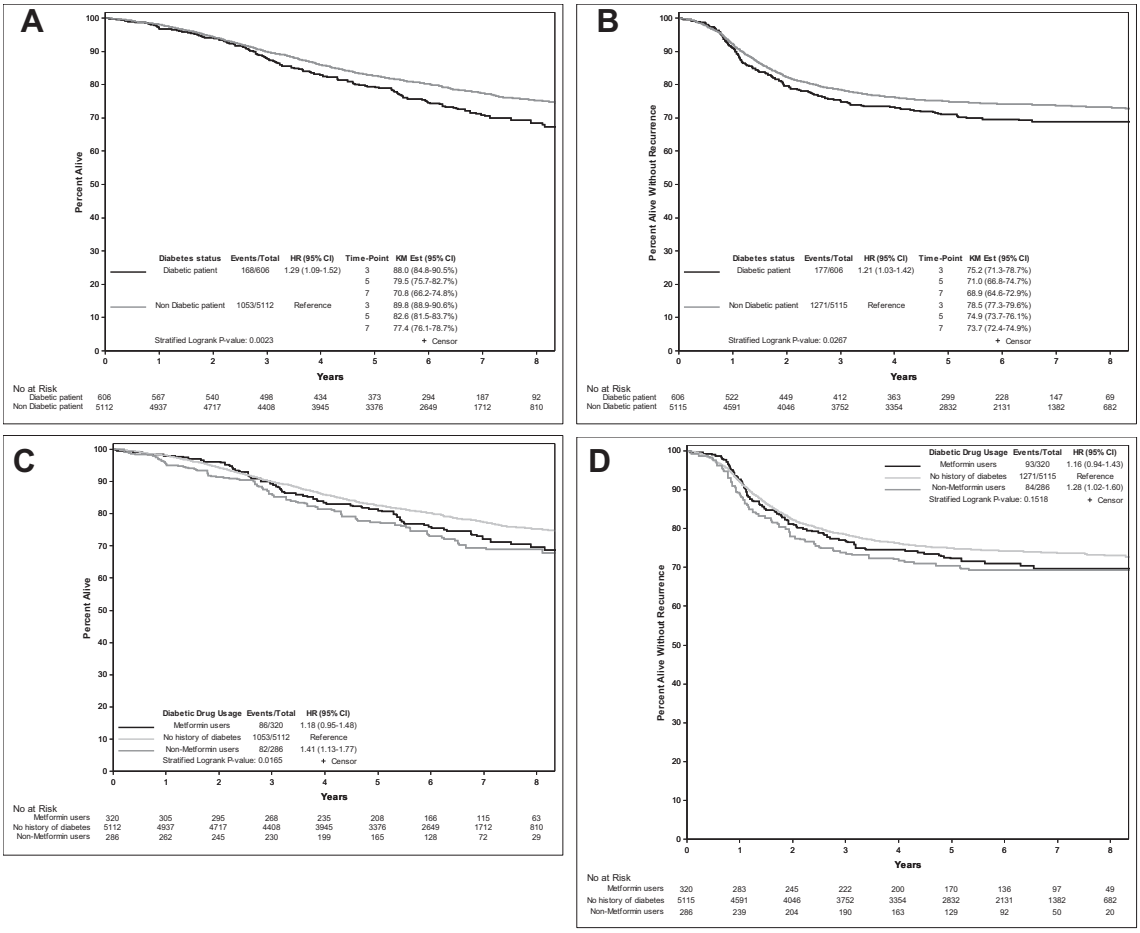

Fig. 2. Kaplan–Meier curves of (A) overall survival (OS) and (B) time to recurrence (TTR) according to diabetic status. Kaplan–Meier curves of (C) OS and (D) TTR according to metformin status. (CI, confidence interval; HR, hazard ratio).

patients, but this difference was not significant for diabetic patients treated with metformin (adjHR: 1.16; 95% CI, 0.94–1.43; $p = 0.168$). Corresponding Kaplan–Meier curves are displayed in Fig. 2C, D. Univariable and multivariable analyses are summarised in Table 3.

