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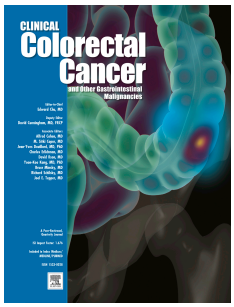

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A Review of the Evolution of Systemic Chemotherapy in the Management of Colorectal Cancer

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Abbreviations used:

5-FU = 5-fluorouracil

ASCO = American Society of Clinical Oncology

CRC = colorectal cancer

DFS = disease-free survival

dTMP = deoxythymidine monophosphate

dUMP = deoxyuridine monophosphate

ECOG = Eastern Cooperative Oncology Group

EGFR = epidermal growth factor receptor

FdUMP = 5-fluorodeoxyuridine monophosphate

FLOX = bolus 5-FU/leucovorin plus oxaliplatin

FOLFIRI = infusional 5-FU/leucovorin plus irinotecan

FOLFOX = 5-FU/leucovorin plus oxaliplatin

FOLFOXIRI = infusional 5-FU/leucovorin, oxaliplatin plus irinotecan

IFL = 5-FU/leucovorin plus irinotecan

IMPACT = International Multicentre Pooled Analysis of Colorectal Cancer Trials

i.v. = intravenous

KRAS = Kirsten rat sarcoma viral oncogene

LV5FU2 = twice-monthly infusion of 5-FU/leucovorin

mCRC = metastatic CRC

mFOLFOX6 = modified FOLFOX

MOF = 5-FU, lomustine and vincristine

MOSAIC = Multicenter International Study of Oxaliplatin/5-FU/Leucovorin in the Adjuvant Treatment of Colon Cancer

MRC = Medical Research Council

MTHF = 5,10-methylenetetrahydrofolate

NCCTG = North Central Cancer Treatment Group

NRAS = neuroblastoma RAS viral oncogene homolog

N/S = non-significant

NSABP = National Surgical Adjuvant Breast and Bowel Project

OS = overall survival

PETACC = Pan-European Trial in Adjuvant Colorectal Cancer

PFS = progression-free survival

RR = response rate

THF = tetrahydrofolate

TRIBE = Combination Chemotherapy and Bevacizumab as First-line Therapy in Treating Patients with Metastatic Colorectal Cancer

TTP = time to progression

UFT = uracil plus the 5-FU prodrug tegafur

Abstract

Here we present a historical review of the development of systemic chemotherapy for colorectal cancer (CRC) in both metastatic and adjuvant treatment settings. We describe the discovery of 5-fluorouracil (5-FU) by Heidelberger and colleagues in 1957, the potentiation of 5-FU cytotoxicity by the reduced folate leucovorin, and the advent of novel cytotoxic agents, including the topoisomerase I inhibitor irinotecan, the platinum-containing agent oxaliplatin, and the 5-FU prodrug capecitabine. The combination therapies FOLFOX (5-FU/leucovorin plus oxaliplatin) and FOLFIRI (5-FU/leucovorin plus irinotecan) have become established as efficacious cytotoxic regimens for the treatment of metastatic CRC, resulting in overall survival times of approximately 2 years. When used as adjuvant therapy, FOLFOX also improves survival and is now the gold standard of care in this setting. Biological agents have been discovered that enhance the effect of cytotoxic therapy, including bevacizumab (a humanized monoclonal antibody that targets vascular endothelial growth factor, a central regulator of angiogenesis) and cetuximab/panitumumab (monoclonal antibodies directed against the epidermal growth factor receptor [EGFR]). Despite the ongoing development of novel anti-tumor agents and therapeutic principles as we enter the era of personalized cancer medicine, systemic chemotherapy involving infusional 5-FU/leucovorin continues to be the cornerstone of treatment for patients with CRC.

Keywords

Colorectal cancer, chemotherapy, 5-fluorouracil, FOLFOX, FOLFIRI, bevacizumab, cetuximab**,** panitumumab, 5,10-methylenetetrahydrofolate.

Introduction

The most recent estimates of the worldwide burden of cancer (GLOBOCAN 2012) indicate that colorectal cancer (CRC) is the third most commonly diagnosed cancer (1.36 million cases, 9.7%) after lung (1.83 million, 13.0%) and breast cancer (1.68 million, 11.9%), and the fourth highest cause of cancer death (694,000 deaths, 8.5%) after lung (1.59 million, 19.4%), liver (746,000, 9.1%), and stomach cancer (723,000, 8.8%).[1] Despite these statistics, most patients (70–80%) newly diagnosed with CRC have localized disease that is amenable to curative (R0) surgical resection.[2] Following R0 resection, adjuvant chemotherapy with cytotoxic agents is recommended as standard clinical practice for patients with stage III CRC.[3] This recommendation is supported by a pooled analysis of data from the National Surgical Adjuvant Breast and Bowel Project (NSABP) trials,[4] which demonstrated significantly improved survival outcomes after surgery and chemotherapy when compared with surgery alone ($P < .0001$).

The remaining 20–30% of newly diagnosed patients present with unresectable metastatic disease. In addition, a considerable proportion (40–50%) patients experience disease recurrence after surgical resection or develop metastatic disease, typically in the liver or lungs.[5] The management of patients with metastatic CRC (mCRC) requires the systemic administration of cytotoxic drugs.[3] Patients with unresectable mCRC receiving supportive care alone have been shown to have a poor prognosis, with a median overall survival (OS) of 5 months.[6] By contrast, patients with mCRC who receive chemotherapy have been shown to have a median OS of more than 2 years.[7]

Here, we present a historical review of systemic chemotherapy in both the adjuvant and metastatic settings, highlighting the key papers that have driven the development of chemotherapy for patients with CRC (Figure 1).

