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Anti-tumour Treatment

A review of the sensitivity of metastatic colorectal cancer patients with deficient mismatch repair to standard-of-care chemotherapy and monoclonal antibodies, with recommendations for future research

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ABSTRACT

In 5% of metastatic colorectal cancer (mCRC) patients, tumours display a deficient mismatch repair (dMMR) system. Immunotherapy is beneficial in dMMR mCRC patients and has recently been approved by the Food and Drug Administration for patients with unresectable or metastatic dMMR CRC. Although dMMR and proficient MMR (pMMR) CRC tumours are biologically distinct, they are commonly treated with the same chemotherapy and monoclonal antibodies. This includes dMMR mCRC patients who did not respond to immunotherapy (20–30%). However, it is unclear if these treatments are equally beneficial in dMMR mCRC. Of note, dMMR mCRC patients have a worse prognosis compared to pMMR, which may in part be caused by a lower response to treatment. To avoid unnecessary exposure to ineffective treatments and their associated toxicity, it is important to identify which systemic treatments are most beneficial in dMMR mCRC patients, thus improving their outcome. Indeed, future treatment strategies are likely to involve combinations of immunotherapy, chemotherapy and monoclonal antibodies. In this evidence-based review, we summarize clinical trials reporting treatment efficacy of different types of chemotherapy and monoclonal antibodies in dMMR mCRC patients. We also review the biological rationale behind a potential differential benefit of chemotherapy with or without monoclonal antibodies in dMMR mCRC patients. A barrier in the interpretation of preclinical results is the choice of model systems. They largely comprise traditional models, including cell lines and xenografts, rather than more representative models, such as patient-derived organoids. We provide concrete recommendations for clinical investigators and fundamental researchers to accelerate research regarding which systemic therapy is most effective in dMMR mCRC patients.

Introduction

In 5% of metastatic colorectal cancer (mCRC) patients, tumours display a deficient mismatch repair (dMMR) system, resulting in accumulation of point-mutations and a microsatellite instability (MSI) phenotype [1,2]. dMMR arises through hereditary or sporadic inactivation of the mismatch repair (MMR) system, resulting in Lynch syndrome or sporadic dMMR CRC, respectively (Fig. 1) [2]. The majority of Lynch syndrome patients inherit a germline mutation in one of the MMR genes, while in sporadic dMMR patients, inactivation is most frequently through epigenetic silencing, but can also occur through somatic mutations or loss of heterozygosity in the MMR system [2-5].

In contrast to early stage CRC, studies have reported a worse survival in patients with dMMR mCRC compared to proficient mismatch repair

Abbreviations: dMMR, Deficient mismatch repair; mCRC, metastatic colorectal cancer; pMMR, proficient mismatch repair.

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(pMMR) mCRC [1,6-8]. Accordingly, stage I – III dMMR CRC patients were less likely to have recurrent disease compared to pMMR CRC patients, however, if disease did recur, OS was worse for dMMR CRC patients compared to pMMR [9]. Likewise, dMMR mCRC population-based patients receiving treatment had a median overall survival (OS) of 16.0 months compared to 23.6 months in pMMR [8]. In a population-based study, the response rate to first-line chemotherapy with or without monoclonal antibodies was lower in dMMR mCRC patients compared to pMMR mCRC patients (5% versus 44%, respectively) [7], suggesting that dMMR mCRC patients may benefit less from chemotherapy with or without monoclonal antibodies. This is supported by the recent KEYNOTE-177 results, where dMMR mCRC patients in the control arm (chemotherapy with/without targeted treatment) showed a lower response rate than expected in first-line mCRC patients of 33.1% and progression-free survival (PFS) of 8.2 months [10]. The worse prognosis in dMMR mCRC patients is likely driven by several factors, including *BRAF* mutational status [11,12], the ability to receive a metastatic resection [8,13-15] and other lesser known factors, such as the programmed cell death ligand 1 (*PD-L1)* gene expression level [16]. To avoid unnecessary exposure to ineffective treatments and their associated toxicity, it is important to identify which systemic treatments are most beneficial in dMMR mCRC patients, thus improving their outcome.

Recent publications report durable responses to immunotherapy in dMMR mCRC patients across all lines of treatment [10,17-19]. Immunotherapy has been approved for dMMR patients with metastatic or unresectable CRC by regulatory authorities (including the Food and Drug Administration (FDA) and the European Medicines Agency (EMA)). Therefore, the standard of care is increasingly becoming immunotherapy. However, chemotherapy with or without monoclonal antibodies will still be relevant in patients with dMMR mCRC. In the first line KEYNOTE-177 trial 29.4% of patients in the pembrolizumab arm had primary progressive disease compared to 12.3% in the chemotherapy control arm [10]. For patients in whom rapid downstaging of disease is desired, e.g. to allow resection of disease or based on poor

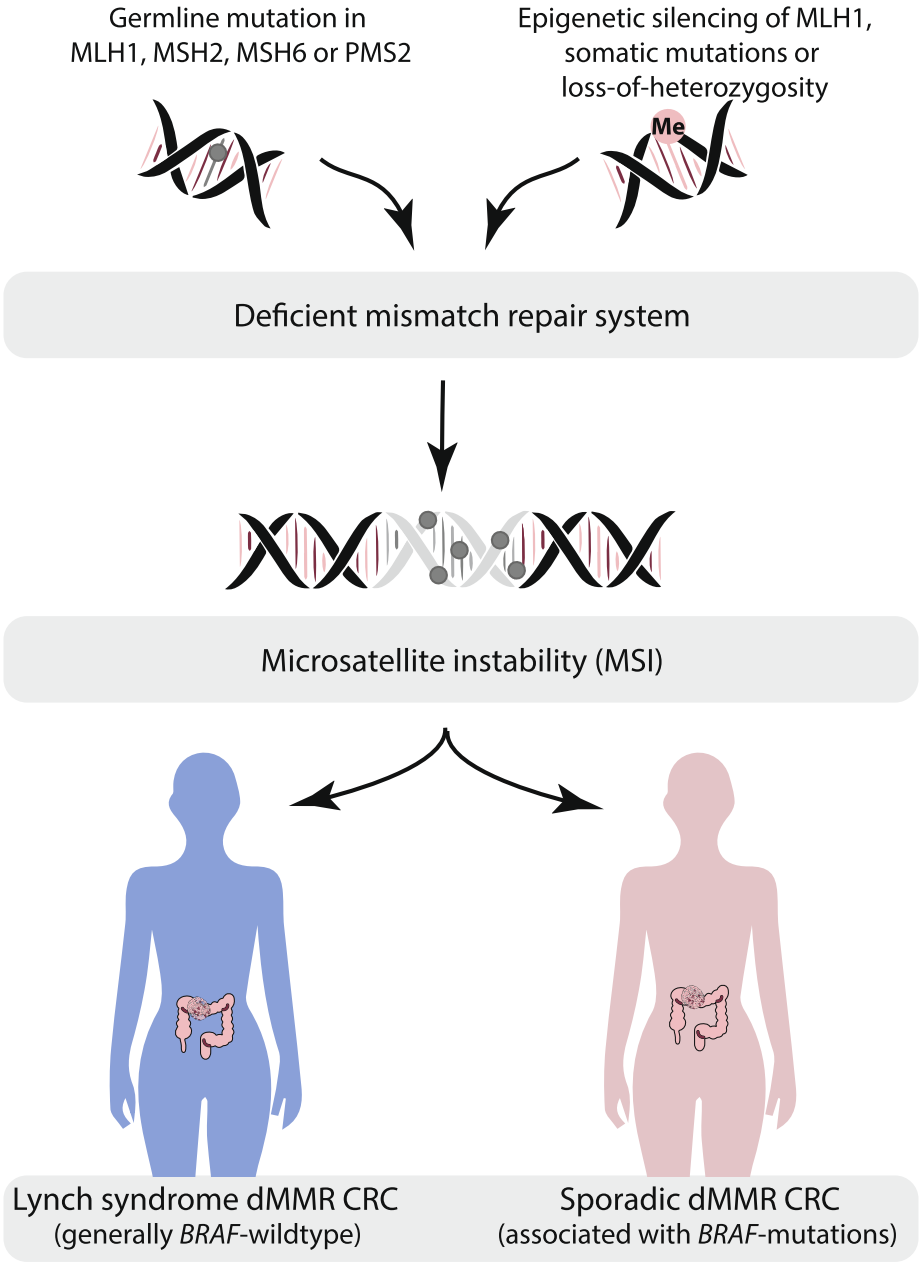

Fig. 1. Mechanisms of carcinogenesis for Lynch syndrome colorectal cancer (CRC) and sporadic deficient mismatch repair (dMMR) CRC.

prognostic features, first-line chemotherapy with monoclonal antibodies may still be preferrable over immunotherapy in selected cases to decrease the chance of having primary progression upon treatment. Furthermore, 12-month PFS rates ranged from 50 to 71% in the immune checkpoint inhibitor trials [10,18-20], with a significant portion of patients requiring an alternative treatment during the course of their disease. Finally, potentially a subgroup of dMMR mCRC patients may benefit from combination chemotherapy and monoclonal treatment with immunotherapy and it is important to find the most effective combination treatment. With the exception of immunotherapy, dMMR mCRC patients receive the same chemotherapy with or without monoclonal antibodies as other mCRC patients. Importantly, it is unclear if these treatments are equally beneficial in dMMR mCRC or if a specific type of chemotherapy or monoclonal antibody may offer increased benefit. Thus, we focused on chemotherapy and/or monoclonal antibodies in this review since dMMR mCRC patients will still be treated with these agents either before or after immunotherapy, or in combination with receiving immunotherapy.

A published *meta*-analysis from 2009 and international CRC guidelines do not provide clarity regarding the choice and efficacy of chemotherapy agents for dMMR mCRC [21,22]. Clinical trials are increasingly reporting MMR molecular analysis subgroup results, warranting a new review of the literature. In this evidence-based review, we assess clinical trials reporting outcome of treatment in dMMR mCRC patients to different types of chemotherapy with or without monoclonal antibodies. Subsequently, to clarify the clinical findings, we summarize the biological rationale for a differential benefit of chemotherapy with or without monoclonal antibodies in dMMR mCRC patients. Finally, we provide recommendations for clinical investigators and researchers.

Efficacy of systemic therapies in dMMR mCRC patients

We performed a systematic literature search (up to April 2020) in the PubMed and Embase databases, which included mCRC clinical trials reporting response rates and/or survival outcomes for dMMR mCRC patients receiving a given treatment. Search terms, inclusion and exclusion criteria are presented with the search results in Fig. 2. We identified 11 trials which examined standard-of-care chemotherapy with or without monoclonal antibodies effectiveness in dMMR/MSI mCRC patients (ancillary studies [1,23-31] and original studies[32-41]). The study characteristics and treatments examined are described in Table 1. All identified studies included patients receiving first-line treatment, often with doublet chemotherapy (5-flourouracil [5-FU] and oxaliplatin or irinotecan) and targeted treatment (anti-epidermal growth factor (EGFR) or anti-vascular endothelial growth factor (VEGF)). Three studies also examined later treatment lines (first, second and third-line) [23,27,32,33,36]. Two studies examined the effect of different first-line maintenance treatments [25,31,34,41], however survival data for dMMR mCRC patients was not reported per type of maintenance treatment and thus could not be analyzed. Immunotherapy trials were excluded as they are not within the scope of this review. The reported treatment efficacy for the different systemic therapy agents are summarized per study (Table 2). We will first discuss the results for studies across multiple treatment lines, subsequently the results for the effectivity of irinotecan-containing treatments, oxaliplatin-containing treatments and lastly, targeted therapies.