3.4. Association of DM with recurrence within distinct molecular subgroups

As colon cancer is no longer considered as a single disease but is now divided into different molecular subtypes, we investigated the impact of DM in patients with KRAS-wild-type and BRAF-wild-type tumours (double WT), KRAS-mutant (mut) or BRAF-mut tumours, proficient mismatch repair (pMMR) tumours and deficient mismatch repair (dMMR) tumours. Molecular annotation was only available within patients of the PETACC8 and N0147 study.

KRAS status was available in 3770 patients, and BRAF status was available in 3569 patients. A total of 1950/3558 patients (54.8%) had double WT tumours, 1203/3558 patients (33.8%) had KRAS-mut tumours and 405/3558 patients (11.4%) had BRAF-mut tumours. In patients with double WT tumours, TTR was significantly shorter in diabetic patients (HR: 1.31; 95% CI,

Table 2
Effect on (A) overall survival (OS) and (B) time to recurrence (TTR) according to diabetic status. Univariable and multivariable stratified by study Cox proportional hazard models. (CI, confidence interval; ECOG PS, ECOG performance status; HR, hazard ratio; IF, individual factor; OS, overall survival; TTR, time to recurrence). Significance is defined by $p < 0.05$.

A: OS	Univariable analysis		Multivariable analysis	
	IF HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Diabetic patients (ref. non-diabetics)	1.37 (1.17–1.61)	**<0.001**	1.29 (1.09–1.52)	**0.003**
Age (ref. <70)				
≥70 years	1.80 (1.58–2.05)	**<0.001**	1.61 (1.41–1.84)	**<0.001**
ECOG PS (ref. 0)				
1	1.52 (1.33–1.74)	**<0.001**	1.37 (1.20–1.57)	**<0.001**
>1	4.33 (2.64–7.12)	**<0.001**	3.64 (2.21–6.00)	**<0.001**
T-stage (ref. T1/T2)				
T3	2.39 (1.86–3.06)	**<0.001**	2.06 (1.59–2.66)	**<0.001**
T4	4.65 (3.57–6.06)	**<0.001**	3.83 (2.90–5.04)	**<0.001**
N-stage (ref. N0)				
N1	1.30 (0.90–1.88)	0.161	1.74 (1.18–2.56)	**0.005**
N2	2.90 (2.00–4.20)	**<0.001**	3.53 (2.39–5.22)	**<0.001**
Histological grade (ref. G1/G2)				
G3/G4	1.55 (1.37–1.75)	**<0.001**	1.23 (1.08–1.40)	**0.002**
Tumour localization (ref. right)				
Left	0.66 (0.59–0.74)	**<0.001**	0.76 (0.67–0.85)	**<0.001**
Both	0.68 (0.42–1.11)	0.120	0.64 (0.38–1.06)	0.084
Therapy regimen (ref. FOLFOX 6 months)				
CAPOX 3 months	0.86 (0.54–1.37)	0.517	0.80 (0.49–1.31)	0.375
CAPOX 6 months	0.80 (0.49–1.29)	0.361	0.86 (0.53–1.40)	0.536
FOLFOX 3 months	1.07 (0.73–1.57)	0.728	1.06 (0.72–1.57)	0.766
FOLFOX + cetuximab 6 months	1.18 (1.05–1.33)	**0.006**	1.18 (1.05–1.33)	**0.007**