5-Fluorouracil and Leucovorin

The German chemist Paul Ehrlich was the first person to coin the term 'chemotherapy' during his work on the use of chemical agents to treat infectious diseases in the early 1900s.[8] However, the evolution of chemotherapy for CRC can be said to have begun with the development of 5-fluorouracil (5-FU) in 1957.[9] Charles Heidelberger and colleagues at the University of Wisconsin observed that tumor tissues preferentially utilized uracil for nucleic acid biosynthesis, and correctly postulated that a fluorouracil analog would inhibit tumor cell division by blocking the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP; thymidylate). Biochemical studies demonstrated that the main route of 5-FU activation proceeds via complex metabolic pathways that result in the formation of 5-fluorodeoxyuridine monophosphate (FdUMP), a potent inhibitor of thymidylate synthase (Figure 2).[10-13] The level of inhibition of thymidylate synthase achieved with FdUMP in patient tumors was shown to correlate with the clinical response to 5-FU treatment.[14,15] Studies of the molecular mechanism of thymidylate formation identified the transient formation of a ternary complex consisting of the substrate dUMP, the folate cofactor 5,10-methylenetetrahydrofolate (MTHF), and thymidylate synthase.[16,17]

The next key advance in the development of 5-FU-based chemotherapy was the finding that inhibition of thymidylate synthase by 5-FU could be potentiated by increased intracellular levels of reduced folates.[12,18-21] At this juncture, it is interesting to note that the anti-tumor activity of folic acid analogs, including aminopterin and amethopterin (methotrexate), was first demonstrated in 1948 by Sidney Farber and Louis Diamond in children with leukemia.[22] The potentiation of 5-FU activity was shown to be mediated by the formation of a stable ternary complex consisting of FdUMP, MTHF, and thymidylate synthase.[10,13,23] Polyglutamate derivatives of MTHF were shown to substantially increase the efficiency of binding of FdUMP to thymidylate synthase compared with monoglutamate derivatives, in both a human colon adenocarcinoma xenograft[24] and human MCF-7 breast cancer cells.[25] In

a pivotal *in vitro* study of the biomodulation of 5-FU activity by the reduced folate leucovorin (5-formyl THF), Ullman et al.[18] reported that 20 µM leucovorin enhanced 5-FU cytotoxicity approximately fivefold in cultured leukemia cells. Following on from this study, the anti-tumor activity of 5-FU/leucovorin and 5-FU/methyl THF was established in a number of studies of tumor cell lines, including those of human origin.[19,21,26-30]

The preclinical data on the biomodulation of 5-FU cytotoxicity by leucovorin led to a large number of phase 1 and 2 clinical studies in the 1980s.[31] In a pooled analysis of 21 phase 2 studies of patients with advanced CRC, conducted by Poon et al. in 1989, the response rate (RR) of tumors to 5-FU/leucovorin was reported to be 23%.[32] The two most commonly used 5-FU/leucovorin treatment regimens in these early studies were those described by Machover et al.[33] and Madajewicz et al.[34] Machover et al. administered 200 mg/m^2 leucovorin by intravenous (IV) bolus and 370 mg/m^2 5-FU by a 15-minuteIV infusion daily for 5 days to patients with gastric cancer and mCRC, with courses repeated at 28-day intervals. Madajewicz administered 500 mg/m^2 leucovorin as a 2-hour infusion to patients with mCRC, with escalating bolus doses of 5-FU up to a maximum of 750 mg/m^2 given 1 hour after the leucovorin infusion; this schedule was repeated weekly for 6 weeks, followed by a 2-week rest period.

Treatment of Metastatic Colorectal Cancer

In 1989, the seminal study of Michael Poon and colleagues[32] showed that there was only a trend towards increased OS with IV bolus 5-FU/leucovorin, but RR and progression-free survival (PFS) were significantly increased, compared with 5-FU alone in patients with metastatic CRC. Median OS was 12.2 months for patients receiving 5-FU plus high-dose (200 mg/m^2) leucovorin and 12.0 months for those taking 5-FU plus low-dose (20 mg/m^2) leucovorin, compared with 7.7 months for 5-FU alone (P = .05, both leucovorin doses). RRs for 5-FU plus high-dose or low-dose leucovorin were 26% (P = .04) and 37% (P < .001),

respectively, compared with 10% for 5-FU alone. The time-to-progression (TTP) rates for 5-FU plus high-dose or low-dose leucovorin were also significantly improved compared with 5-FU alone *(P* = .015 and *P* = .007, respectively).

Another important study, carried out by Petrelli et al.[35], demonstrated that the RR for 5-FU plus high-dose leucovorin (48%) was significantly higher than that with 5-FU alone (11%) or 5-FU plus methotrexate (5%; overall *P* = .0009). In a subsequent phase III study that compared 5-FU plus high-dose or low-dose leucovorin with 5-FU alone, Petrelli et al. [36] reported RRs of 12% for 5-FU alone, 30% for 5-FU plus high-dose leucovorin (*P* < .01), and 18.8% for 5-FU plus low-dose leucovorin (*P* = N/S).

A meta-analysis of 19 randomized trials[37], involving 3338 patients, reported a twofold increase in RR with 5-FU/leucovorin (21%) compared with 5-FU alone (11%; *P* < .0001) and a small but statistically significant OS benefit for 5-FU/leucovorin over 5-FU alone (11.7 vs 10.5 months, respectively; *P* = .004).

Key developments in the early 2000s included the introduction of the topoisomerase I inhibitor irinotecan and the platinum-containing agent oxaliplatin as components of cytotoxic combination therapy for mCRC. Irinotecan was first discovered and synthesized in Japan by Yakult Honsha Ltd. in 1983.[38] It is a prodrug analog (7-ethyl-10-piperidino-piperidino-carbonyloxy derivative) of the alkaloid camptothecin that is converted to the active metabolite SN-38 by liver carboxylesterases.[39] Oxaliplatin was also discovered in Japan at Nagoya City University by Yoshinori Kidani in 1976 by testing the anti-tumor activity of various platinum(II) complexes of 1,2-diaminocyclohexane isomers.[40]

Saltz et al.[41] found that treatment with bolus 5-FU/leucovorin plus irinotecan (IFL) resulted in significantly longer PFS (7.0 vs 4.3 months; *P* = .004), higher RR (39% vs 21%; *P* < 0.001), and longer OS (14.8 vs 12.6 months; *P* = 0.04) than 5-FU/leucovorin alone as first-line

therapy for patients with mCRC. In the Intergroup trial N9741,[42] the efficacy of 5-FU/leucovorin plus oxaliplatin (FOLFOX) was significantly better than that of IFL with regards to OS (19.5 vs 15.0 months, respectively; *P* < .0001, TTP (8.7 vs 6.9 months; *P* = .0014), and RR (45% vs 31%; *P* = .002). The FOLFOX regimen was also associated with significantly lower rates of severe nausea, vomiting, diarrhea, and febrile neutropenia than was the IFL regimen (all, *P* < .001). The unfavorable toxicity profile of the IFL regimen led to the development of a regimen comprising infusional 5-FU/leucovorin plus irinotecan (FOLFIRI). The Gruppo Oncologico Dell'Italia Meridionale (GOIM) study[43] and the Groupe Coopérateur Multidisciplinaire en Oncologie (GERCOR) crossover study[44] each showed similar efficacy for the FOLFIRI and FOLFOX regimens. The GOIM study reported RRs of 31% and 34% (*P* = N/S), OS rates of 14 and 15 months (*P* = N/S), and median TTPs of 7 months (both, *P* = N/S) for FOLFIRI and FOLFOX, respectively. The GERCOR study demonstrated OS rates of 21.5 months in patients allocated to FOLFIRI then FOLFOX, and 20.6 months in those treated with FOLFOX then FOLFIRI (*P* = N/S). As first-line therapy, FOLFIRI achieved an RR of 56% and PFS of 8.5 months, while for FOLFOX the RR was 54% (*P* = N/S) and the PFS was 8.0 months (*P* = N/S).