Studies across multiple treatment lines

Three studies reported outcomes of multiple treatment lines [23,27,32,33,36]. We first assess the results of OS for the different treatment regimens (sequential versus combination regimens in the CAIRO and FOCUS studies and doublet versus triplet in the TRIBE2 study), while later examining the PFS of different treatment types in the corresponding sections for each treatment.

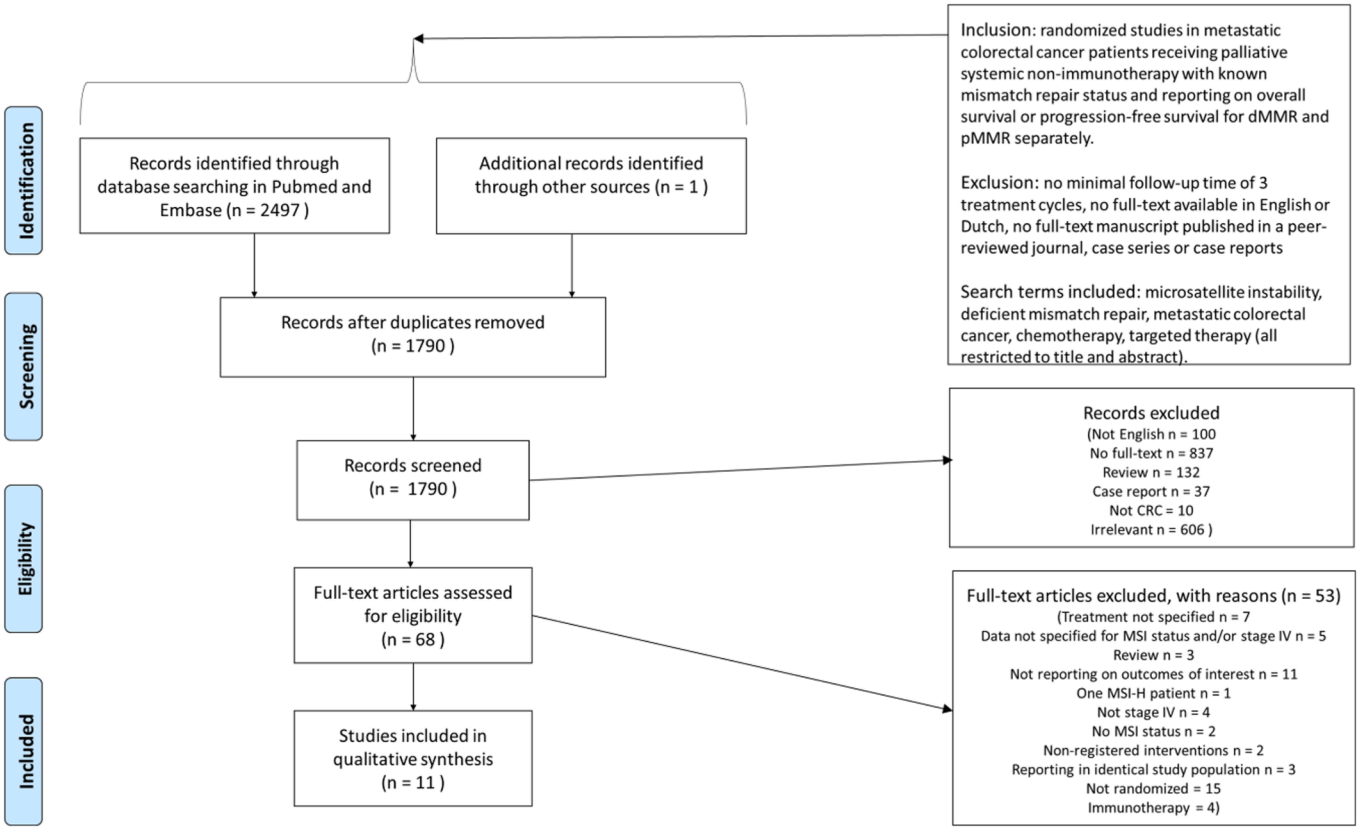

Fig. 2. PRISMA flow diagram.

Table 1
Description of included clinical studies in evidence-based review.

Author	Study Design	mCRC (*n*)	dMMR (*n*)	pMMR (*n*)	Method MMR/MSI	Intervention
Braun [23] 2008	Phase III RCT (**FOCUS** [33])	1313	58	1252	IHC (MLH1, MSH2)	5-FU, FOLFOX, FOLFIRI; sequential versus combination A) **1st line** 5-FU, **2nd line** irinotecan; B) **1st line** 5-FU, **2nd line** FOLFIRI or FOLFOX; C) **1st line** FOLFIRI or FOLFOX.
Chua [24] 2009	Phase II trials (3 studies [42-44])	118	2	116	PCR-based (BAT-26)	**1st line** FOLFOX
Cremolini [32] 2020	Phase III RCT (**TRIBE2** [32])	679	26	528	IHC (MLH1, PMS2, MSH2, MSH6)	A) **1st line** FOLFOX-B (8 cycles) then 5-FU-B maintenance, **2nd line** FOLFIRI-B B) **1st line** FOLFOXIRI-B (8 cycles) then 5-FU-B maintenance, **2nd line** FOLFOXIRI-B
Goey [25] 2017	Phase III RCT (**CAIRO3** [34])	558	4	275	IHC (MLH1, PMS2, MSH2, MSH6)	**1st line** CAPOX-B +/- CAP-B maintenance, reintroduction CAPOX-B upon progression
Innocenti [26] 2019	Phase III RCT **(CALGB** [35]**)**	1585	52	755	PCR-based (Bethesda panel)	**1st line** FOLFOX or FOLFIRI plus bevacizumab, cetuximab or both.
Koopman [27] 2009	Phase III RCT (**CAIRO** [36])	515	18	497	1) All: IHC (MLH1, MSH2, PMS2, MSH6); 2) if uninterpretable IHC or negative staining $\geq$ 1 protein, PCR-based (BAT-25, BAT-26); 3) if $\geq$ 1 instable, extended PCR (BAT-40, D2S123, D5S346, D17S250).	*Sequential:* **1st line** capecitabine (**2nd line** irinotecan, **3rd line** CAPOX) *Combination:* **1st line** CAPIRI (**2nd line** CAPOX)
Nopel [28] 2014	Phase III RCT (**AIO** [37])	229	14	190	PCR-based (1st BAT-26, then if unstable full 5 Bethesda panel); IHC (MLH1, MSH2, MSH6)	**1st line** FOLFOX or CAPOX
Smith [29] 2013	Phase III RCT (**COIN** [38])	2445	66	1499	PCR-based (BAT-25, BAT-26)	**1st line** CAPOX or FOLFOX A) continuous; B) continuous + cetuximab; C) intermittent (if no progression at 12-wk scan, then chemotherapy-free interval until progression, then re-start)
Venderbosch [1] 2014	Pooled analysis of 4 Phase III trials (**CAIRO** [36]**, CAIRO2** [39]**, COIN** [38]**, FOCUS** [33])	3063	153	2910	See CAIRO [27], CAIRO2 (conform CAIRO), COIN [29], FOCUS [23].	**1st line** Type treatment: see original studies for CAIRO, COIN and FOCUS. *CAIRO2:* ***1st line*** CAPOX-B versus **1st-line** CAPOX-B with cetuximab
Omrčen [30] 2019	Phase II trial [40]	40	2	38	IHC (MLH1, MSH2, MSH6)	**1st line** Capecitabine + bevacizumab (elderly patients, age $\geq$ 70 years)
Morano [31] 2019	Phase II, randomized, open-label trial (**Valentino** [41])	199	5	194	PCR-based (BAT-25, BAT-26, NR-21, NR-24, MONO-27)	**1st line** Induction FOLFOX + panitumumab with maintenance panitumumab (Arm A) or 5-FU + panitumumab (Arm B)

Abbreviations: 5-flourouracil (5-FU), 5-fluorouracil + irinotecan (FOLFIRI), 5-fluorouracil + irinotecan + bevacizumab (FOLFIRI-B), 5-flourouracil + oxaliplatin (FOLFOX), 5-flourouracil + oxaliplatin + bevacizumab (FOLFOX-B), 5-fluorouracil + oxaliplatin + irinotecan + bevacizumab (FOLFOXIRI-B), capecitabine + bevacizumab (CAP-B), capecitabine + irinotecan (CAPIRI), capecitabine + oxaliplatin + bevacizumab (CAPOX-B), deficient mismatch repair (dMMR), immunohistochemistry (IHC), metastatic colorectal cancer (mCRC), microsatellite instability (MSI), mismatch repair (MMR), polymerase chain reaction (PCR), proficient mismatch repair (pMMR). 1st or 2nd refer to first or second-line systemic therapy. Trial and protein abbreviations are not elaborated.

Combination versus sequential treatment

The CAIRO and FOCUS studies examined sequential versus combination treatment [23,27,36]. The CAIRO trial randomized patients between first-line capecitabine, second-line irinotecan and third-line capecitabine + oxaliplatin (CAPOX; sequential treatment arm) treatment versus first-line capecitabine + irinotecan (CAPIRI), and second-line CAPOX (combination treatment arm) [36]. The FOCUS trial randomized patients for 3 treatment arms: first-line 5-FU/capecitabine monotherapy and second-line irinotecan (arm A); first-line 5-FU/capecitabine monotherapy and second-line combination chemotherapy consisting of 5-FU with irinotecan or oxaliplatin (FOLFIRI or FOLFOX; arm B), or first- and second-line combination chemotherapy consisting of FOLFIRI or FOLFOX (arm C) [33].

In the CAIRO trial, OS was lower in dMMR mCRC patients treated with combination treatment compared to sequential treatment, however with a low number of patients per group and large survival confidence intervals (6.2 months, 3.6–31.3, $n = 8$ versus 12.7 months, 7.4–22.2, $n = 7$) [27,36]. However, in the FOCUS trial, there was a non-significant trend to longer OS in dMMR mCRC patients receiving combination treatment versus sequential treatment (HR 0.66, 95%C.I. 0.37–1.17, n = 58) [23,33]. In pMMR mCRC patients, OS was similar with combination and sequential treatment (HR 0.94, 95%C.I. 0.83–1.00, $n = 1252$) [23,33]. With contrasting non-significant results and a low number of dMMR patients per study, no firm conclusions can be drawn regarding the benefit of combination versus sequential treatment. Upfront combination treatment regimens may be beneficial rather than sequential treatments, since the response rates of dMMR mCRC patients to traditional chemotherapy and targeted treatment are low and thus patients may not be able to receive later-line treatments.