B: TTR	Univariable analysis		Multivariable analysis	
	IF HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Diabetes patients (ref. non-diabetics)	1.20 (1.02–1.40)	**0.024**	1.21 (1.03–1.42)	**0.017**
Age (ref. <70)				
≥70 years	1.18 (1.03–1.35)	**0.014**	1.09 (0.95–1.25)	0.206
ECOG PS (ref. 0)				
1	1.25 (1.10–1.42)	**0.001**	1.16 (1.02–1.33)	**0.025**
>1	2.70 (1.56–4.67)	**<0.001**	2.44 (1.40–4.23)	**0.002**
T-stage (ref. T1/T2)				
T3	2.31 (1.85–2.89)	**<0.001**	2.10 (1.66–2.65)	**<0.001**
T4	4.66 (3.67–5.91)	**<0.001**	4.20 (3.27–5.38)	**<0.001**
N-stage (ref. N0)				
N1	1.41 (1.04–1.90)	**0.026**	1.90 (1.38–2.60)	**<0.001**
N2	3.14 (2.32–4.26)	**<0.001**	3.90 (2.84–5.37)	**<0.001**
Histological grade (ref. G1/G2)				
G3/G4	1.32 (1.17–1.48)	**<0.001**	1.11 (0.99–1.25)	0.082
Tumour localization (ref. right)				
Left	0.87 (0.78–0.96)	**0.006**	0.94 (0.85–1.05)	0.292
Both	0.74 (0.47–1.15)	0.177	0.75 (0.48–1.19)	0.219
Therapy regimen (ref. FOLFOX 6 months)				
CAPOX 3 months	1.11 (0.77–1.60)	0.584	1.08 (0.74–1.57)	0.703
CAPOX 6 months	1.11 (0.77–1.61)	0.568	1.12 (0.77–1.64)	0.559
FOLFOX 3 months	1.28 (0.92–1.78)	0.137	1.26 (0.90–1.76)	0.178
FOLFOX + cetuximab 6 months	1.04 (0.93–1.16)	0.502	1.03 (0.92–1.16)	0.581

1.01–1.69; $p = 0.040$) compared to non-diabetic patients (Fig. 3A). TTR in patients with KRAS-mut and BRAF-mut tumours was not different according to diabetic status (HR: 1.12; 95% CI, 0.83–1.50; $p = 0.468$ and HR: 1.01; 95% CI, 0.60–1.71; $p = 0.968$) (Fig. 3B,C). The interaction between KRAS/BRAF status and diabetic status was $p_{interaction} = 0.78$. MMR status was available in 3626 patients. However, 3225/3626 tumours were classified as pMMR and 401/3626 as dMMR. While a trend to a worse TTR was observed in

Table 3
Effect on (A) overall survival (OS) and (B) time to recurrence (TTR) according to metformin use. Univariable and multivariable stratified by study Cox proportional hazard models. (CI, confidence interval; ECOG PS, ECOG performance status; HR, hazard ratio; IF, individual factor; OS, overall survival; TTR, time to recurrence). Significance is defined by $p < 0.05$.