The combination of infusional 5-FU/leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) was compared with FOLFIRI in two randomized, phase III trials. Souglakos et al.[45] reported no significant differences in OS, TTP or RR between the two treatment regimens. Falcone et al.[46] showed a significantly higher RR for patients treated with FOLFOXIRI than for those treated with a modified FOLFIRI regimen containing 400–600 mg/m^2 5-FU (60% vs 34%, respectively; *P* < .0001). PFS (9.8 vs 6.9 months; *P* = .0006) and OS (22.6 vs 16.7 months; *P* = .032) were also significantly improved in the FOLFOXIRI arm compared with the modified FOLFIRI arm, but at the cost of a significant (*P* < .001) increase in toxicity, in terms of increased grades of peripheral neurotoxicity (P < .001), and neutropenia (P < .001).

The idea of targeting angiogenesis as an anti-cancer therapy was first proposed by Judah Folkman and colleagues in 1971.[47] However, it was not until 2004 that the pivotal AVF2107 phase III trial[48] evaluated the humanized monoclonal antibody bevacizumab, which inhibits the action of vascular endothelial growth factor evaluation of bevacizumab. In this trial, patients were randomized to IFL plus bevacizumab or IFL alone. The addition of bevacizumab significantly improved OS (20.3 vs 15.6 months, respectively; $P < .001$), PFS (10.6 vs 6.2 months; $P < .001$), and RR (44.8% vs 34.8%; $P = .004$) compared with IFL alone. In another key trial, the Eastern Cooperative Oncology Group (ECOG) 3200 study[49] enrolled patients previously treated with IFL and found that OS (12.9 vs 10.8 months, respectively; $P < .0011$), PFS (7.3 vs 4.7 months; $P < .0001$), and RR (22.7% vs 8.6%; $P < .0001$) were all significantly improved with bevacizumab plus FOLFOX treatment compared with FOLFOX alone.

In 1983–1984, John Mendelsohn and Gordon Sato proposed EGFR as a novel target for cancer therapy, based on observations that EGFR was frequently overexpressed in epithelial tumors and that monoclonal antibodies directed against EGFR inhibited the growth of cancer cells.[50-53] The anti-EGFR monoclonal antibodies cetuximab and panitumumab were the first therapeutic agents targeted at a specific molecular pathology: EGFR-positive tumors expressing wild-type Kirsten rat sarcoma viral oncogene homolog (KRAS).[54] The efficacy of cetuximab in the treatment of patients with mCRC was evaluated in the Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer (CRYSTAL) study[55,56] in which patients with EGFR-positive tumors were randomized to receive FOLFIRI alone or FOLFIRI plus cetuximab. FOLFIRI plus cetuximab marginally improved PFS compared with FOLFIRI alone (8.9 vs 8.0 months, respectively; $P = .048$), but there was no significant difference in OS between the two treatments (19.9 vs 18.6 months; P = N/S). In a subset analysis of patients with wild-type KRAS (63%), FOLFIRI plus cetuximab significantly improved OS (23.5 vs 20.0 months; $P = .01$), PFS (9.9 vs 8.4

months; *P* = .001), and RR (57.3% vs 39.7%; *P* = .001) compared with FOLFIRI alone. No significant difference in efficacy was evident in patients with mutant KRAS.

In the Panitumumab Randomized Trial in Combination With Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy (PRIME),[57] patients were randomized to FOLFOX with or without panitumumab, regardless of EGFR or KRAS status. In the subset with wild-type KRAS (60% of the study population), panitumumab plus FOLFOX significantly improved PFS compared with FOLFOX alone (9.6 vs 8.0 months, respectively; *P* = 0.02), but did not lead to a significant improvement in OS (23.9 vs 19.7 months; *P* = N/S).

The UK Medical Research Council (MRC) Continuous Chemotherapy plus Cetuximab or Intermittent Chemotherapy (COIN) trial was a three-arm randomized controlled trial in which patients were randomized to continuous FOLFOX, continuous FOLFOX plus cetuximab, or intermittent FOLFOX alone. Maughan et al.[58] reported the results for two of these regimens: FOLFOX plus cetuximab increased RR compared with FOLFOX alone (59% vs 50%, respectively; *P* = .015), but there was no evidence of improved PFS or OS in patients with wild-type KRAS.

Patients in the Nordic-VII study[59] were randomized to receive bolus 5-FU/leucovorin plus oxaliplatin (Nordic FLOX), Nordic FLOX plus cetuximab, or intermittent Nordic FLOX plus cetuximab. OS, PFS, and RR were similar in the three treatment arms (OS: 20.4, 19.7, and 20.3 months, respectively (*P* = N/S); PFS: 7.9, 8.3, and 7.3 months (*P* = N/S); and RR: 41%, 49%, and 47% (*P* = N/S). In patients with wild-type KRAS, cetuximab did not provide any additional benefit compared with Nordic FLOX alone for PFS, OS, or RR.

Findings of several key studies presented at the 2013 Annual Meeting of the American Society of Clinical Oncology (ASCO) provided important updates to the current picture. In the FIRE-3 trial,[60] patients with wild-type KRAS were randomized to receive first-line

FOLFIRI plus cetuximab or FOLFIRI plus bevacizumab. The primary endpoints of overall RR (62% vs 58%, respectively) and PFS (10.0 vs 10.3 months, respectively) were not significantly different in the two treatments arms. However, FOLFIRI plus cetuximab provided a statistically significant improvement in OS compared with FOLFIRI plus bevacizumab (28.7 vs 25.0 months, respectively; *P* = .017). A further important contribution to the ongoing first-line therapy debate in mCRC was the Triplet Chemotherapy plus Bevacizumab (TRIBE) trial.[61] This trial, evaluating FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab, showed a significant difference in the primary endpoint of PFS (12.1 vs 9.7 months, respectively; *P* = .006). The phase II Panitumumab Efficacy in Combination with mFOLFOX6 Against Bevacizumab plus mFOLFOX6 in mCRC Subjects with Wild-Type *KRAS* Tumors (PEAK) trial[62] randomized patients with wild-type RAS (KRAS or neuroblastoma RAS) to first-line panitumumab plus FOLFOX or bevacizumab plus FOLFOX. PFS for panitumumab plus FOLFOX was 13.1 months, compared with 9.5 months for bevacizumab plus FOLFOX (*P* = .02). OS for the panitumumab arm was not reached at the time of reporting, but was 29 months for the bevacizumab arm. At ASCO 2012, PEAK data were reported which suggested that the panitumumab regimen had an adverse effect on PFS in patients with mutated compared with wild-type KRAS, although the effect was not significant (15.5 vs 19.3 months, respectively; *P* = N/S).[63] Although not validated, the PEAK results suggest that panitumumab should not be used for the treatment of mCRC in patients with KRAS mutations or in whom the KRAS status is unknown.