Doublet versus triplet treatment

In the TRIBE2 trial, patients were randomized for first-line doublet versus triplet treatment with first-line FOLFOX-bevacizumab and 5-FU-bevacizumab (5-FU-B) maintenance followed by second-line FOLFIRI-bevacizumab in the doublet arm versus first-line 5-FU, oxaliplatin, irinotecan + bevacizumab (FOLFOXIRI-B) and 5-FU-B maintenance followed by second-line reintroduction FOLFOXIRI-B in the triplet arm [32]. This study showed no benefit of triplet compared to doublet

Table 2
Results with treatment efficacy for PFS, OS and response rate, for different systemic therapy agents, per study.

Article	Treatment	Overall Survival (OS)		Progression-free survival (PFS)		Response rate		Hazard Ratio (HR)	
		dMMR	pMMR	dMMR	pMMR	dMMR	pMMR	dMMR	pMMR
Braun [23] 2008	*Arm A:* **1st line** 5-FU, **2nd line** irinotecan; *Arm B:* **1st line** 5-FU, **2nd line** FOLFIRI or FOLFOX; *Arm C:* **1st line** FOLFIRI or FOLFOX.							**PFS for 1st line** ($n = 58$): 5-FU HR PFS 1.00 FOLFIRI HR PFS 0.93 (0.45–1.91) FOLFOX HR PFS 0.68 (0.35–1.31) **OS** ($n = 58$): Sequential HR OS 1.00 Combination HR OS 0.66 (0.37–1.17)	**PFS for 1st line**($n = 1252$): 5-FU HR PFS 1.00 FOLFIRI HR PFS 0.76 (0.65–0.89) FOLFOX HR PFS 0.72 (0.62–0.84) **OS** ($n = 1262$): Sequential HR OS 1.00 Combination HR OS 0.94 (0.83–1.00)
Koopman [27] 2009	*Sequential:* **1st line** capecitabine (**2nd line** irinotecan, **3rd line** CAPOX)	12.7 months (7.4–22.2, $p = 0.47$, $n = 7$)	17.2 months (14.7–18.8, $n = 230$)	4.2 months (2.2–10.6, $p = 0.72$, $n = 7$)	5.8 months (4.9–6.3, $n = 247$)	**ORR** 25% (2/8) (3–65, $p = 0.57$) **DCR** 50% (4/8) (16–84, $p = 0.09$)	**ORR** 17% (41/239) (13–23) **DCR** 76% (182/239) (70–81)		
	Combination: **1st line** CAPIRI (**2nd line** CAPOX)	6.2 months (3.6–31.3, $n = 8$, $p = 0.58$)	18.3 months (16.2–20.6, $n = 210$)	4.0 months (2.3–6.5, $p = 0.02$, $n = 10$)	8.3 months (7.6–8.7, $n = 243$)	**ORR** 25% (2/8) (3–65%, $p = 0.30$) **DCR** 63% (5/8) (25–92, $p = 0.048$).	**ORR** 46% (98/214) (39–53%). **DCR** 90% (193/214) (85–93).		
Cremolini [32] 2020	*Doublet:* **1st line** FOLFOX-B + 5-FU-B maintenance, **2nd line** FOLFIRI-B *Triplet:* **1st line** FOLFOXIRI-B + 5-FU-B maintenance, **2nd line** FOLFOXIRI-B							**PFS (doublet versus triplet)** ($n = 26$): FOLFOXIRI-B HR PFS1 0.97 (0.39–2.38, *p for interaction* = 0.410); HR PFS2 1.11 (0.44–2.81, *p for interaction* = 0.319) **OS doublet versus triplet**: FOLFOXIRI-B HR 1.28 (0.45–3.63, *p for interaction* = 0.330)	**PFS (doublet versus triplet)** ($n = 528$): FOLFOXIRI-B HR PFS1 0.66 (0.55–0.79); HR PFS2 0.68 (0.57–0.83) **OS doublet versus triplet**: FOLFOXIRI-B HR 0.73 (0.60–0.90)
Chua [24] 2009	**1st line** FOLFOX					**ORR** 50% (1/2), OR 0.79 (0.05–12.97, $n = 2$)	**ORR** OR 1.00 ($p = 0.09$, $n = 114$)	($n = 2$) HR OS 0.40 (0.06–2.92, $p = 0.4$). HR PFS 0.36 (0.05–2.62, $p = 0.3$)	($n = 114$) Reference
Goey [25] 2017	**1st line** CAPOX-B (CAIRO3)	13.6 months (9.5–17.7, $n = 4$).	21.4 months (19.1–23.7, $p = 0.040$, $n = 275$).	PFS1 2.1 months (0.0–11.6, $n = 4$). PFS2 8.0 months (0.0–17.4, $n = 4$).	PFS1 5.7 months (4.9–6.6, $p = 0.748$, $n = 275$). PFS2 10.7 months (9.4–12.0, $p = 0.268$, $n = 275$).				
Nopel [28] 2015	**1st line** CAPOX/FOLFOX	24 months (95%CI 19.1–29.5, $n = 14$).	17.5 months (95%CI 15.5–19.7, $p = 0.228$, $n = 190$).	4.5 months (95%CI 1.8–7.2, $n = 14$).	7.5 months (95%CI 6.7–8.4, $p = 0.431$, $n = 190$).	**ORR** 50% (7/14) **DCR** 64.3% (9/14)	**ORR** 56.3% (107/190), $p = 0.782$. **DCR**		

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Table 2 (*continued*)

Article	Treatment	Overall Survival (OS)		Progression-free survival (PFS)		Response rate		Hazard Ratio (HR)	
		dMMR	**pMMR**	**dMMR**	**pMMR**	**dMMR**	**pMMR**	**dMMR**	**pMMR**
							85.3% (162/190), p = 0.055.		
Smith [29] 2013	**1st line** COIN CAPOX/ FOLFOX +/- Cetux	For any type chemotherapy: HR 1.27 (95% CI 0.65–2.45; p = 0.49, n = 45).		Cetuximab, any type chemotherapy: HR 1.26 (95% CI 0.68–2.34; p = 0.47, n = 45); **BRAF-wt** HR 0.98 (0.49–1.95; p = 0.95, n = 36). Cetuximab, for FOLFOX: HR 3.59 (0.43–29.7; p = 0.24, n = 9); **BRAF-wt** HR 1.89 (0.21–17.3; p = 0.57, n = 7).	Cetuximab, any type chemotherapy: HR 0.98 (0.86–1.12; p = 0.80, n = 977). Cetuximab, for FOLFOX: HR 0.83 (0.66–1.04; p = 0.11, n = 337).			Adjusted HR (for somatic mutation status, treatment arm, chemotherapy regimen) PFS HR 1.66 (95% CI, 1.21–2.27; p = 1.6 x10-3, n = 66). **BRAF-wt subgroup** HR PFS 1.85 (95% CI, 1.31–2.61; p = 0.0005, n = 36), OS HR 1.60 (95% CI 1.14–2.24; p = 0.0066, n = 66); **BRAF-wt subgroup** OS HR 1.89 (95% CI, 1.30–2.76; p = 0.00085, n = 36).	Reference.
Innocenti [26] 2019	**1st line** CALGB Bevacizumab Beva + Cetux Cetuximab	Bevacizumab 30.0 months (23.6-NE); Beva + Cetux 21.5 months (16.4–41.1); Cetuximab OS 11.9 months (10.3–24.6); p = 0.0014.	Bevacizumab 30.3 months (27.3–34.3) Beva + Cetux 26.2 months (22.6–29.7) Cetuximab 30.7 months (27.6–35.0); p = 0.2182	Bevacizumab (n = 21) 9.3 months (5.4–29.0); Beva + Cetux (n = 15) 7.7 months (6.6–17.6); Cetuximab (n = 16) 5.4 months (4.1–8.6).	Bevacizumab (n = 285) 11.2 months (10.3–12.5) Beva + Cetux (n = 189) 10.9 months (9.8–12.8) Cetuximab (n = 301) 10.9 months (9.5–12.8)			Adjusted HR (see article). Cetuximab (n = 16) ref. Bevacizumab (n = 21) PFS HR 0.16 (0.07–0.37), p < 0.001; Beva + Cetux (n = 15) PFS HR 0.44 (0.20–0.99), p = 0.046. Bevacizumab OS HR 0.13 (0.06–0.30), p < 0.001; Beva + Cetux OS HR 0.37 (0.16–0.85), p = 0.018. **MSI-H vs MSS** PFS HR 1.02 (0.71–1.47, p = 0.912). OS HR 0.87 (0.60–1.28p = 0.491).	Adjusted HR (see article). Cetuximab (n = 301) ref. Bevacizumab (n = 285) PFS HR 0.93 (0.77–1.12, p = 0.439) Beva + Cetux (n = 189) PFS HR 0.98 (0.80–1.21, p = 0.881) Bevacizumab OS HR 1.06 (0.87–1.29, p = 0.539) Beva + Cetux OS HR 1.18 (0.95–1.47, p = 0.134).
Venderbosch [1] 2014	**1st line** CAIRO2 (CAPOX-B +- Cetuximab)	15.6 months (12.9–22.3, n = 29).	22.0 (20.3–24.1, n = 487).	7.5 months (6.4–10.5, n = 29).	10.5 months (9.6–11.4, n = 487).			PFS HR 1.66 (1.13–2.45); OS HR 1.60 (1.07–2.40).	Reference.
Omrčen [30] 2019	**1st line** Capecitabine + Bevacizumab	**BRAF-m** (n = 2) 6.9 months & 13 months (each patient)	**Whole cohort** 20.5 months (range 3.5–134, n = 40)	Patient 1: 2.3 months	**Whole cohort** 9.8 months (range 3.5 – 22, n = 40)				
Morano [31] 2019	**1st line** FOLFOX + panitumumab	***BRAF*-wt** 2 year OS-rate 60.0%	***BRAF*-wt** 2 year OS-rate 62.9%	**BRAF-wt** 4.1 months (95%	**BRAF-wt** 11.1 months (95%			HR PFS 3.03 (95% C.I. 1.24–7.42, p =	Reference.