A: OS	Univariable analysis		Multivariable analysis	
	IF HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Metformin users	1.31 (1.05–1.62)	**0.015**	1.19 (0.95–1.48)	0.127
Non-metformin users (ref. non-diabetics)	1.45 (1.16–1.82)	**0.001**	1.41 (1.13–1.77)	**0.003**
Age (ref. <70)				
≥70 years	1.80 (1.58–2.05)	**<0.001**	1.61 (1.41–1.84)	**<0.001**
ECOG PS (ref. 0)				
1	1.52 (1.33–1.74)	**<0.001**	1.37 (1.20–1.57)	**<0.001**
>1	4.33 (2.64–7.12)	**<0.001**	3.68 (2.23–6.07)	**<0.001**
T-stage (ref. T1/T2)				
T3	2.39 (1.86–3.06)	**<0.001**	2.06 (1.59–2.66)	**<0.001**
T4	4.65 (3.57–6.06)	**<0.001**	3.83 (2.90–5.04)	**<0.001**
N-stage (ref. N0)				
N1	1.30 (0.90–1.88)	0.161	1.73 (1.18–2.55)	**0.005**
N2	2.90 (2.00–4.20)	**<0.001**	3.53 (2.39–5.21)	**<0.001**
Histological grade (ref. G1/G2)				
G3/G4	1.55 (1.37–1.75)	**<0.001**	1.23 (1.08–1.40)	**0.002**
Tumour localization (ref. right)				
Left	0.66 (0.59–0.74)	**<0.001**	0.76 (0.67–0.85)	**<0.001**
Both	0.68 (0.42–1.11)	0.120	0.64 (0.38–1.07)	0.086
Therapy regimen (ref. FOLFOX 6 months)				
CAPOX 3 months	0.86 (0.54–1.37)	0.517	0.80 (0.49–1.31)	0.377
CAPOX 6 months	0.80 (0.49–1.29)	0.361	0.85 (0.52–1.39)	0.530
FOLFOX 3 months	1.07 (0.73–1.57)	0.728	1.06 (0.72–1.57)	0.769
FOLFOX + cetuximab 6 months	1.18 (1.05–1.33)	**0.006**	1.18 (1.05–1.33)	**0.007**
B: TTR	**Univariable analysis**		**Multivariable analysis**	
	IF HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Metformin users	1.15 (0.94–1.42)	0.180	1.16 (0.94–1.43)	0.168
Non-metformin users (ref. non-diabetics)	1.25 (1.00–1.55)	**0.047**	1.28 (1.02–1.60)	**0.032**
Age (ref. <70)				
≥70 years	1.18 (1.03–1.35)	**0.014**	1.09 (0.95–1.25)	0.204
ECOG PS (ref. 0)				
1	1.25 (1.10–1.42)	**0.001**	1.16 (1.02–1.33)	**0.025**
>1	2.70 (1.56–4.67)	**<0.001**	2.44 (1.41–4.24)	**0.001**
T-stage (ref. T1/T2)				
T3	2.31 (1.85–2.89)	**<0.001**	2.10 (1.66–2.65)	**<0.001**
T4	4.66 (3.67–5.91)	**<0.001**	4.20 (3.27–5.38)	**<0.001**
N-stage (ref. N0)				
N1	1.41 (1.04–1.90)	**0.026**	1.90 (1.38–2.60)	**<0.001**
N2	3.14 (2.32–4.26)	**<0.001**	3.90 (2.84–5.37)	**<0.001**
Histological grade (ref. G1/G2)				
G3/G4	1.32 (1.17–1.48)	**<0.001**	1.11 (0.99–1.25)	0.081
Tumour localization (ref. right)				
Left	0.87 (0.78–0.96)	**0.006**	0.94 (0.85–1.05)	0.296
Both	0.74 (0.47–1.15)	0.177	0.75 (0.48–1.19)	0.220
Therapy regimen (ref. FOLFOX 6 months)				
CAPOX 3 months	1.11 (0.77–1.60)	0.584	1.08 (0.74–1.57)	0.704
CAPOX 6 months	1.11 (0.77–1.61)	0.568	1.12 (0.76–1.64)	0.564
FOLFOX 3 months	1.28 (0.92–1.78)	0.137	1.26 (0.90–1.76)	0.179
FOLFOX + cetuximab 6 months	1.04 (0.93–1.16)	0.502	1.03 (0.92–1.15)	0.586