Orally administered 5-FU prodrugs were developed to provide a convenient alternative to treatment regimens requiring IV infusion of 5-FU. An example of such an oral regimen is the combination of uracil and the 5-FU prodrug tegafur in a 4:1 molar ratio (UFT). Uracil competitively inhibits dihydropyrimidine dehydrogenase, the main catabolic enzyme of 5-FU (Figure 2). In a meta-analysis of five randomized controlled trials comparing UFT/leucovorin with bolus 5-FU/leucovorin, Bin et al.[64] reported that there were no significant differences in OS and RR between the two regimens; however, UFT/leucovorin had significantly lower

toxicity than bolus 5-FU/leucovorin (*P* < .001 for stomatitis/mucositis, grade 1–4 leucopenia, febrile neutropenia, and infection). These findings are consistent with a pooled efficacy analysis from two phase III studies comparing capecitabine (another oral 5-FU prodrug) with bolus 5-FU/leucovorin.[65] A statistically significant difference in RR was reported for capecitabine compared with 5-FU plus leucovorin (26% vs 17%, respectively; *P* < .0002), whereas OS (12.9 vs 12.8 months; *P* = N/S) and TTP (4.6 vs 4.7 months; *P* = N/S) were equivalent in the two treatment groups. Table 1 summarizes the findings of the key mCRC studies described in this section. Figure 3 shows the temporal trend of OS in these studies. It can be seen that median OS increased sharply from 12.0 months in the early studies of Petrelli[35] and Poon,[32] to 21.5 months in the GERCOR study,[44] and except for the GOIM study[43] has remained at 18–24 months in recent, large phase III trials.

Adjuvant Treatment of Colorectal Cancer

In the 1970s and 1980s, the anti-helminthic drug levamisole attracted interest as a possible chemotherapeutic agent because of its putative immunomodulatory activity.[66,67] In 1989, the North Central Cancer Treatment Group (NCCTG) reported that treatment with levamisole plus 5-FU led to a significant reduction in cancer recurrence (*P* = .003) and a significant increase in OS (*P* = .03) when compared with no adjuvant therapy.[68] In 1990, Charles Moertel and colleagues [69] published the results of their seminal study of the efficacy of 5-FU plus levamisole versus no adjuvant therapy in patients with stage II or III CRC. 5-FU plus levamisole reduced the risk of cancer recurrence by 41% (*P* < .0001) and the overall death rate by 33% (*P* = .006) when compared with observation alone. Interestingly, treatment with levamisole alone had no effect. These findings led to the acceptance of 5-FU plus levamisole as the standard adjuvant therapy in the 1990s.[70]

The next stage in the evolution of adjuvant therapy involved the evaluation of 5-FU plus leucovorin in several key trials. The NSABP C-03 study[71] reported a 3-year disease-free

survival (DFS) rate of 73% for patients receiving 5-FU/leucovorin, compared with a rate of 64% for those receiving a combination of the alkylating nitrosourea lomustine, the alkaloid vincristine, and 5-FU (MOF; *P* = .0004). The International Multicenter Pooled Analysis of Colorectal Cancer Trials (IMPACT)[72] pooled data from three randomized trials investigating high-dose 5-FU/leucovorin compared with no adjuvant therapy. 5-FU/leucovorin reduced mortality by 22% (*P* = .029) and CRC events by 35% (*P* < .0001) compared with no adjuvant therapy.

A number of randomized trials evaluated the efficacy and safety of the most commonly used 5-FU/leucovorin treatment regimens in the adjuvant setting. The INT-0089 study[73] set out to evaluate four regimens: (1) the Mayo Clinic regimen, comprising a daily 20 mg/m^2 (low-dose) IV bolus of leucovorin and 425 mg/m^2 IV bolus of 5-FU for 5 consecutive days, repeated every 4–5 weeks; (2) the Roswell Park regimen, consisting of a weekly 500 mg/m^2 (high-dose) IV bolus of leucovorin and 500 mg/m^2 IV bolus of 5-FU for 6 weeks, repeated every 8 weeks; (3) low-dose 5-FU/leucovorin plus levamisole; and (4) levamisole alone. The main finding was that there were no statistically significant differences among the treatment arms in DFS (9.4, 7.9, 9.2, and 7.1 months, respectively) or OS (11.5, 10.7, 11.4, and 10.3 months, respectively). The MRC study[74] evaluated 3 months of continuous infusion of 5-FU and a 6-month course of the Mayo clinic regimen. There was no statistically significant difference between the two arms in terms of OS (87.9% vs 83.2%, respectively; *P* = N/S). However, patients in the Mayo Clinic regimen arm had significantly lower rates of PFS compared with those receiving continuous infusion 5-FU (69% vs 80%, respectively; *P* = .02). In terms of safety, the frequency of grades 3–4 neutropenia, diarrhea, stomatitis, and severe alopecia were significantly lower (*P* < .0001), and global quality of life scores significantly better (*P* < .001), for patients in the continuous infusion arm compared with the Mayo Clinic regimen arm. The GERCOR C96.1 study[75] compared the Mayo Clinic regimen with a twice-monthly IV infusion of 5-FU/leucovorin (LV5FU2; de Gramont regimen[76]). There were no statistically significant differences between the two arms in terms of DFS (*P* = N/S)

or OS (*P* = N/S), but the de Gramont regimen was significantly less toxic than the Mayo Clinic regimen (*P* = .001).

In the Xeloda in Adjuvant Colon Cancer Therapy (X-ACT) trial [77] patients were randomized to capecitabine or the Mayo Clinic regimen. There were no statistically significant differences between the two arms in terms of DFS (64.2% vs 60.6%, respectively; *P* = N/S) or OS (81.3 % and 77.6%; *P* = .05). However, capecitabine was associated with significantly fewer adverse events than the Mayo Clinic regimen (*P* < .001). The NSABP C-06 study[78], which compared tegafur plus leucovorin with the Roswell Park regimen, reported that 5-year DFS (68.2% vs 67.0%, respectively; *P* = N/S) and OS (78.7% vs 78.5%; *P* = N/S) were similar for the two treatments.