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Table 2 (*continued*)

Article	Treatment	Overall Survival (OS)		Progression-free survival (PFS)		Response rate		Hazard Ratio (HR)	
		dMMR	pMMR	dMMR	pMMR	dMMR	pMMR	dMMR	pMMR
	(pan) with maintenance pan (Arm B) or 5-FU + pan (Arm B)	(29.3–100, *n* = 5)	(56.0–70.6, *n* = 194)	C.I. 4.0-NA, *n* = 5)	C.I. 10.4–13.2, *n* = 194)			0.015) HR 2-year OS rate 1.23 (95% C.I. 0.38–3.92, *p* = 0.732) Adjusted HR for PFS (see article): HR 1.28 (0.47–3.47, *p* = 0.626)	

Abbreviations: 5-flourouracil (5-FU), 5-fluorouracil + irinotecan (FOLFIRI), 5-fluorouracil + irinotecan + bevacizumab (FOLFIRI-B), 5-flourouracil + oxaliplatin (FOLFOX), 5-flourouracil + oxaliplatin + bevacizumab (FOLFOX-B), 5-fluorouracil + oxaliplatin + irinotecan + bevacizumab (FOLFOXIRI-B), bevacizumab (beva), BRAF mutant (BRAF-mt), BRAF wildtype (BRAF-wt), capecitabine + bevacizumab (CAP-B), capecitabine + irinotecan (CAPIRI), capecitabine + oxaliplatin + bevacizumab (CAPOX-B), cetuximab (cetux), deficient mismatch repair (dMMR), disease control rate (DCR), first progression-free survival (PFS1), microsatellite instability high (MSI-H), microsatellite stable (MSS), objective response rate (ORR), proficient mismatch repair (pMMR), second progression-free survival (PFS2). 1st refers to first-line systemic therapy. Trial abbreviations are not elaborated.

therapy in dMMR mCRC patients in terms of PFS1 (HR 0.97, 95%C.I. 0.39–2.38, *n = 26*), PFS2 (HR 1.11, 95%C.I. 0.44–2.81) or OS (HR 1.28, 95%C.I. 0.45–3.63) [32]. Whereas pMMR mCRC patients showed a clear benefit of triplet compared to doublet therapy in PFS1 (HR 0.66, 95%C.I. 0.55–0.79), PFS2 (HR 0.68, 95%C.I. 0.57–0.83), and OS (HR 0.73, 95% C.I. 0.60–0.90, *n = 528*) [32]. Although no clear benefit for triplet versus doublet treatment in first-line setting can be seen for dMMR mCRC patients, the results reflect subgroup analysis with a low number of patients and thus may not have enough power to detect such a difference.

Irinotecan-containing systemic therapy versus 5-FU monotherapy

The CAIRO and FOCUS randomized studies also report PFS for 5-FU monotherapy versus irinotecan-containing systemic therapy in first-line setting in mCRC patients [23,27,33,36]. In the CAIRO trial, dMMR patients receiving capecitabine and irinotecan doublet therapy (CAPIRI) had a similar progression-free survival (PFS) and ORR as dMMR patients receiving mono capecitabine; PFS 4.0 months (95%C.I. 2.3–6.5, n = 10) versus 4.2 months (95%C.I. 2.2–10.6, n = 7), respectively [27]. As a reference, the PFS and ORR were higher in pMMR patients receiving CAPIRI compared to mono capecitabine; PFS 8.3 months (95%C.I. 7.6–8.7, n = 243) versus 5.8 months (95%C.I. 4.9–6.3, n = 247), respectively [27]. The overall response rate (ORR) in dMMR mCRC patients receiving CAPIRI compared to mono capecitabine were also similar (25% in both treatment lines) [27]. Likewise, dMMR patients in the FOCUS trial receiving irinotecan-containing therapy, versus 5-FU/capecitabine monotherapy, did not have a significantly different PFS with a hazard ratio (HR) of 0.93 (95%C.I. 0.45–1.91) [23]. In the FOCUS trial, the HR for PFS was non-significantly lower for oxaliplatin-containing therapy (HR 0.68, 95%C.I. 0.35–1.31) compared to 5-FU monotherapy in dMMR patients [23]. There was a trend to a better PFS in pMMR patients receiving irinotecan-containing therapy versus 5-FU/capecitabine; HR 0.76 (95%C.I. 0.65–0.89) [23].

Thus, the FOCUS and CAIRO trials report a similar PFS in dMMR patients receiving 5-FU monotherapy compared to irinotecan doublet therapy, while pMMR patients have a trend to increased PFS upon irinotecan doublet therapy compared to 5-FU monotherapy. However this should be interpreted with caution due to small numbers and results being subgroup analysis. These results may suggest that 5-FU monotherapy may be better suited by offering similar survival benefit but less toxicity compared to irinotecan doublet therapy, however this should be validated prior to implementation, given the weak evidence. This is in contrast to the preclinical study results, summarized later in this review, which showed that dMMR CRC tumours are sensitive to irinotecan therapy.

Oxaliplatin-containing systemic therapy

Seven studies examined the efficacy of first-line oxaliplatin-containing systemic treatment (5-FU and oxaliplatin [FOLFOX] or capecitabine and oxaliplatin [CAPOX]) in dMMR mCRC patients (ancillary studies: [1,23-25,28,29] and original studies: [32-34,37-39,41-44]). The FOCUS trial was the only trial to examine treatment arms with and without oxaliplatin [23,33], while the CAIRO3 trial examined maintenance treatment [25,34].

In the FOCUS trial, dMMR mCRC patients receiving FOLFOX had a trend to better PFS (HR 0.68, 95%C.I. 0.35–1.31) compared to patients receiving 5-FU or 5-FU and irinotecan doublet therapy (FOLFIRI) (HR 0.93, 95%C.I. 0.45–1.91) [23]. In comparison, FOCUS pMMR patients had similar PFS with FOLFOX (HR 0.72, 95%C.I. 0.62–0.84) and FOLFIRI (HR 0.76, 95%C.I. 0.65–0.89) compared to 5-FU [23,33]. The remaining studies reported the efficacy of first-line oxaliplatin-containing systemic therapy in dMMR mCRC patients, without a comparison arm [1,24,25,28]. The AIO trial reported a comparable ORR in 14 dMMR patients compared to 190 pMMR patients (ORR 50% versus 56.3%) receiving oxaliplatin doublet therapy [28]. Three studies [1,25,28] did not find a statistical difference in PFS for dMMR and pMMR mCRC patients receiving oxaliplatin doublet therapy, although the studies may not have been powered to detect a survival difference, considering the small group sizes. Of note, the PFS was shorter in dMMR mCRC versus pMMR mCRC patients across all three studies. In the AIO trial, the median PFS was 4.5 months (95%C.I. 1.8–7.2, *n = 14*) versus 7.5 months (95%C.I. 6.7–8.4, *n = 190, p* = 0.431) respectively [28]. In the CAIRO2 trial, dMMR mCRC patients had a higher, but non-significant, risk for shorter PFS (HR 1.66 (95%C.I. 1.13–2.45), with an observed median PFS of 7.5 months (95%C.I. 6.4–10.5, *n = 29*) in dMMR mCRC patients compared to 10.5 months (95%C.I. 9.6–11.4, *n = 407*) in pMMR mCRC patients [1]. Lastly, the PFS1 in CAIRO3 patients was similar regardless of MMR status, with an observed PFS1 of 2.1 months (95%C.I. 0.0–11.6, *n = 4*) in dMMR mCRC patients versus PFS1 of 5.7 months (95%C.I. 4.9–6.6, *n = 275*, *p* = 0.748) in pMMR mCRC patients [25]. The median OS ranged from 13.6 to 24.0 months (dMMR) versus 17.5 to 22.0 months (pMMR), which was significantly different between dMMR and pMMR in the CAIRO3 trial, but not in the AIO trial [1,25,28]. Of note is the low percentage of dMMR mCRC patients in the CAIRO3 trial (1%), lower than the expected 5% prevalence, potentially indicating a patient selection due to poor treatment response as all CAIRO3 patients were required to have at least stable disease after 6 cycles of induction CAPOX-B prior to inclusion [25].

Targeted therapy (anti-EGFR and anti-VEGF)

In mCRC patients, primary tumour location 'sidedness' and *RAS*-mutational status are predictive biomarkers for treatment response to anti-EGFR treatment, with a worse outcome for patients with a right-sided and/or *RAS*-mutant tumour [35,45,46]. The predictive value of *BRAF*-mutational status in mCRC patients receiving anti-EGFR is inconclusive. The BEACON trial indicates that combination treatment with anti-EGFR, MEK and BRAF inhibitors in later-line setting provides survival benefit, while other studies indicate that monotherapy anti-EGFR treatment in pre-treated patients should be avoided [47,48]. In analyzing the trial results for dMMR mCRC patients receiving anti-EGFR treatment, we also analyze available results for relevant prognostic subgroups.

In the COIN, Valentino, CALGB, CAIRO3 trials and Omrčen *et al.* study, mCRC patients received first-line treatment containing chemotherapy with cetuximab or bevacizumab or combined cetuximab/bevacizumab treatment (ancillary studies: [25,26,29-31] and original trials: [34,35,38,40,41]). The CAIRO3 trial results (where all patients received oxaliplatin doublet and bevacizumab targeted treatment) is discussed in detail above. Given the low number of patients, we shall not discuss the Omrčen *et al.* study in detail [30,40].

COIN study patients received CAPOX or FOLFOX treatment and were randomized to receive cetuximab [29,38]. In 45 dMMR mCRC patients receiving CAPOX/FOLFOX with cetuximab, there was a trend towards worse PFS (HR 1.26, 95%C.I. 0.68–2.34, p = 0.47) compared to dMMR mCRC patients without cetuximab [29]. The trend was not present when analyzing the dMMR mCRC *BRAF*-wildtype subgroup (HR 0.98, 95%C.I. 0.49–1.95, p = 0.95, n = 36) [29]. No subgroup data results are available regarding the effect of primary tumour sidedness or *RAS*-mutational status in dMMR mCRC patients receiving cetuximab. In comparison, pMMR mCRC patients had similar PFS with or without cetuximab, including the pMMR / *BRAF*-wildtype subgroup [29]. The adjusted HR for PFS and OS, for somatic mutation status, treatment arm and chemotherapy regimen, were significantly worse in dMMR patients compared to pMMR patients; PFS HR 1.66 (95%C.I. 1.21–2.27, p = 0.0016) and OS HR 1.60 (95%C.I. 1.14–2.24, p = 0.0066) [29].

In the Valentino trial, *BRAF/RAS* wildtype mCRC patients were randomized between 5-FU and panitumumab maintenance treatment (arm A) and panitumumab (arm B), after induction first-line treatment consisting of FOLFOX plus panitumumab [31,41]. PFS was significantly lower (unadjusted HR 3.03, 95%C.I. 1.24–7.42, p = 0.015) in dMMR versus pMMR mCRC, with a median PFS of 4.1 months (95%C.I. 4.0-not reached, *n = 5*) versus 11.1 months (95%C.I. 10.4–13.2, *n = 194*) respectively [31]. The 2-year OS rate is lower in dMMR mCRC patients (60.0%, 95%C.I. 9.3–100) versus pMMR mCRC patients (62.9%, 95%C.I. 56.0–70.6) with an unadjusted HR of 1.23 (95%C.I. 0.38–3.92, p = 0.732) [31]. Of note, 40% (2/5) of the dMMR mCRC patients had molecular alterations associated with anti-EGFR primary resistance (*PI3KCA* exon 20 mutation and *PTEN* inactivating mutation) [31]. In multivariable analysis, dMMR status was not associated with PFS when adjusted for other clinical factors (HR 1.28, 95%C.I. 0.47–3.47, p = 0.626), however this might be limited by the low number (5) of dMMR patients in the trial [31]. Thus, the survival of dMMR mCRC patients receiving first-line panitumumab (with FOLFOX and maintenance treatment) is lower than in pMMR mCRC patients, but MMR status was not independently prognostic for survival during anti-EGFR treatment when adjusted for other clinical variables in the Valentino trial.