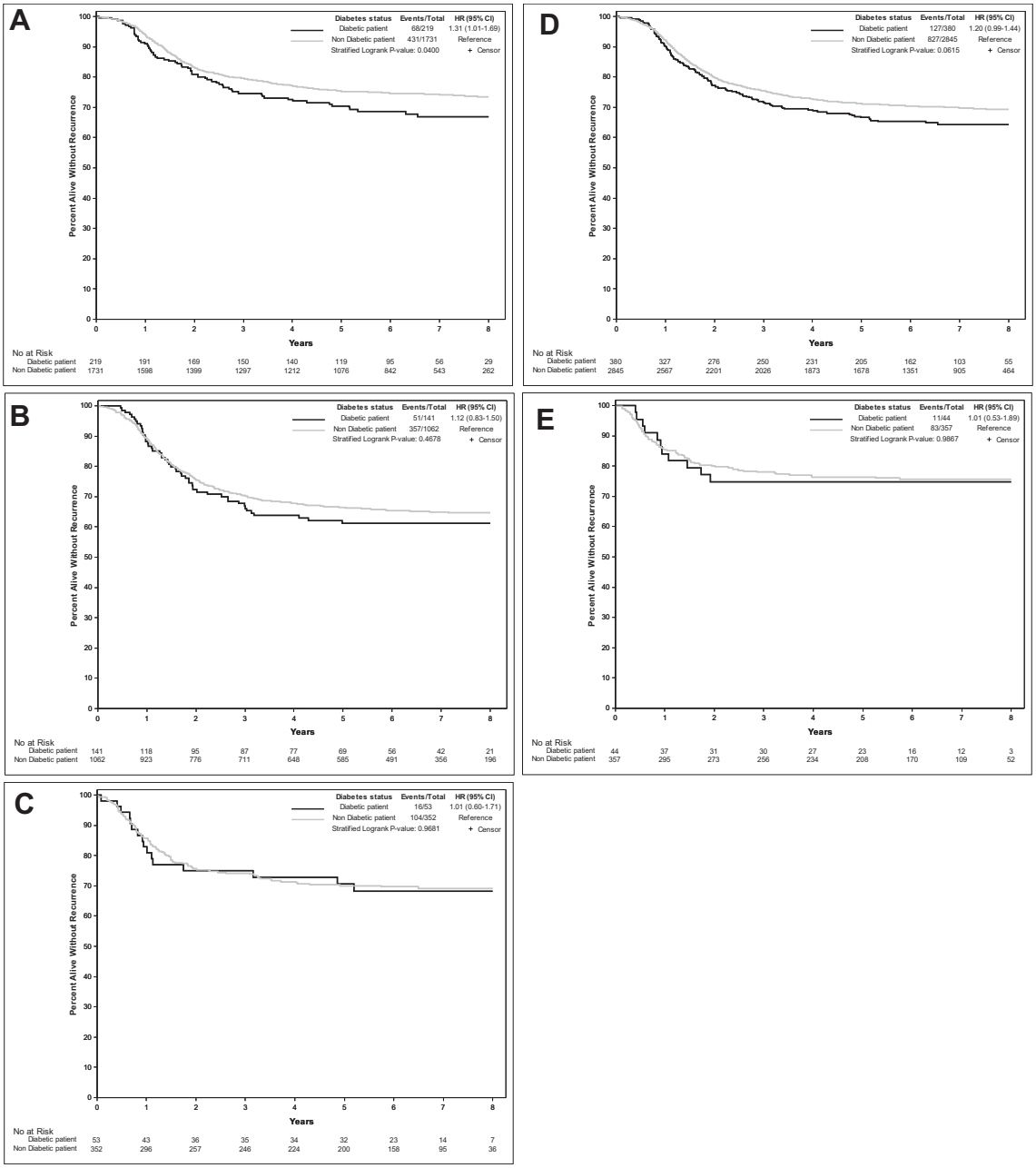

Fig. 3. Kaplan–Meier curves of time to recurrence (TTR) according to diabetic status in patients with (A) KRAS + BRAF-wild-type (wt) tumours (double wt), (B) KRAS-mutated (mut) tumours, (C) BRAF-mut tumours, (D) proficient mismatch repair (pMMR) tumours, and (E) deficient mismatch repair (dMMR) tumours (CI, confidence interval; HR, hazard ratio).

pMMR diabetic patients (HR: 1.20; 95% CI, 0.99–1.44; $p = 0.062$), TTR in dMMR patients was not different between diabetic and non-diabetic patients (HR: 1.01; 95% CI, 0.53–1.89; $p = 0.987$) (Fig. 3D,E). The interaction between MMR status and diabetic status was $p_{interaction} = 0.72$.

OS was significantly shorter in diabetic compared to non-diabetic patients within the double WT (HR: 1.66; 95% CI, 1.28–2.14; $p < 0.001$), KRAS-mut (HR: 1.50; 95% CI, 1.13–2.00; $p = 0.005$) and pMMR subgroup (HR: 1.55; 95% CI, 1.29–1.86; $p < 0.000$). Kaplan–Meier curves of OS according to diabetic status within molecular subtypes are illustrated in Supplementary Fig. 1.