In 2004, an interim analysis of data from the pivotal Multicenter International Study of Oxaliplatin/5-FU/Leucovorin in the Adjuvant Treatment of Colon Cancer (MOSAIC)[79] showed that FOLFOX significantly improved 3-year DFS compared with 5-FU/leucovorin (FL regimen: 2-hour IV infusion of 200 mg/m^2 of leucovorin followed by an IV bolus of 400 mg/m^2 of 5-FU and then a 22-hour IV infusion of 600 mg/m^2 of 5-FU given on 2 consecutive days every 14 days). FOLFOX, 78.2% vs FL, 72.9%, respectively (*P* = .002) in patients with stage III CRC, although neutropenia (grades 3–4) was significantly more frequent with FOLFOX than with FL (41.1% vs 4.7%; *P* < .001). The final analysis of data from MOSAIC in 2009[80] confirmed statistically significant improvements in both DFS and OS for FOLFOX compared with FL (5-year DFS: 73.3% vs 67.4%, respectively [*P* = .003] and 6-year OS: 78.5% vs 76.0% [*P* = .046]). No survival benefit was detected in patients with stage II disease. The MOSAIC findings established FOLFOX as the standard adjuvant therapy for resected stage III CRC, and, in so doing, suggested that treatments with proven efficacy in the management of mCRC could also be effective in the adjuvant setting. Unfortunately, negative results from a number of large multicenter trials have shown these hopes to be unfounded.

The Pan-European Trial in Adjuvant Colorectal Cancer (PETACC)-3[81] compared FOLFIRI and 5-FU/leucovorin (de Gramont regimen) in patients with stage III disease. FOLFIRI did not produce significant improvements compared with 5-FU/leucovorin in either DFS (56.7% vs 54.3%, respectively; *P* = N/S) or OS (73.6% vs 71.3%; *P* = N/S). These findings corroborated those of the Cancer and Leukemia Group B (CALGB) 89803[82] and ACCORD02[83] trials. The CALGB study reported that there was no significant difference in 3-year DFS, the primary endpoint of the trial, between 5-FU/leucovorin and IFL (60% vs 63%, respectively; *P* = N/S). The main ACCORD02 findings were that 5-year OS rates for 5-FU/leucovorin and FOLFIRI were 67% and 61%, respectively (*P* = N/S), and 3-year DFS rates were 60% and 51% (*P* = N/S).

Much effort has been expended in investigating the efficacy of bevacizumab and cetuximab in the adjuvant setting. In the NSABP C-08 trial[84], carried out in patients with stage II or III CRC, treatment with FOLFOX plus bevacizumab showed no significant improvement in 3-year DFS compared with FOLFOX alone (77.4% vs 75.5%, respectively; *P* = N/S). In the NCCTG/Intergroup N0147 trial[85], patients with resected stage III CRC and wild-type KRAS were randomly assigned to receive modified FOLFOX (mFOLFOX6) plus cetuximab or mFOLFOX6 alone. The trial was terminated when the prespecified interim analysis demonstrated that there was no benefit in terms of the primary endpoint of 3-year DFS from the addition of cetuximab to mFOLFOX6 (74.6% with mFOLFOX6 alone vs 71.5% with mFOLFOX6 plus cetuximab; *P* = N/S). Table 2 summarizes the findings of the key adjuvant studies described in this section.

Conclusions

The evolution of chemotherapy for patients with CRC has involved a series of landmark advances, including the discovery of 5-FU, the identification of the reduced folate leucovorin

as a clinical potentiator of 5-FU cytotoxicity, and the advent of novel cytotoxic and biological agents. As we move into the era of personalized cancer medicine, systemic chemotherapy involving infusional 5-FU/leucovorin remains the cornerstone of treatment for patients with CRC, but there is a need for empirical studies that explore how current treatment regimens can be optimized for individual patients.

Conflict of interest statement

Isofol Medical AB, Gothenburg, Sweden, is currently evaluating a reduced folate (Modufolin®) together with 5-FU or methotrexate as treatment for colorectal cancer in clinical trials. Bengt Gustavsson is a director of Isofol Medical AB. Fernando Gibson is an employee of PharmaGenesis London Ltd which received payment from Isofol Medical AB for this work.

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Table 1 Key clinical studies in the development of therapy for patients with mCRC

Therapy	Study	Publication date	Study objective	Patients (n)	Key efficacy results
5-FU/LV	Petrelli et al.[35]	1987	To compare the efficacy of 5-FU + high-dose LV (500 mg/m^2), 5-FU + methotrexate and 5-FU alone	74	OS: 12, 10, 11 months, respectively (*P* = N/S) RR: 48%, 5%, 11%, respectively (*P* = .0009)
	Petrelli et al.[36]	1989	To determine whether 5-FU + high-dose (500 mg/m^2) or low-dose (25 mg/m^2) LV increases efficacy compared with 5-FU alone	343	OS: 13.8, 11.3, 11.5, months, respectively (*P* = N/S) RR: 30%, 19%, 12%, respectively (*P* < .01)
	Poon et al.[32]	1989	To evaluate the efficacy of 5-FU + high-dose (200 mg/m^2) LV, 5-FU + low-	429	OS: 12.2, 12.0, 7.7 months, respectively (adjusted *P* = .05, both LV doses) RR: 26% (*P* = .04), 37% (*P* < .001), 10%,

			dose (20 mg/m^2) LV, and 5-FU alone		respectively
5-FU/LV, IFL	Saltz et al.[41]	2000	To compare the efficacy of IFL vs 5-FU/LV alone	683	OS: 14.8 vs 12.6 months (*P* = .04) PFS: 7.0 vs 4.3 months (*P* = .004) RR: 39% vs 21%; (*P* < .001)
FOLFOX, IFL	Intergroup N9741[42]	2004	To compare the efficacy and toxicity of FOLFOX vs IFL regimens	795	OS: 19.5 vs 15.0 months (*P* < .0001) TTP: 8.7 vs 6.9 months (*P* = .0014) RR: 45% vs 31% (*P* = .002)
FOLFIRI, FOLFOX	GERCOR[44]	2004	A crossover study to investigate the efficacy of FOLFIRI followed by FOLFOX vs FOLFOX followed by FOLFIRI	222	OS: 21.5 vs 20.6 months (*P* = N/S) PFS: 8.5 vs 8.0 months (*P* = N/S) RR: 56% vs 54% (*P* = N/S)
	GOIM[43]	2005	To compare the efficacy of FOLFIRI vs FOLFOX regimens	360	OS: 14 vs 15 months (*P* = N/S) RR: 31% vs 34% (*P* = N/S) TTP: 7 vs 7 months (*P* = N/S)
FOLFIRI,	Souglakos et al.[45]	2006	To compare the efficacy	283	OS: 19.5 vs 21.5 months (*P* = N/S)