Patients in the CALGB trial were randomized to receive bevacizumab, cetuximab or both in combination with first-line chemotherapy (FOLFOX or FOLFIRI) [26,35]. An adjusted HR for PFS and OS was calculated, adjusting for several factors, including primary tumour location (right versus transverse vs left-sidedness), *BRAF* and *RAS* mutational status [26]. CALGB patients with dMMR tumours receiving cetuximab versus bevacizumab had a significantly worse adjusted HR for PFS and OS, while pMMR patients showed comparable adjusted HR for PFS and OS in all treatment arms [26]. The adjusted HR for PFS in dMMR mCRC patients receiving bevacizumab, combined bevacizumab/cetuximab and cetuximab was HR 0.16 (95%C.I. 0.07–0.37, n = 21, p < 0.001) versus HR 0.44 (95%C.I. 0.20–0.99, n = 15, p = 0.046) versus HR 1.0 (n = 16), respectively [26]. The reported PFS in dMMR patients receiving chemotherapy + cetuximab is 5.4 months (4.1–8.6, n = 16) versus 9.3 months (5.4–29.0, n = 21) for chemotherapy + bevacizumab [26].

Biological rationale for differential sensitivity of dMMR and pMMR CRC tumours to systemic therapy

Assessment of available models used in pre-clinical studies

Preclinical studies have extensively examined the sensitivity, and mechanisms involved, of dMMR CRC to chemotherapy with or without monoclonal antibodies, as outlined in recent reviews [49-52]. An extensive literature review was provided in the recent reviews, as such, we briefly touch on the relevant findings below. These preclinical findings have not resulted in a clear benefit in clinical trials. A potential barrier to interpreting the available pre-clinical data is the choice of model systems used, which traditionally have included cell lines with a dMMR system [53-57] and xenograft models [52,54].

The use of dMMR CRC cell lines in preclinical studies has key limitations. Cell lines are genetically unstable *in vitro* and acquire a more aggressive subclone through repeated passaging while culturing, thus no longer reflecting the heterogeneity of the original tumours [58]. The dMMR preclinical studies often chose cell lines with only one mutational type (e.g. *hMLH1* deficient cell lines), not accurately reflecting the mutational landscape of dMMR CRC patients. Preclinical studies should comprise *BRAF*-wildtype and *BRAF*-mutant models (to reflect Lynch syndrome and sporadic dMMR patients), and where applicable, confirm results in epigenetically silenced *hMLH1* models. Moreover, dMMR CRC cell lines should display genomic instability reflecting the genomic landscape of dMMR CRC tumours. Due to non-functional mismatch repair machinery, dMMR CRC tumours accumulate somatic mutations in specific oncogenes and tumour suppressor genes, including *BRAF, MRE11A, KRAS, TGFβrII* and *IGF2R* [59,60]. The choice of cell line can affect preclinical results, as was seen in irinotecan preclinical studies where cell lines without secondary *MRE11A* mutations had a different irinotecan sensitivity compared to *MRE11A*-mutant cell lines [50,57,61,62].

Recent studies have shown that immunotherapy can effectively produce anti-tumour immune responses in dMMR CRC tumours, highlighting the crucial role of the tumour microenvironment (TME) [17-19]. In addition to immunotherapy, the TME also affects the sensitivity of other treatments [63]. Thus, when studying dMMR mCRC sensitivity to treatment it is important to use models with TME components where possible. Patient-derived xenograft models, which have stromal components, display intra-tumoral heterogeneity and long-term genomic stability. However, they are limited in clinical applicability since they require substantial patient tumour substrate, animal models and months to establish [64-66]. Humanized mouse models have been used to model PD-1 blockade using xenografts for non-colorectal cancers [67]. The models would lend well to preclinical dMMR CRC treatment studies, since the mice have a full human immune system as well as the engrafted xenograft. Patient-derived organoids (PDOs) may be a more accurate model for dMMR CRC, since organoids maintain intra-tumoral heterogeneity, have long-term phenotypical and genetical stability and can be easily propagated from patient tissues [68,69]. Organoids allow researchers to perform library drug screens on dMMR mCRC PDOs, revealing the sensitivity of patients to different treatments in one experiment [70]. Co-cultures with TME components can be established using PDOs; moreover, these co-culture models have altered treatment sensitivity compared to PDOs alone [71,72]. Drug screen data derived from CRC PDOs is highly correlated to patient response in the clinic,

showing that organoids may be used to predict treatment response [73-75].

Key preclinical findings concerning altered sensitivity of dMMR CRC tumours to chemotherapy with or without monoclonal antibodies

To clarify the clinical findings, we summarize the biological rationale for a differential benefit of chemotherapy with or without monoclonal antibodies in dMMR mCRC patients. Preclinical studies have examined the response to the main backbones of chemotherapy (5-FU, irinotecan and oxaliplatin) in dMMR CRC. Chemotoxicity induced by 5-FU treatment is thought to be partially mediated by the MMR pathway [76]. The hMutSα heterodimer, consisting of the MMR proteins hMSH6 and hMSH2, binds and recognizes 5-FU-modified DNA [77]. Although not all studies agree [78], preclinical studies have demonstrated that dMMR CRC cell lines are at least 18-fold more resistant to 5-FU than pMMR cell lines, and that the resistance is reversed when the MMR deficiency is corrected [55,56,79]. Regarding topoisomerase inhibitor sensitivity, dMMR CRC tumours are thought to be more sensitive to irinotecan since these tumours often have a secondary frameshift mutation in *MRE11A*, part of the double strand break repair (dsBR) complex [57,80,81]. CRC cell lines which were hMLH1-deficient, especially cell lines with *MRE11A* mutations, were more sensitive to irinotecan than pMMR cell lines, and accordingly, restoring hMLH1 induced resistance to irinotecan [57,61,62]. Other studies contradict the above results, possibly due to using dMMR cell lines without secondary mutations [50,53]. Thirdly, dMMR CRC cell lines are resistant to cisplatin and carboplatin, but not to oxaliplatin, when compared to pMMR CRC cell lines [54,78,82]. Oxaliplatin forms a 1,2-diaminocyclohexane ligand, which is not recognized by the MMR pathway when incorporated into DNA [83], thus dMMR CRC tumours do not have a different sensitivity to oxaliplatin compared to pMMR tumours [50]. Altogether, preclinical studies suggest that dMMR mCRC, compared to pMMR mCRC, may be more resistant to 5-FU, more sensitive to irinotecan, but not have an altered sensitivity to oxaliplatin. This in contrast to the trends observed in clinical studies, where 5-FU + irinotecan doublet therapy did not have an increased benefit over 5-FU monotherapy or oxaliplatin doublet treatment.

Regarding the effectivity of targeted treatment, to our knowledge no studies have investigated the efficacy of anti-EGFR or anti-VEGF treatment in preclinical dMMR CRC models. dMMR mCRC patients may be less sensitive to anti-EGFR treatment due to the higher prevalence of *BRAF*-mutations, increased right-sided primary tumour location and decreased EGFR ligand expression [84,85]. For anti-VEGF targeted treatment, contradictory hypotheses exist: mucinous dMMR CRC tumours have decreased *VEGF* expression and decreased microvessel density, which would support lower sensitivity to anti-VEGF targeted treatment [86], however, the immunostimulatory effect associated with anti-VEGF treatment may potentiate the immunostimulatory environment in dMMR CRC tumours, improving sensitivity [87]. In clinical trials, there is a suggestion of better efficacy of bevacizumab targeted therapy in dMMR patients compared to cetuximab targeted therapy and of reduced benefit to cetuximab targeted therapy in general, which may be explained by the above mechanisms.

Conclusions and future perspectives

This evidence-based review cannot draw a definitive conclusion regarding the relative efficacy of different systemic therapy agents within dMMR mCRC patients, due to the low amount of clinical trials and low number of dMMR patients, despite an increasing number of trials reporting subgroup data for dMMR mCRC patients. We have identified three trends. These trends need to be confirmed by more studies prior to implementation of altered treatment strategies in clinical practice since there are too few trials available which support these results. Firstly, dMMR patients receiving 5-FU + irinotecan doublet therapy did not have an increased benefit over 5-FU monotherapy, suggesting that patients may not benefit from the addition of irinotecan [23,27]. Secondly, one study reported a trend to better efficacy of oxaliplatin-containing doublet treatment versus irinotecan or 5-FU monotherapy in dMMR mCRC patients, suggesting that dMMR mCRC may benefit more from oxaliplatin doublet therapy compared to irinotecan doublet or 5-FU monotherapy [23]. Lastly, there was a suggestion of better efficacy of bevacizumab targeted therapy in dMMR patients compared to cetuximab targeted therapy [26], and of no significant benefit in PFS of cetuximab (versus no targeted treatment) in *BRAF*-wildtype dMMR mCRC patients [29]. Despite our review, it is difficult to establish the exact predictive value of MMR for each treatment strategy considering the low numbers of patients and heterogeneous studies. Given the sparse clinical evidence supporting each trend, more studies are needed to confirm the findings prior to implementation in clinical practice. Likewise, no conclusion can be drawn about the effect of other relevant factors including sidedness and mutational status.

The strength of this review lies in including all current available evidence, linking the results to preclinical studies and aiming to identify solutions to accelerate research on this topic. Our analysis was restricted by the quality of data available, which is limited by a low number of studies, small number of dMMR mCRC patients per treatment and heterogeneity in study designs. Furthermore, there may be a selection bias in the available evidence since we could only include studies where MMR status was known. As well, a time bias is likely, given that the prognostic value of MMR status in mCRC patients was not known prior to 2009 [27]. The majority of included studies were ancillary studies, based on subgroup analysis for which the trials were not powered, which limits the conclusions which can be drawn based on the studies alone. A *meta*-analysis of the data could provide clarity. However, due to the limited data available, a *meta*-analysis was impossible and we could not compare treatment results for dMMR mCRC subgroups (e.g. Lynch syndrome versus sporadic dMMR). The limited amount of available data affirms the urgent need for trials examining which systemic treatment is most beneficial in dMMR mCRC patients.

Due to the low prevalence of dMMR in the metastatic CRC population, available trials only identify a small number of dMMR mCRC patients. We advise clinical trial researchers and clinicians to publish molecular analysis results and deposit patient-level data in data repositories. This will enable *meta*-analyses, hopefully increasing our knowledge of the most effective treatment in dMMR mCRC patients. If relevant clinical parameters are shared, a comparison of relevant dMMR mCRC patient subgroups (e.g. Lynch syndrome) and adjustment of prognostic clinical parameters is possible. Furthermore, we advise clinicians to test all mCRC patients for MMR status to guide treatment, but also to enable CRC patient registries to register population-based data for research, which is often more reflective of patients in the clinic than trials. Effective preclinical studies can help to confirm differential treatment sensitivity and to elucidate the molecular pathways involved in dMMR CRC. Compared to other preclinical models, patient-derived organoids can accelerate research regarding which systemic therapy is most effective in dMMR mCRC patients, as discussed previously. This technique is especially applicable to low prevalence diseases, such as dMMR mCRC, where patients are under-represented in clinical trials. Cumulatively, these efforts will help future research for dMMR mCRC sensitivity to treatment, as shown in Fig. 3.