3.5. Association of metformin use with recurrence within distinct molecular subgroups

We compared the effect of metformin use in diabetic to non-diabetic patients on TTR according to molecular features such as KRAS/BRAF mutational status and MMR status. When comparing non-diabetic with non-metformin diabetic patients, a shorter TTR was seen in diabetic patients when their tumours were double WT

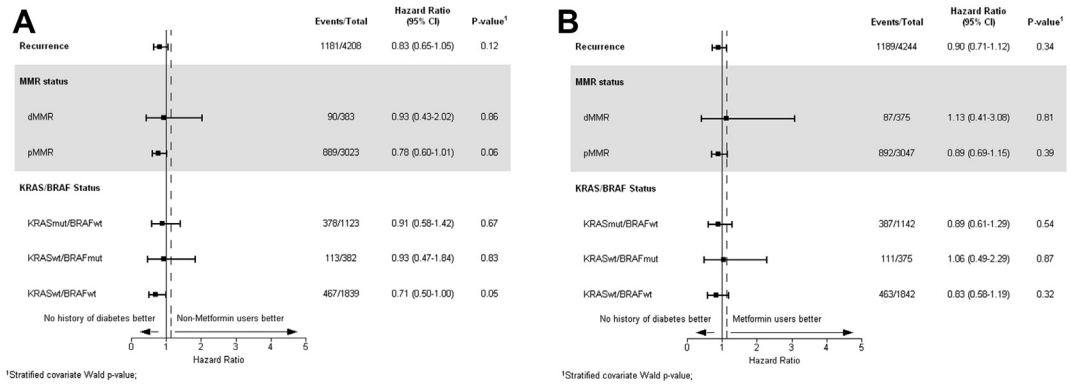

Fig. 4. Forest plots for time to recurrence (TTR) of (A) non-diabetics vs. non-metformin users ($P_{interact}$ MMR status = 0.80; $p_{interact}$ KRAS/BRAF status = 0.61) and (B) non-diabetics vs. metformin users according to molecular tumour characteristics ($P_{interact}$ MMR status = 0.71; $p_{interact}$ KRAS/BRAF status = 0.88) (CI, confidence interval; dMMR, deficient mismatch repair; MMR, mismatch repair; pMMR, proficient mismatch repair).

(HR: 0.71; 95% CI, 0.50–1.00; $p = 0.05$) and a trend was observed in patients with pMMR (HR: 0.78; 95% CI, 0.60–1.0; $p = 0.06$; $p_{interact}$ MMR status = 0.80; $p_{interact}$ KRAS/BRAF status = 0.61) (Fig. 4A). When comparing non-diabetic with metformin-taking diabetic patients, no significant difference in TTR could be detected within molecular subgroups ($p_{interact}$ MMR status = 0.71; $p_{interact}$ KRAS/BRAF status = 0.88) (Fig. 4B). Kaplan–Meier curves of TTR according to metformin use within molecular subtypes are illustrated in Supplementary Fig. 2.

OS was significantly longer in patients with double WT, KRAS-mut and pMMR tumours if they had no history of diabetes (HR: 0.57; 95% CI, 0.41–0.81; $p = 0.000$; HR: 0.65; 95% CI, 0.43–1.00; $p = 0.020$; and HR: 0.58; 95% CI, 0.45–0.74; $p < 0.001$, respectively) compared to non-metformin diabetics, but this difference was not significant for diabetics treated with metformin (HR: 0.90; 95% CI, 0.56–1.44; HR: 0.97; 95% CI, 0.57–1.65 and HR: 0.80; 95% CI, 0.57–1.12, respectively). Kaplan–Meier curves of OS according to metformin use within molecular subtypes are illustrated in Supplementary Fig. 3.

4. Conclusions

Our pooled analysis of randomised clinical trials (RCTs) assessed the impact of DM on recurrence and survival of stage II and III CC patients treated by surgery performed with curative intent followed by adjuvant chemotherapy. We further evaluated the influence on metformin therapy in these patients.