FOLFOXIRI			and toxicity of FOLFIRI vs FOLFOXIRI regimens		TTP: 6.9 vs 8.4 months (*P* = N/S) RR: 34% vs 43% (*P* = N/S)
	Falcone et al.[46]	2007	To compare the efficacy and toxicity of FOLFOXIRI vs FOLFIRI regimens	244	OS: 22.6 vs 16.7 months (*P* = .032) RR: 60% vs 34% (*P* < .0001) PFS: 9.8 vs 6.9 months (*P* = .0006)
Bevacizumab	AVF 2107[48]	2004	To determine whether bevacizumab + IFL improves survival vs IFL alone	813	OS: 20.3 vs 15.6 months (*P* < .001) PFS: 10.6 vs 6.2 months (*P* < .001) RR: 44.8% vs 34.8% (*P* = .004)
	ECOG 3200[49]	2007	To determine the effect of bevacizumab + FOLFOX on survival duration vs FOLFOX alone	829	OS: 12.9 vs 10.8 months; (*P* < .0011) PFS: 7.3 vs 4.7 months (*P* < .0001) RR: 22.7% vs 8.6% (*P* < .0001)
Cetuximab, panitumumab	CRYSTAL[55,56]	2009, 2011	To investigate the efficacy of cetuximab +	1198	OS: 19.9 vs 18.6 months (*P* = N/S) PFS: 8.9 vs 8.0 months (*P* = .048).

			FOLFIRI vs FOLFIRI alone; and the association between tumor KRAS mutation status and clinical response to cetuximab		In patients with wild-type KRAS (63%), OS: 23.5 vs 20.0 months (*P* = .01) PFS: 9.9 vs 8.4 months (*P* = .001) RR: 57.3% vs 39.7% (*P* = .001) No significant difference in efficacy was evident in patients with mutant KRAS
	PRIME[57]	2010	To evaluate the efficacy and safety of panitumumab + FOLFOX vs FOLFOX alone	1183	In patients with wild-type KRAS (60%) OS: 23.9 vs 19.7 months (*P* = N/S) PFS: 9.6 vs 8.0 months (*P* = .02)
	COIN[58]	2011	To assess the efficacy of cetuximab + FOLFOX vs FOLFOX alone	1630	OS: 17.0 vs 17.9 months (*P* = N/S) RR: 59% vs 50% (*P* = 0.015) No evidence of improved PFS or OS in patients with wild-type KRAS
	Nordic-VII[59]	2012	To investigate the efficacy of Nordic FLOX, cetuximab + Nordic	571	OS: 20.4, 19.7, 20.3 months , respectively (*P* = N/S) PFS: 7.9, 8.3, 7.3 months, respectively (*P* = N/S)

			FLOX, and cetuximab + intermittent Nordic FLOX		RR: 41%, 49%, 47%, respectively (*P* = N/S) In patients with *KRAS* mutations, no significant differences were detected

Abbreviations: 5-FU = 5-fluorouracil; COIN = Continuous Chemotherapy plus Cetuximab or Intermittent Chemotherapy; CRYSTAL = Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer; ECOG = Eastern Cooperative Oncology Group; FLOX = bolus 5-FU/LV plus oxaliplatin; FOLFIRI = infusional 5-FU/LV plus irinotecan; FOLFOX = 5-FU/LV plus oxaliplatin; FOLFOXIRI = 5-FU/LV plus oxaliplatin plus irinotecan; GERCOR = Groupe Coopérateur Multidisciplinaire en Oncologie; GOIM = Gruppo Oncologico dell'Italia Meridionale; IFL = bolus 5-FU/LV plus irinotecan; KRAS = Kirsten rat sarcoma viral oncogene homolog; LV = leucovorin; mCRC = metastatic colorectal cancer; N/S = non-significant; OS = overall survival; PFS = progression-free survival; PRIME = Panitumumab Randomized Trial in Combination With Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy; RR = response rate; TTP = time to progression

Table 2 Key clinical studies in the development of adjuvant therapy for patients with CRC

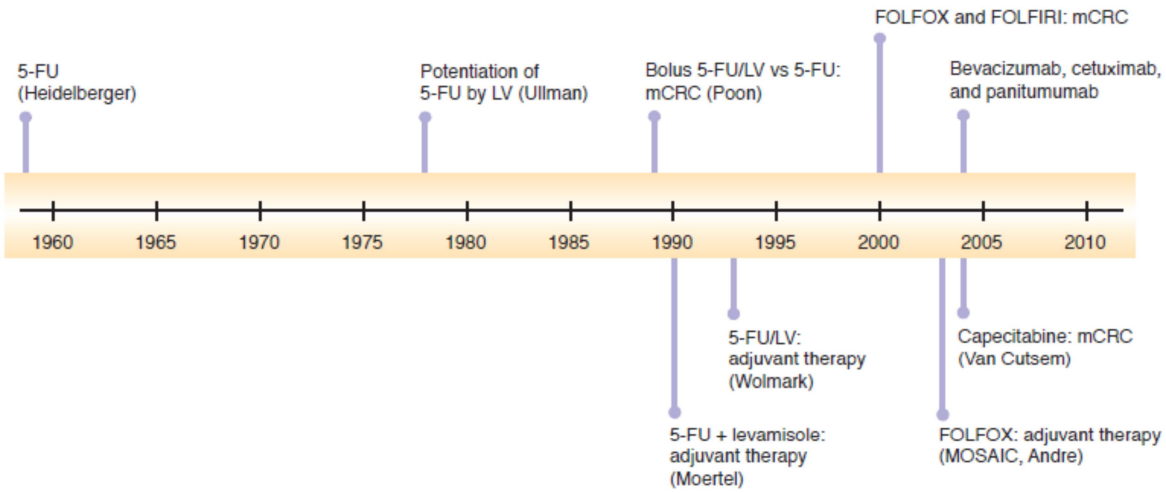
Therapy	Study	Publication date	Study objective	Patients (n)	Key efficacy results
5-FU + levamisole	Moertel et al.[69]	1990	To compare the efficacy of 5-FU + levamisole vs observation only in patients with stage II or III CRC	1296	3.5-year OS: 71% vs 55% Cancer recurrence rate: −41% (*P* < .0001) Overall death rate: −33% (*P* = .006)
5-FU/LV	NSABP C-03[71]	1993	To evaluate the efficacy of 5-FU/LV vs 5-FU + lomustine + vincristine (MOF) in patients with stage II or III CRC	1081	3-year OS: 84% vs 77% (*P* = .007)
	IMPACT[72]	1995	Pooled analysis of three randomized trials to investigate the efficacy of high-dose 5-FU/LV vs no adjuvant therapy in patients with stage II or III CRC	1493	3-year OS: 83% vs 78% Overall death rate: −22% (*P* = .029) and CRC events: −35% (*P* < .0001)