Despite advances in immune checkpoint inhibitor treatment, it is anticipated that dMMR mCRC patients will continue to receive chemotherapy with or without monoclonal antibodies during their treatment, prior to receiving immunotherapy, upon progression during immunotherapy or in combination with immunotherapy. One strategy to overcome primary resistance to immunotherapy is to combine immune checkpoint inhibitors with chemotherapy and/or targeted treatment [88]. Chemotherapy can increase the efficacy of immunotherapy through promoting tumour antigen release, antigen presentation and

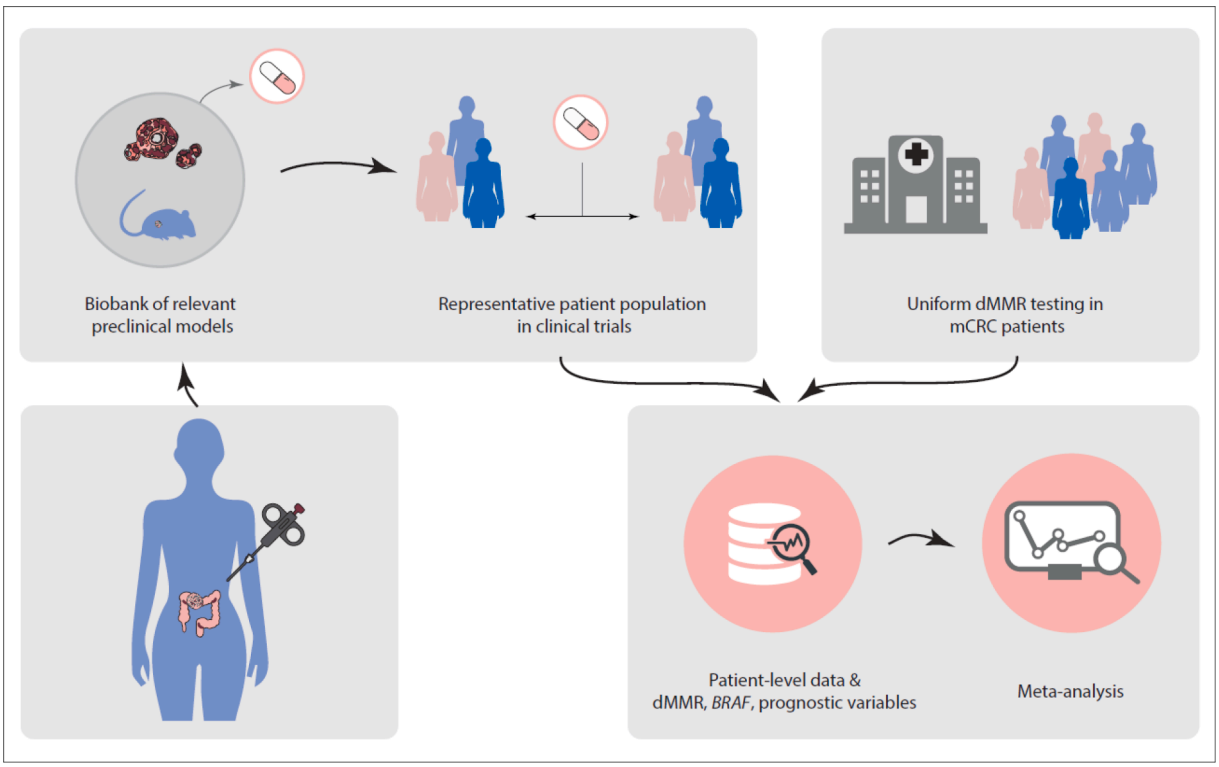

Fig. 3. Accelerating research for dMMR mCRC treatment. To determine which treatment is most effective in dMMR mCRC patients, representative preclinical models for the dMMR CRC population should be used (e.g. patient-derived organoids). In clinical trials, the patient population should reflect the clinical population (e.g. a mix of germline and sporadic dMMR) and for patient-level data, dMMR status, mutational status and relevant prognostic factor should be made available in depositories so that patient-level *meta*-analysis is possible.

stimulating immune effectors, while certain targeted treatments decrease tumour characteristics which have been associated with decreased immunotherapy effectivity [88]. Several phase III trials of combination treatment with immunotherapy in dMMR CRC patients are ongoing, including the COMMIT trial (NCT02997228; first-line FOLFOX, bevacizumab with atezolizumab versus atezolizumab single agent treatment for mCRC patients), the A021502 trial (NCT02912559; chemotherapy combined with atezolizumab versus chemotherapy as adjuvant therapy) and the CheckMate-142 trial (NCT02060188; which includes an intervention arm with combination nivolumab + ipilimumab and cobimetinib treatment). These and other ongoing studies will help clarify the role of chemotherapy and targeted treatment in increasing the efficacy of immunotherapy.

The available evidence highlights differences in sensitivity to systemic therapy in patients with dMMR versus pMMR mCRC. However, no definitive conclusion can be drawn regarding which chemotherapy with or without monoclonal antibodies is more effective in dMMR mCRC patients. A better understanding of which chemotherapy with or without monoclonal antibodies is most effective in dMMR mCRC patients is urgently needed. Several strategies can help accelerate research into this field: using preclinical models which more accurately reflect dMMR CRC patients (e.g. PDOs comprising multiple molecular subgroups), publishing patient-level data to allow for *meta*-analyses and increased testing of MMR status in patients in daily clinical care to increase known dMMR mCRC patients in population registries. With these solutions, clinicians and researchers will hopefully be able to reveal how best to treat dMMR mCRC patients, improving their prognosis.

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CRediT authorship contribution statement

Emerens Wensink: Conceptualization, Data curation, Formal analysis, Methodology, Writing - original draft. **Marinde Bond:** Conceptualization, Data curation, Formal analysis, Methodology, Writing - original draft. **Emre Kucukkose:** Conceptualization, Methodology. **Anne May:** Conceptualization, Methodology. **Geraldine Vink:** Conceptualization, Methodology. **Miriam Koopman:** Conceptualization, Formal analysis, Methodology, Supervision. **Onno Kranenburg:** Conceptualization, Formal analysis, Methodology, Supervision. **Jeanine Roodhart:** Conceptualization, Formal analysis, Methodology, Supervision.

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References

[1] Venderbosch S, Nagtegaal ID, Maughan TS, Smith CG, Cheadle JP, Fisher D, et al. Mismatch repair status and BRAF mutation status in metastatic colorectal cancer patients: A pooled analysis of the CAIRO, CAIRO2, COIN, and FOCUS studies. Clin Cancer Res 2014;20:5322–30. https://doi.org/10.1158/1078-0432.CCR-14-0332.

[2] Gelsomino F, Barbolini M, Spallanzani A, Pugliese G, Cascinu S. The evolving role of microsatellite instability in colorectal cancer: A review. Cancer Treat Rev 2016; 51:19–26. https://doi.org/10.1016/j.ctrv.2016.10.005.

[3] Weisenberger DJ, Siegmund KD, Campan M, Young J, Long TI, Faasse MA, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. Nat Genet 2006;38: 787–93. https://doi.org/10.1038/ng1834.

[4] Mensenkamp AR, Vogelaar IP, Van Zelst-Stams WAG, Goossens M, Ouchene H, Hendriks-Cornelissen SJB, et al. Somatic mutations in MLH1 and MSH2 are a frequent cause of mismatch-repair deficiency in lynch syndrome-like tumors. Gastroenterology 2014;146(643–646):e8. https://doi.org/10.1053/j.gastro.2013.12.002.

[5] Børresen A-L, Lothe RA, Meling GI, Lystad S, Morrison P, Lipford J, et al. Somatic mutations in the hMSH2 gene in microsatellite unstable colorectal carcinomas. Hum Mol Genet 1995;4:2065–72. https://doi.org/10.1093/hmg/4.11.2065.

[6] Tran B, Kopetz S, Tie J, Gibbs P, Jiang Z. Impact of BRAF Mutation and Microsatellite Instability on the Pattern of Metastatic Spread and Prognosis in Metastatic Colorectal Cancer. Cancer 2011;117:4623–32. https://doi.org/10.1002/cncr.26086.

[7] Aasebø K, Dragomir A, Sundström M, Mezheyeuski A, Edqvist PH, Eide GE, et al. Consequences of a high incidence of microsatellite instability and BRAF-mutated tumors: A population-based cohort of metastatic colorectal cancer patients. Cancer Med 2019;8:3623–35. https://doi.org/10.1002/cam4.2205.

[8] Wensink GE, Elferink MAG, May AM, Mol L, Hamers PAH, Bakker SD, et al. Survival of patients with deficient mismatch repair metastatic colorectal cancer in the pre-immunotherapy era. Br J Cancer 2021;124:399–406. https://doi.org/10.1038/s41416-020-01076-0.

[9] Kim CG, Ahn JB, Jung M, Beom SH, Kim C, Kim JH, et al. Effects of microsatellite instability on recurrence patterns and outcomes in colorectal cancers. Br J Cancer 2016;115:25–33. https://doi.org/10.1038/bjc.2016.161.

[10] André T, Shiu K-K, Kim TW, Jensen BV, Jensen LH, Punt C, et al. Pembrolizumab in Microsatellite-Instability–High Advanced Colorectal Cancer. NEJM 2020;383:2207–18. https://doi.org/10.1056/NEJMoa2017699.

[11] Taieb J, Shi Q, Pederson L, Alberts S, Wolmark N, Van Cutsem E, et al. Prognosis of microsatellite instability and / or mismatch repair deficiency stage III colon cancer patients after disease recurrence following adjuvant treatment: results of an ACCENT pooled analysis of seven studies. Ann Oncol 2019;30:1466–71. https://doi.org/10.1093/annonc/mdz208.

[12] Chong LC, Townsend AR, Young J, Roy A, Piantadosi C, Hardingham JE, et al. Outcomes for Metastatic Colorectal Cancer Based on Microsatellite Instability: Results from the South Australian Metastatic Colorectal Cancer Registry. Target Oncol 2019;14:85–91. https://doi.org/10.1007/s11523-018-0615-9.

[13] Goldstein J, Tran B, Ensor J, Gibbs P, Wong HL, Wong SF, et al. Multicenter retrospective analysis of metastatic colorectal cancer (CRC) with high-level microsatellite instability (MSI-H). Ann Oncol 2014;25:1032–8. https://doi.org/10.1093/annonc/mdu100.

[14] Cohen R, Buhard O, Cervera P, Hain E, Dumont S, Andre T. Clinical and molecular characterisation of hereditary and sporadic metastatic colorectal cancers harbouring microsatellite instability/DNA mismatch repair deficiency. Eur J Cancer 2017;86:266–74. https://doi.org/10.1016/j.ejca.2017.09.022.