Within our study, we could demonstrate that DM is associated with not only an impaired OS probably caused by DM-related mortality but also a significantly shorter TTR suggesting a direct association between DM and cancer recurrence. Within mechanisms underlying DM-induced cancer genesis and development, constant hyperglycemia as present in DM patients leads to increased levels of insulin as well as subsequently stimulated production of insulin-like growth factors (IGFs) [5]. Consequently, several oncogenic pathways associated with cancer cell growth and angiogenesis are being activated potentially leading to an enhanced cancer risk and progression [6]. In line with that, recent studies reported an increased risk of 30% for CRC in patients with DM [7,8]. In a post hoc analysis of a large randomised trial of metastatic CRC patients, DM was associated with a significantly shorter PFS [8] in line with results in other tumour entities pointing into the same direction [9–11].

To overcome this tumour-promoting effect of DM, distinct antidiabetic drugs lowering systemic glucose levels as well as influencing insulin resistance seem of major interest. Here, metformin acts mainly by reducing gluconeogenesis in the liver and possibly by enhancing glucose uptake in the skeletal muscle, which consequently leads to reduced levels of insulin and IGFs [12]. Regarding molecular cancer-protective mechanisms of metformin, different *in vitro* and *in vivo* studies have been conducted over the last years. Metformin was described to reduce epithelial–mesenchymal transition (EMT) with an increase of E-cadherin membrane expression [13,14] via PI3K/Akt/mTOR pathway but also by repressing the Wnt/beta-catenin pathway, thus reducing tumour progression by inhibiting tumour metastasis and angiogenesis [15]. Moreover, systemic inflammation, which has been well described to promote cancer progression [16], might also be influenced by metformin treatment as displayed by lower levels of C-reactive protein (CRP) and pro-inflammatory cytokines in metformin-treated patients [17–19]. Finally, in the specific setting of a population receiving adjuvant treatment, metformin has been described to potentially improve 5FU and oxaliplatin efficacy in preclinical models and may thus enhance the benefit of the

standard adjuvant 5FU + oxaliplatin-based chemotherapy used in stage III CC [20].

In the present work, the population of patients with DM who were not receiving metformin treatment had significantly worse TTR as well as OS. Typically, reasons leading to the use of antidiabetic drugs other than metformin relate to renal or liver insufficiency. Therefore, it is likely that patients with DM using medications other than metformin have an impaired prognosis compared to DM patients with metformin due to comorbidities associated with DM. However, the significantly shorter TTR, which has been obtained through a multivariate analysis confirming the independent poor prognostic value of DM in CC patients if treated without metformin, supports the theory of an insulin-related cancer recurrence and even possibly a tumour-promoting effect mediated by other antidiabetic drugs. Most interestingly, the use of metformin in DM patients of our study seems to attenuate the detrimental effect of DM as highlighted by an—albeit non-significant—improved TTR and OS compared to patients without metformin treatment. However, according to previously conducted retrospective and registry-based trials, the role of metformin in the adjuvant setting remains controversial [21–24]. A positive prognostic effect of metformin had so far mainly been observed in large meta-analyses [4,25,26]. However, patient populations were extremely heterogeneous and cancer-specific outcomes often were not investigated. Within the sub-studies of the N0147 and TOSCA trial included in this pooled analysis, no significant impact of metformin on disease-free survival (DFS), recurrence-free survival (RFS) and OS could be observed [27,28]. Interestingly, merging this data with another randomised clinical trial within this pooled analysis yielded a significantly worse TTR in DM patients not treated with metformin. Increasing the number of patients might thus overcome the lack of power of smaller previously published trials assuming that diabetic patients generally account for around 10% of adjuvant CC populations.

Another very important factor to consider between diabetic and non-diabetic patients, in general, may represent adherence to treatment in the curative as well as palliative setting. Since diabetic patients and especially those without metformin usually present with a higher number of comorbidities, chemotherapy-related toxicities and drug interactions may lead more frequently to treatment discontinuations as was observed within the present study as well. Here, an intensified and interdisciplinary patient follow-up may be beneficial.

CRC is no longer considered as a single disease entity. From the introduction of RAS assessment to predict the efficacy of anti-EGFR treatments [29] in the 2010s, we have moved to a current landscape with specific therapeutic approaches for many different molecular subgroups such as BRAF V600E mutant and microsatellite instable (MSI) CC patients [30,31]. To our knowledge, the prognostic implications of metformin treatment in diabetic patients as compared to diabetic patients who are not treated with metformin and non-diabetic patients with regard to tumour molecular profile have never been investigated to date. We thus decided to analyse the prognostic impact of DM and metformin use in each specific molecular subtype for the more than 3500 patients for which molecular annotations were available. We found worse TTR and OS in diabetic patients with double WT, KRAS-mut and pMMR tumours (Fig. 3). When looking at patients resected from a BRAF-mut and/or dMMR tumour, similar outcomes were found in diabetic and non-diabetic patients. However, when looking at non-diabetic patients, metformin-treated diabetic patients and diabetics patients who did not receive metformin, the same trends were observed, with a detrimental effect of DM only in patients who were not using metformin (Supplementary Fig. 2). Surprisingly, in KRAS-mut CC patients, we observed similar outcomes in diabetic patients with or without metformin use through several pre-clinical studies suggesting an enhanced anti-cancer effect of metformin in RAS-mut cell lines [32]. However, this result remains non-significant and should be tested in larger samples of patients.

The main strengths of our study are the fact that the large adjuvant trials that we used had well-defined study populations, who were treated with standard adjuvant treatments. The databases have high-quality clinical and pathological annotations and long follow-up. In addition, our study includes patients from many western countries and represents one of the largest cohorts of early-stage CC patients being investigated for DM and metformin impacts. The main study limitations are that information regarding diabetic status and antidiabetic treatment were assessed retrospectively, without any possibility to obtain more specific details. Information about other antidiabetic drugs would have been indeed of great interest to further evaluate their specific effects. In addition, the imbalance in treatment discontinuation observed between metformin users (20%) and non-metformin users (32%) may also have marginally impacted our results as complete treatment stop has been reported to impact both DFS and OS [33]. Further, the molecular profile of patients was available in only two out of three trials. Therefore, the number of patients in some investigated subgroups remains limited.

Within this large, pooled analysis of RCTs comprising early CC patients, DM was associated not only with a significantly impaired survival but also TTR supporting the idea of a tumour-promoting impact of hyperglycemia or hyperinsulinemia. Metformin may attenuate the detrimental impact of DM. Further translational studies to better understand the translational background of the influence of DM as well as

metformin and other antidiabetic drugs on cancer genesis are warranted to optimise treatment decisions in this distinct patient cohort.

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Author contributions

Conceptualization: NC, ESB, KLM, CC, JT; Data curation: KLM; Formal analysis: KLM; Funding acquisition: JT; Investigation: NC, ESB, KLM, CC, JT; Methodology: NC, ESB, KLM, JT; Project administration: JT; Resources: KLM, MDB, FaG, FrG, RL, QS, SRA, RMG, CL, FAS, JT; Software: KLM; Supervision: JT; Validation: MDB, FaG, FrG, RL, QS, SRA, RMG, CL, FAS, JT; Visualization: ESB, KLM; Writing: original draft: NC, ESB, KLM, JT; Writing: review & editing: NC, ESB, KLM, CC, MDB, FaG, FrG, RL, QS, SRA, RMG, CL, FAS, JT.

Conflict of interest statement

MDB received honoraria as a speaker and/or in an advisory role of BMS, MSD, Lilly, Servier, Astra Zeneca. QS reports consulting/advisory role from Yiviva Inc, Boehringer Ingelheim Pharmaceuticals, Inc, Regeneron Pharmaceuticals, Inc., Hoosier Cancer Research Network (to himself), Honorarium/speaker role from Chugai Pharmaceutical Co., Ltd, stocks from Johnson & Johnson, Amgen and Merck & CO (to himself) research funds from Celgene/BMS, Roche/Genentech, Janssen, Novartis (to institution). JT has received honoraria as a speaker and/or in an advisory role from Merck KGaA, Sanofi, Roche Genentech, MSD, Astra Zeneca, Servier, Novartis, Pierre Fabre, HallioDx and Amgen. All other authors declare no conflicts of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2022.02.005.

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