	INT-0089[73]	2005	To assess the relative efficacy of 5-FU/LV (Mayo), 5-FU/LV (Roswell Park), Mayo + levamisole, and 5-FU + levamisole in patients with stage II or III CRC	3794	5-year OS: 66%, 66%, 64%, and 54% (*P* = N/S)
	X-ACT[77]	2005	To evaluate the efficacy of capecitabine vs 5-FU/LV (Mayo) in patients with stage III CRC	1987	3-year OS: 81% vs 78% (*P* = .05) 3-year DFS: 64% vs 61% (*P* = N/S)
	NSABP C-06[78]	2006	To compare the efficacy of tegafur + LV vs 5-FU/LV (Roswell Park) in patients with stage II or III CRC	1608	5-year OS: 79% vs 79% (*P* = N/S) 5-year DFS: 68% vs 67% (*P* = N/S)
	GERCOR C96.1[75]	2007	To compare the efficacy of the de Gramont vs Mayo Clinic regimens of 5-FU/LV in	905	6-year OS: 76% vs 78% (*P* = N/S) 6-year DFS: 66% vs 65% (*P* = N/S)

			patients with stage II or III CRC		
FOLFOX, FOLFIRI	MOSAIC[79,80]	2004, 2009	To evaluate the efficacy of FOLFOX vs 5-FU/LV in patients with stage II or III CRC	2246	6-year OS: 79% vs 76% (*P* = .046) 5-year DFS: 73% vs 67% (*P* = .003)
	PETACC-3[81]	2009	To investigate the efficacy of FOLFIRI vs the de Gramont 5-FU/LV regimen in patients with stage III CRC	2094	5-year OS: 73.6% vs 71.3% (*P* = N/S) 5-year DFS: 56.7% vs 54.3% (*P* = N/S)
Bevacizumab	NSABP C-08[84]	2011	To investigate the efficacy and safety of bevacizumab + FOLFOX vs FOLFOX in patients with stage II or III CRC	2672	3-year DFS: 77% vs 76% (*P* = N/S)
Cetuximab	NCCTG/Intergroup N0147[85]	2012	To assess the benefit of cetuximab + mFOLFOX6 vs mFOLFOX6 in wild-type KRAS patients with stage III CRC	2686	3-year OS: 87% vs 86% (*P* = N/S) 3-year DFS: 75% vs 72% (*P* = N/S) (pre-specified interim analysis)

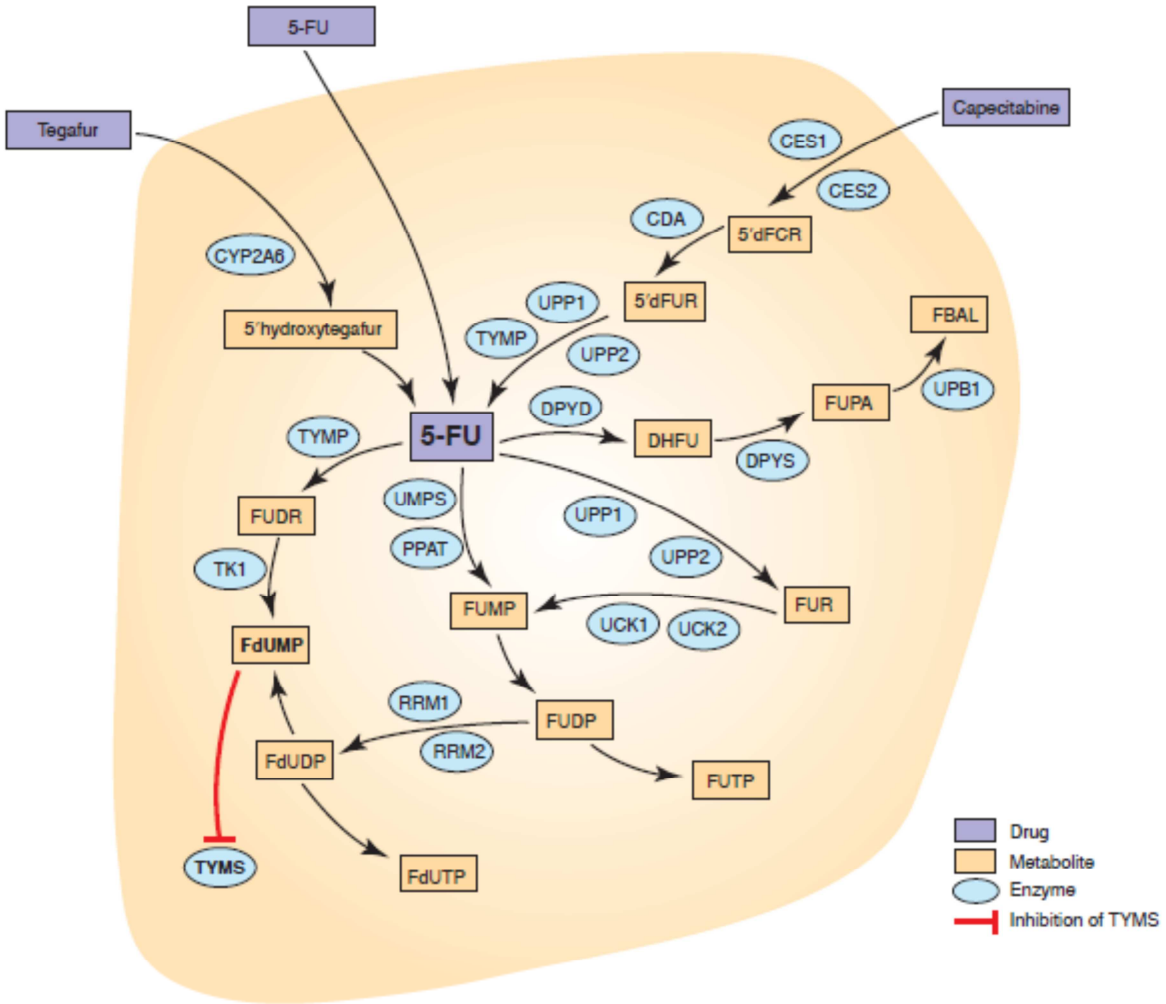
Abbreviations: 5-FU = 5-fluorouracil; CRC = colorectal cancer; DFS = disease-free survival; FOLFIRI = infusional 5-FU/LV plus irinotecan; FOLFOX = 5-FU/LV plus oxaliplatin; GERCOR = Groupe Coopérateur Multidisciplinaire en Oncologie; IMPACT = International Multicentre Pooled Analysis of Colorectal Cancer Trials; KRAS = Kirsten rat sarcoma viral oncogene homolog; LV = leucovorin; MOF = 5-FU plus lomustine plus vincristine; MOSAIC = Multicenter International Study of Oxaliplatin/5-FU/Leucovorin in the Adjuvant Treatment of Colon Cancer; N/S = non-significant; NCCTG = North Central Cancer Treatment Group; NSABP = National Surgical Adjuvant Breast and Bowel Project; OS = overall survival; PETACC = Pan-European Trial in Adjuvant Colorectal Cancer; X-ACT = Xeloda in Adjuvant Colon Cancer Therapy