[15] Boeckx N, Janssens K, Van Camp G, Rasschaert M, Papadimitriou K, Peeters M, et al. The predictive value of primary tumor location in patients with metastatic colorectal cancer: A systematic review. Crit Rev Oncol Hematol 2018;121:1–10. https://doi.org/10.1016/j.critrevonc.2017.11.003.

[16] Marisa L, Svrcek M, Collura A, Becht E, Cervera P, Wanherdrick K, et al. The Balance Between Cytotoxic T-cell Lymphocytes and Immune Checkpoint Expression in the Prognosis of Colon Tumors. JNCI 2018;110:68–77. https://doi.org/10.1093/jnci/djx136.

[17] Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. NEJM 2015;372:2509–20. https://doi.org/10.1056/NEJMoa1500596.

[18] Overman MJ, Mcdermott R, Leach JL, Lonardi S, Lenz H, Morse MA, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. Lancet 2017;18:1182–91. https://doi.org/10.1016/S1470-2045(17)30422-9.

[19] Overman MJ, Lonardi S, Yeung K, Wong M, Lenz H, Gelsomino F, et al. Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair–Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer. JCO 2018;36:773–9. https://doi.org/10.1200/JCO.2017.76.9901.

[20] Le DT, Kim TW, Van Cutsem E, Geva R, Jäger D, Hara H, et al. Phase II Open-Label Study of Pembrolizumab in Treatment-Refractory, Microsatellite Instability–High/Mismatch Repair-Deficient Metastatic Colorectal Cancer: KEYNOTE-164. J Clin Oncol 2020;38:11–9. https://doi.org/10.1200/JCO.19.02107.

[21] Guetz GDES, Uzzan B, Nicolas P, Schischmanoff O, Perret G, Morere J. Microsatellite Instability does not Predict the Efficacy of Chemotherapy in Metastatic Colorectal Cancer. A Systematic Review and Meta-analysis. Anticancer Res 2009;29:1615–20.

[22] Van Cutsem E, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol 2016;27:1386–422. https://doi.org/10.1093/annonc/mdw235.

[23] Braun MS, Richman SD, Quirke P, Daly C, Adlard JW, Elliott F, et al. Predictive Biomarkers of Chemotherapy Efficacy in Colorectal Cancer: Results From the UK MRC FOCUS Trial. J Clin Oncol 2008;26:2690–8. https://doi.org/10.1200/JCO.2007.15.5580.

[24] Chua W, Goldstein D, Lee CK, Dhillon H, Michael M, Mitchell P, et al. Molecular markers of response and toxicity to FOLFOX chemotherapy in metastatic colorectal cancer. Br J Cancer 2009;101:998–1004. https://doi.org/10.1038/sj.bjc.6605239.

[25] Goey KKH, Elias SG, van Tinteren H, Laclé MM, Willems SM, Offerhaus GJA, et al. Maintenance treatment with capecitabine and bevacizumab versus observation in metastatic colorectal cancer: Updated results and molecular subgroup analyses of the phase 3 CAIRO3 study. Ann Oncol 2017;28:2128–34. https://doi.org/10.1093/annonc/mdx322.

[26] Innocenti F, Ou F-S, Qu X, Zemla TJ, Niedzwiecki D, Tam R, et al. Mutational Analysis of Patients With Colorectal Cancer in CALGB/SWOG 80405 Identifies New Roles of Microsatellite Instability and Tumor Mutational Burden for Patient Outcome. J Clin Oncol 2019;37:1217–27. https://doi.org/10.1200/JCO.18.01798.

[27] Koopman M, Kortman GAM, Mekenkamp L, Ligtenberg MJL, Hoogerbrugge N, Antonini NF, et al. Deficient mismatch repair system in patients with sporadic advanced colorectal cancer. Br J Cancer 2009;100:266–73. https://doi.org/10.1038/sj.bjc.6604867.

[28] Nöpel-Dünnebacke S, Schulmann K, Reinacher-Schick A, Porschen R, Schmiegel W, Tannapfel A, et al. Prognostic value of microsatellite instability and p53 expression in metastatic colorectal cancer treated with oxaliplatin and fluoropyrimidine-based chemotherapy. Z Gastroenterol 2014;52:1394–401. https://doi.org/10.1055/s-0034-1366781.

[29] Smith CG, Fisher D, Claes B, Maughan TS, Idziaszczyk S, Peuteman G, et al. Somatic profiling of the epidermal growth factor receptor pathway in tumors from patients with advanced colorectal cancer treated with chemotherapy ± cetuximab. Clin Cancer Res 2013;19:4104–13. https://doi.org/10.1158/1078-0432.CCR-12-2581.

[30] Omrčen T, Katić A, Tomić S, Eterović D, Vrdoljak E. Predictors of outcome in elderly patients with metastatic colorectal cancer: the final results of a prospective phase II study of bevacizumab in combination with capecitabine as first-line treatment. Anticancer Drugs 2020;31:518–22. https://doi.org/10.1097/CAD.0000000000000892.

[31] Morano F, Corallo S, Lonardi S, Raimondi A, Cremolini C, Rimassa L, et al. Negative hyperselection of patients with RAS and BRAF wild-type metastatic colorectal cancer who received panitumumab-based maintenance therapy. J Clin Oncol 2019;37:3099–110. https://doi.org/10.1200/JCO.19.01254.

[32] Cremolini C, Antoniotti C, Rossini D, Lonardi S, Loupakis F, Pietrantonio F, et al. Upfront FOLFOXIRI plus bevacizumab and reintroduction after progression versus mFOLFOX6 plus bevacizumab followed by FOLFIRI plus bevacizumab in the treatment of patients with metastatic colorectal cancer (TRIBE2): a multicentre, open-label, phase 3, rand. Lancet Oncol 2020;21:497–507. https://doi.org/10.1016/S1470-2045(19)30862-9.

[33] Seymour MT, Maughan TS, Ledermann JA, Topham C, James R, Gwyther SJ, et al. Different strategies of sequential and combination chemotherapy for patients with poor prognosis advanced colorectal cancer (MRC FOCUS): a randomised controlled trial. Lancet 2007;370:143–52.

[34] Simkens LHJ, Van Tinteren H, May A, Ten Tije AJ, Creemers GJM, Loosveld OJL, et al. Maintenance treatment with capecitabine and bevacizumab in metastatic colorectal cancer (CAIRO3): A phase 3 randomised controlled trial of the Dutch Colorectal Cancer Group. Lancet 2015;385:1843–52. https://doi.org/10.1016/S0140-6736(14)62004-3.

[35] Venook AP, Niedzwiecki D, Lenz HJ, Innocenti F, Fruth B, Meyerhardt JA, et al. Effect of first-line chemotherapy combined with cetuximab or bevacizumab on overall survival in patients with KRAS wild-type advanced or metastatic colorectal cancer a randomized clinical trial. JAMA 2017;317:2392–401. https://doi.org/10.1001/jama.2017.7105.

[36] Koopman M, Antonini NF, Douma J, Wals J, Honkoop AH, Erdkamp FLG, et al. Sequential versus combination chemotherapy with capecitabine, irinotecan, and oxaliplatin in advanced colorectal cancer (CAIRO): a phase III randomised controlled trial. Lancet 2007;370:135–42. https://doi.org/10.1016/S0140-6736(07)61086-1.

[37] Porschen R, Arkenau HT, Kubicka S, Greil R, Seufferlein T, Freier W, et al. Phase III study of capecitabine plus oxaliplatin compared with fluorouracil and leucovorin plus oxaliplatin in metastatic colorectal cancer: A final report of the AIO colorectal study group. J Clin Oncol 2007;25:4217–23. https://doi.org/10.1200/JCO.2006.09.2684.

[38] Maughan TS, Adams RA, Smith CG, Meade AM, Seymour MT, Wilson RH, et al. Addition of cetuximab to oxaliplatin-based fi rst-line combination chemotherapy for treatment of advanced colorectal cancer : results of the randomised phase 3 MRC COIN trial. Lancet 2011;377:2103–14. https://doi.org/10.1016/S0140-6736(11)60613-2.

[39] Tol J, Koopman M, Rodenburg CJ, Cats A, Creemers GJ, Schrama JG, et al. A randomised phase III study on capecitabine, oxaliplatin and bevacizumab with or without cetuximab in first-line advanced colorectal cancer, the CAIRO2 study of the Dutch Colorectal Cancer Group (DCCG). An interim analysis of toxicity. Ann Oncol 2008;19:734–8. https://doi.org/10.1093/annonc/mdm607.

[40] Vrdoljak E, Omrčen T, Boban M, Hrabar A. Phase II study of bevacizumab in combination with capecitabine as first-line treatment in elderly patients with metastatic colorectal cancer. Anticancer Drugs 2011;22:191–7. https://doi.org/10.1097/CAD.0b013e3283417f3e.

[41] Pietrantonio F, Morano F, Corallo S, Miceli R, Lonardi S, Raimondi A, et al. Maintenance Therapy with Panitumumab Alone vs Panitumumab Plus Fluorouracil-Leucovorin in Patients with RAS Wild-Type Metastatic Colorectal Cancer: A Phase 2 Randomized Clinical Trial. JAMA Oncol 2019;5:1268–75. https://doi.org/10.1001/jamaoncol.2019.1467.

[42] Goldstein D, Mitchell P, Michael M, Beale P, Friedlander M, Zalcberg J, et al. Australian experience of a modified schedule of FOLFOX with high activity and tolerability and improved convenience in untreated metastatic colorectal cancer patients. Br J Cancer 2005;92:832–7. https://doi.org/10.1038/sj.bjc.6602426.

[43] Clarke S, Goldstein D, Mitchell P, Michael M, Beale P, Friedlander M, et al. Modification of leucovorin dose within a simplified FOLFOX regimen improves tolerability without compromising efficacy. Clin Colorectal Cancer 2007;6:578–82. https://doi.org/10.3816/CCC.2007.n.025.

[44] Mitchell PL, Goldstein D, Michael M, Beale P, Friedlander M, Zalcberg J, et al. Addition of gabapentin to a modified FOLFOX regimen does not reduce oxaliplatin-induced neurotoxicity. Clin Colorectal Cancer 2006;6:146–51. https://doi.org/10.3816/CCC.2006.n.032.

[45] Tejpar S, Stintzing S, Ciardiello F, Tabernero J, van Cutsem E, Beier F, et al. Prognostic and Predictive Relevance of Primary Tumor Location in Patients With RAS Wild-Type Metastatic Colorectal Cancer Retrospective Analyses of the CRYSTAL and FIRE-3 Trials. JAMA Oncol 2017;3:194–201. https://doi.org/10.1001/jamaoncol.2016.3797.

[46] Van Cutsem E, Köhne CH, Hitre E, Zaluski J, Chien CRC, Makhson A, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 2009;360:1408–17. https://doi.org/10.1056/NEJMoa0805019.

[47] Taieb J, Lapeyre-Prost A, Laurent Puig P, Zaanan A. Exploring the best treatment options for BRAF-mutant metastatic colon cancer. Br J Cancer 2019;121:434–42. https://doi.org/10.1038/s41416-019-0526-2.

[48] Kopetz S, Grothey A, Yaeger R, Van Cutsem E, Desai J, Yoshino T, et al. Encorafenib, binimetinib, and cetuximab in BRAF V600E–mutated colorectal cancer. N Engl J Med 2019;381:1632–43. https://doi.org/10.1056/NEJMoa1908075.

[49] Zhang CM, Lv JF, Gong L, Yu LY, Chen XP, Zhou HH, et al. Role of deficient mismatch repair in the personalized management of colorectal cancer. Int J Environ Res Public Health 2016;13:892. https://doi.org/10.3390/ijerph13090892.

[50] Hewish M, Lord CJ, Martin SA, Cunningham D, Ashworth A. Mismatch repair deficient colorectal cancer in the era of personalized treatment. Nat Rev Clin Oncol 2010;7:197–208. https://doi.org/10.1038/nrclinonc.2010.18.

[51] Valentini AM, Armentano R, Pirrelli M, Caruso ML. Chemotherapeutic agents for colorectal cancer with a defective mismatch repair system: The state of the art. Cancer Treat Rev 2006;32:607–18. https://doi.org/10.1016/j.ctrv.2006.08.001.

[52] Lee K, Tosti E, Edelmann W. Mouse models of DNA mismatch repair in cancer research. DNA Repair (Amst) 2016;38:140–6. https://doi.org/10.1016/j.dnarep.2015.11.015.

[53] Fedier A, Schwarz VA, Walt H, Carpini RD, Haller U, Fink D. Resistance to topoisomerase poisons due to loss of DNA mismatch repair. Int J Cancer 2001;93:571–6. https://doi.org/10.1002/ijc.1356.

[54] Fink D, Zheng H, Nebel S, Norris PS, Aebi S, Lin TP, et al. In vitro and in vivo resistance to cisplatin in cells that have lost DNA mismatch repair. Cancer Res 1997;57:1841–5.

[55] Meyers M, Wagner MW, Hwang HS, Kinsella TJ, Boothman DA. Role of the hMLH1 DNA mismatch repair protein in fluoropyrimidine-mediated cell death and cell cycle responses. Cancer Res 2001;61:5193–201.

[56] Carethers JM, Chauhan DP, Fink D, Nebel S, Bresalier RS, Howell SB, et al. Mismatch repair proficiency and in vitro response to 5-fluorouracil. Gastroenterology 1999;117:123–31.

[57] Vilar E, Scaltriti M, Balmãa J, Saura C, Guzman M, Arribas J, et al. Microsatellite instability due to hMLH1 deficiency is associated with increased cytotoxicity to irinotecan in human colorectal cancer cell lines. Br J Cancer 2008;99:1607–12. https://doi.org/10.1038/sj.bjc.6604691.

[58] Pampaloni F, Reynaud EG, Stelzer EHK. The third dimension bridges the gap between cell culture and live tissue. Nat Rev Mol Cell Biol 2007;8:839–45. https://doi.org/10.1038/nrm2236.

[59] Vilar E, Gruber SB. Microsatellite instability in colorectal cancer-the stable evidence. Nat Rev Clin Oncol 2010;7:152–62. https://doi.org/10.1038/nrclinonc.2009.237.

[60] Duval A, Hamelin R. Mutations at coding repeat sequences in mismatch repair-deficient human cancers: Toward a new concept of target genes for instability. Cancer Res 2002;62:2447–54.

[61] Jacob S, Aguado M, Fallik D, Praz F. The role of the DNA mismatch repair system in the cytotoxicity of the topoisomerase inhibitors camptothecin and etoposide to human colorectal cancer cells. Cancer Res 2001;61:6555–62.

[62] Magrini R, Bhonde MR, Hanski ML, Notter M, Scherbl H, Richard Boland C, et al. Cellular effects of CPT-11 on colon carcinoma cells: Dependence on p53 and hMLH1 status. Int J Cancer 2002;101:23–31. https://doi.org/10.1002/ijc.10565.

[63] Son B, Lee S, Youn H, Kim E, Kim W, Youn B. The role of tumor microenvironment in therapeutic resistance. Oncotarget 2017;8:3933–45. https://doi.org/10.1007/978-3-319-67577-0_4.

[64] Kopetz S, Lemos R, Powis G. The promise of patient-derived xenografts: The best laid plans of mice and men. Clin Cancer Res 2012;18:5160–2. https://doi.org/10.1158/1078-0432.CCR-12-2408.

[65] Bertotti A, Migliardi G, Galimi F, Sassi F, Torti D, Isella C, et al. A molecularly annotated platform of patient- derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov 2011;1:508–23. https://doi.org/10.1158/2159-8290.CD-11-0109.

[66] Hidalgo M, Bruckheimer E, Rajeshkumar NV, Garrido-Laguna I, De Oliveira E, Rubio-Viqueira B, et al. A pilot clinical study of treatment guided by personalized tumorgrafts in patients with advanced cancer. Mol Cancer Ther 2011;10:1311–6. https://doi.org/10.1158/1535-7163.MCT-11-0233.

[67] Wang M, Yao LC, Cheng M, Cai D, Martinek J, Pan CX, et al. Humanized mice in studying efficacy and mechanisms of PD-1-targeted cancer immunotherapy. FASEB J 2018;32:1537–49. https://doi.org/10.1096/fj.201700740R.

[68] Sato T, Vries RG, Snippert HJ, Van De Wetering M, Barker N, Stange DE, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009;459:262–5. https://doi.org/10.1038/nature07935.

[69] Huch M, Gehart H, Van Boxtel R, Hamer K, Blokzijl F, Verstegen MMA, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell 2015;160:299–312. https://doi.org/10.1016/j.cell.2014.11.050.

[70] Van De Wetering M, Francies HE, Francis JM, Bounova G, Iorio F, Pronk A, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell 2015;161:933–45. https://doi.org/10.1016/j.cell.2015.03.053.

[71] Dijkstra KK, Cattaneo CM, Weeber F, Chalabi M, van de Haar J, Fanchi LF, et al. Generation of Tumor-Reactive T Cells by Co-culture of Peripheral Blood Lymphocytes and Tumor Organoids. Cell 2018;174(1586–1598):e12. https://doi.org/10.1016/j.cell.2018.07.009.

[72] Tsai S, McOlash L, Palen K, Johnson B, Duris C, Yang Q, et al. Development of primary human pancreatic cancer organoids, matched stromal and immune cells and 3D tumor microenvironment models. BMC Cancer 2018;18:1–13. https://doi.org/10.1186/s12885-018-4238-4.

[73] Ooft SN, Weeber F, Dijkstra KK, McLean CM, Kaing S, van Werkhoven E, et al. Patient-derived organoids can predict response to chemotherapy in metastatic colorectal cancer patients. Sci Transl Med 2019;11.. https://doi.org/10.1126/scitranslmed.aay2574.

[74] Ganesh K, Wu C, O'Rourke KP, Szeglin BC, Zheng Y, Sauvé C-EG, et al. A rectal cancer organoid platform to study individual responses to chemoradiation. Nat Med 2019;(25):1607–14. https://doi.org/10.1038/s41591-019-0584-2.

[75] Vlachogiannis G, Hedayat S, Vatsiou A, Jamin Y, Fernández-mateos J, Khan K, et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. Science 2018;359:920–6. https://doi.org/10.1126/science.aao2774 Cancer.

[76] Tokunaga E, Oda S, Fukushima M, Maehara Y, Sugimachi K. Differential growth inhibition by 5-fluorouracil in human colorectal carcinoma cell lines. Eur J Cancer 2000;36:1998–2006. https://doi.org/10.1016/S0959-8049(00)00200-8.

[77] Tajima A, Hess MT, Cabrera BL, Kolodner RD, Carethers JM. The mismatch repair complex hMutSα recognizes 5-fluorouracil-modified DNA: Implications for chemosensitivity and resistance. Gastroenterology 2004;127:1678–84. https://doi.org/10.1053/j.gastro.2004.10.001.

[78] Aebi S, Fink D, Gordon R, Kim HK, Zheng H, Fink JL, et al. Resistance to cytotoxic drugs in DNA mismatch repair-deficient cells. Clin Cancer Res 1997;3:1763–7.

[79] Arnold CN, Goel A, Boland CR. Role of hMLH1 promoter hypermethylation in drug resistance to 5-fluorouracil in colorectal cancer cell lines. Int J Cancer 2003;106:66–73. https://doi.org/10.1002/ijc.11176.

[80] Pommier Y. Topoisomerase I inhibitors: Camptothecins and beyond. Nat Rev Cancer 2006;6:789–802. https://doi.org/10.1038/nrc1977.

[81] Rodriguez R, Hansen LT, Phear G, Scorah J, Spang-Thomsen M, Cox A, et al. Thymidine selectively enhances growth suppressive effects of camptothecin/irinotecan in MSI+ cells and tumors containing a mutation of MRE11. Clin Cancer Res 2008;14:5476–83. https://doi.org/10.1158/1078-0432.CCR-08-0274.

[82] Fink D, Nebel S, Aebi S, Zheng H, Cenm B, Nehmä A, et al. The Role of DNA Mismatch Repair in Platinum Drug Resistance. Cancer Res 1996;56:4881–6.

[83] Scheeff ED, Briggs JM, Howell SB. Molecular modeling of the intrastrand guanine-guanine DNA adducts produced by cisplatin and oxaliplatin. Mol Pharmacol 1999;56:633–43. https://doi.org/10.1124/mol.56.3.633.

[84] Lee MS, McGuffey EJ, Morris JS, Manyam G, Baladandayuthapani V, Wei W, et al. Association of CpG island methylator phenotype and EREG/AREG methylation and expression in colorectal cancer. Br J Cancer 2016;114:1352–61. https://doi.org/10.1038/bjc.2016.87.

[85] Pentheroudakis G, Kotoula V, De Roock W, Kouvatseas G, Papakostas P, Makatsoris T, et al. Biomarkers of benefit from cetuximab-based therapy in metastatic colorectal cancer: Interaction of EGFR ligand expression with RAS/RAF, PIK3CA genotypes. BMC Cancer 2013;13:1. https://doi.org/10.1186/1471-2407-13-49.

[86] Wendum D, Boëlle PY, Rigau V, Sebbagh N, Olschwang S, Mourra N, et al. Mucinous colon carcinomas with microsatellite instability have a lower microvessel density and lower vascular endothelial growth factor expression. Virchows Arch 2003;442:111–7. https://doi.org/10.1007/s00428-002-0737-3.

[87] Tian L, Goldstein A, Wang H, Lo HC, Kim IS, Welte T, et al. Mutual regulation of tumour vessel normalization and immunostimulatory reprogramming. Nature 2017;544:250–4. https://doi.org/10.1038/nature21724.

[88] Meric-Bernstam F, Larkin J, Tabernero J, Bonini C. Enhancing anti-tumour efficacy with immunotherapy combinations. Lancet 2020;(6736). https://doi.org/10.1016/s0140-6736(20)32598-8.