Figure 1 Landmark advances in the evolution of systemic chemotherapy for patients with CRC



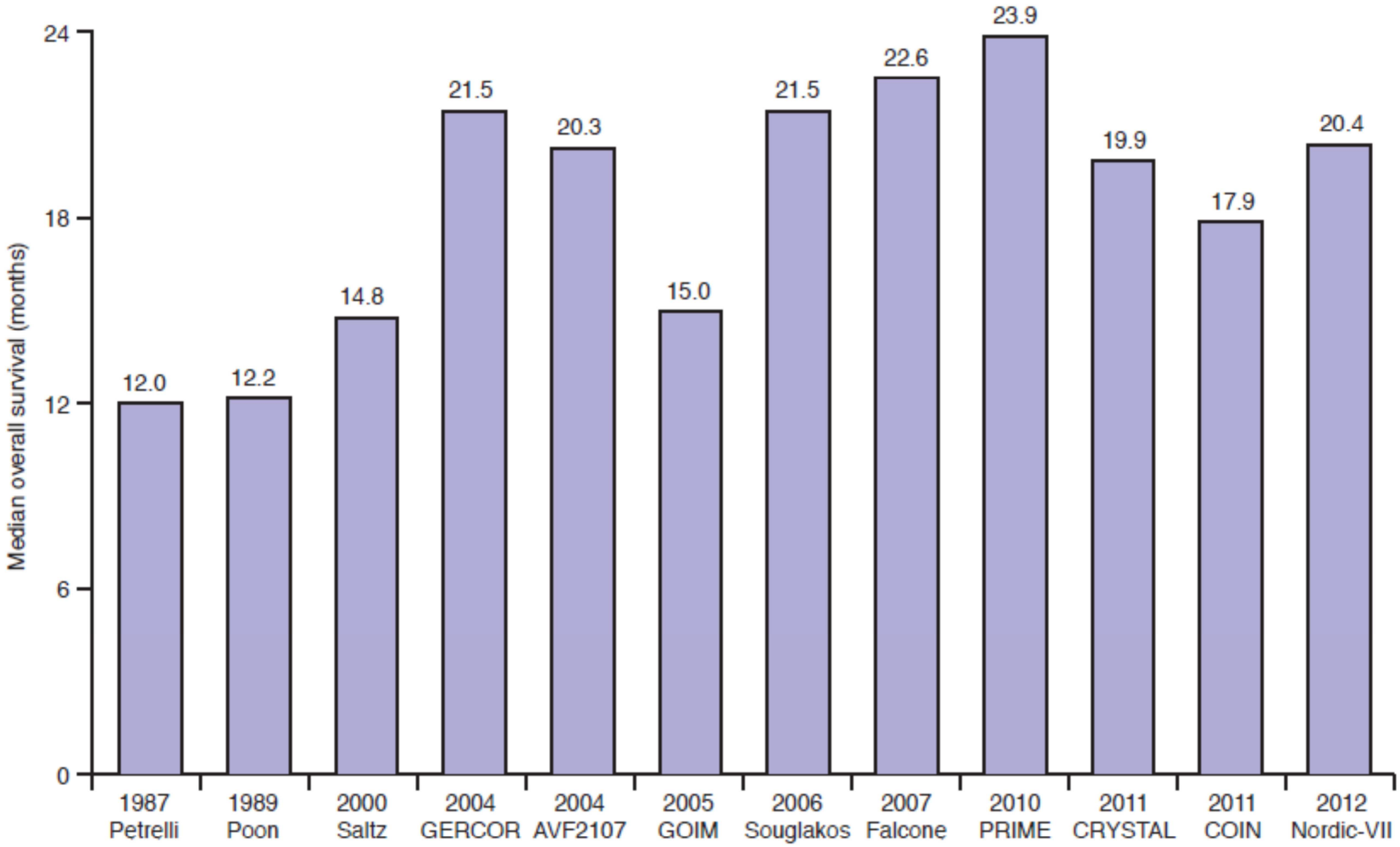
Abbreviations: 5-FU = 5-fluorouracil; CRC = colorectal cancer; FOLFIRI = infusional 5-FU/LV plus irinotecan; FOLFOX = 5-FU/LV plus oxaliplatin; LV = leucovorin; mCRC = metastatic colorectal cancer

Figure 2 5-fluorouracil metabolism



Abbreviations: 5′-dFCR = 5′-deoxy-5-fluorocytidine; 5′-dFUR = 5′-deoxy-5-fluorouridine; 5-FU = 5-fluorouracil; CDA = cytidine deaminase; CES = carboxylesterase; DHFU = dihydrofluorouracil; DPYD = dihydropyrimidine dehydrogenase; DPYS = dihydropyrimidinease; FBAL = fluoro-β-alanine; FdUDP = 5-fluorodeoxyuridine diphosphate; FdUMP = 5-fluorodeoxyuridine monophosphate; FdUTP = 5-fluorodeoxyuridine triphosphate; FUDP = fluorouridine diphosphate; FUDR = fluorodeoxyuridine; FUMP = fluorouridine monophosphate; FUPA = fluoro-β-ureidopropionate; FUR = fluorouridine; FUTP = fluorouridine triphosphate; PPAT = phosphoribosyl pyrophosphate amidotransferase; RRM = ribonucleotide reductase M; TK = thymidine kinase; TYMP = thymidylate phosphorylase; TYMS = thymidylate synthase; UCK = uridine–cytidine kinase; UMPS = uridine monophosphate synthase; UPB = β-ureidopropionase; UPP = uridine phosphorylase. This figure is based on the PharmGKB fluoropyrimidine cycle diagram[86] © PharmGKB and Stanford University, Stanford, CA, USA

Figure 3 Temporal trend of median overall survival in key mCRC clinical trials



The OS values shown for each study represent the treatment arm with the longest median survival

Abbreviations: COIN = Continuous Chemotherapy plus Cetuximab or Intermittent Chemotherapy; CRYSTAL = Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer; GERCOR = Groupe Coopérateur Multidisciplinaire en Oncologie; GOIM = Gruppo Oncologico dell'Italia Meridionale; PRIME = Panitumumab Randomized Trial in Combination With Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy