

Evidence-based efficacy of methotrexate in adult Crohn's disease in different intestinal and extraintestinal indications

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Abstract

Introduction: Methotrexate (MTX) is included in the therapeutic armamentarium of Crohn's disease (CD), although its positioning is currently uncertain in an era in which many effective biological drugs are available. No systematic reviews or meta-analysis have stratified the clinical outcomes of MTX according to the specific clinical scenarios of its use.

Methods: Medline, PubMed and Scopus were used to extract eligible studies, from database inception to May 2021. A total of 163 studies were included. A systematic review was performed by stratifying the outcomes of MTX according to formulation, clinical indication and criteria of efficacy.

Results: The use of MTX is supported by randomized clinical trials only in steroid-dependent CD, with similar outcomes to thiopurines. The use of MTX in patients with steroid-refractoriness, failure of thiopurines or in combination with biologics is not supported by high levels of evidence. Combination therapy with biologics can optimize the immunogenic profile of the biological drug, but the impact on long-term clinical outcomes is described only in small series with anti-TNF α . Other off-label uses, such as fistulizing disease, mucosal healing, postoperative prevention and extraintestinal manifestations, are described in small uncontrolled series. The best performance in most indications was shown by parenteral MTX, favouring higher doses (25 mg/week) in the induction phase.

Discussion: Evidence from high-quality studies in favour of MTX is scarce and limited to the steroid-dependent disease, in which other drugs are the leading players today. Many limitations on study design have been found, such as the prevalence of retrospective underpowered studies and the lack of stratification of outcomes according to specific types of patients and formulations of MTX.

Conclusion: MTX is a valid option as steroid-sparing agent in steroid-dependent CD. Numerous other clinical scenarios require well-designed clinical studies in terms of patient profile, drug formulation and dosage, and criteria of efficacy.

Keywords: Crohn's disease, extraintestinal manifestations, methotrexate

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Introduction

Methotrexate (MTX) has a long history as an effective therapy of oncological diseases, such as acute leukaemia, or rheumatologic diseases, such as rheumatoid arthritis and psoriasis. Over the past 30 years, several studies have also evaluated

its efficacy in the treatment of inflammatory bowel disease (IBD).

The first description of MTX use in Crohn's disease (CD) dates back to 1989 with the pilot study by Kozarek *et al.*¹ Since then, MTX has been

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included in international therapeutic guidelines,²⁻⁵ although its positioning is currently uncertain in an era in which new and effective drugs are available, in particular the anti-TNF α (infliximab – IFX, adalimumab – ADA, certolizumab pegol – CZP) or anti-integrin (vedolizumab – VDZ) and anti-IL12/23 (ustekinumab – UST) biologics.

Despite this, new interest has been raised about MTX in CD. In fact, the literature has been enriched with new studies aimed at delineating the best patient profile to benefit from this therapy according to age, concomitant or previous therapies, comorbidities and disease behaviour.

In this review, we will focus on the adult literature, although it should be noted that MTX is also emerging in the paediatric literature as the preferred immunosuppressant compared with thiopurines⁶ because of concerns about rare cases of lymphoma in young males, treated with thiopurines in combination with anti-TNF α .⁷ A recent systematic review of observational studies demonstrated the ability of MTX to induce clinical remission of disease in paediatric patients with CD in nearly 60% of cases at 3–6 months.⁸ Thus, it is expected that more patients on MTX therapy will transit in the hands of the adult gastroenterologist.

Finally, MTX has been studied in the treatment of ulcerative colitis (UC),^{9,10} but the recent METEOR and MERIT-UC trials failed to demonstrate the efficacy of MTX in inducing and maintaining disease remission at 24 and 54 weeks, respectively.^{11,12}

Methods

Medline, PubMed and Scopus were used to extract eligible studies, from database inception to May 2021. The terms ‘methotrexate’ AND (IBD OR Crohn’s) AND (combination therapy OR biologics OR infliximab OR adalimumab OR golimumab OR certolizumab OR vedolizumab OR ustekinumab OR extraintestinal manifestations OR erythema nodosum OR pyoderma gangrenosum OR arthritis OR spondylitis OR uveitis OR sclerosing cholangitis) were matched. The initial search yielded 1096 results, which were extracted by three independent gastroenterologists (A.C., M.P. and C.C.C.). Further selection was performed by the specialists in rheumatology

(A.B.), dermatology (M.L.) and ophthalmology (P.R.) for each respective field of expertise, finding an additional 87 studies. After the exclusion of duplicates, overlapping and inappropriate records, a total of 163 studies were included (Figure 1). All authors finally analyzed the specific indications for which MTX was used. For each indication, formulation and dosage, we performed a systematic review following the rules of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) Statement. Clinical use of topical MTX was not included.

Results

Pharmacokinetics, formulation and dosage

The formulation and dosage of MTX may influence the clinical efficacy of the drug. MTX can be administered through oral, subcutaneous, intramuscular and intravenous routes. Its individual bioavailability varies from 45% to 100% depending not only on the route of administration but also on various patient-dependent factors and the indication for treatment (type, extent and activity of disease). For example, it is not clear whether established pharmacokinetic data demonstrated in non-gastroenterological (eg, rheumatologic) case series can be transferred to patients with CD, depending on the site and inflammatory activity of the disease, particularly with regard to small bowel disease.¹³

In general, the bioavailability of the oral route is thought to be slightly lower than that of the parenteral route.¹⁴ After oral intake, MTX is absorbed in the proximal jejunum by a saturable, dose-dependent process.¹⁵ It follows that the bioavailability of the oral form is higher at low doses (up to 15 mg), whereas intestinal absorption may be relatively lower with higher doses of the drug. However, in patients with proximal small bowel CD, the absorption and, therefore, the bioavailability of the drug might be reduced, but specific studies are lacking.

In quiescent CD, two studies described a bioavailability of 73–86% for the oral form compared with the subcutaneous form.^{16,17} Despite this lower bioavailability than the parenteral formulation, some authors believe that, at least in patients with CD in remission, the oral form (preferred by patients for convenience, if tolerated) should not be discarded

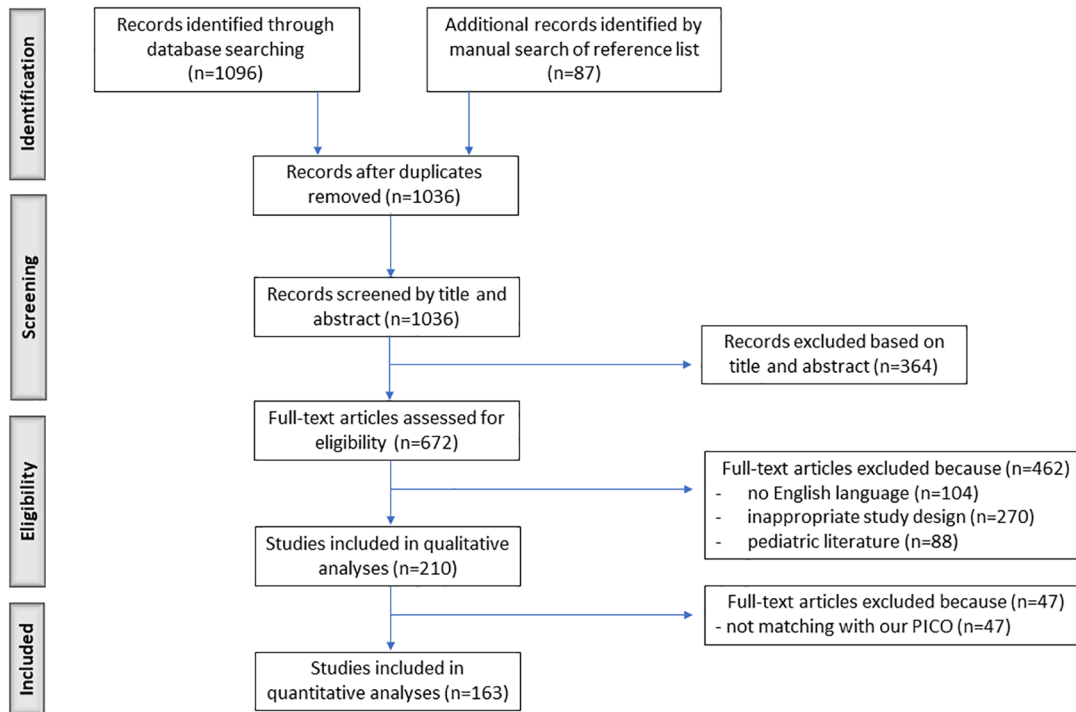


Figure 1. PRISMA flow diagram of study screening and selection.

a priori, according to some positive results in uncontrolled series. However, as it will be shown below, the oral formulation is not supported by randomized controlled trials (RCTs) in CD.

To improve the bioavailability of the oral formulation of MTX, double split dose administration of oral MTX (halved doses 8 h apart, dose ranging 25–35 mg/week) has been proposed in rheumatoid arthritis,¹⁸ but this strategy has not yet been tested in IBD. More recently, several formulations for targeted release have been developed exploiting the lymphatic route to increase drug bioavailability and thus reduce side effects, using micelles,^{19,20} microspheres,²¹ nanoparticles,^{22,23} liposomes,²⁴ polymersomes,²⁵ nanoemulsions²⁶ and glucan particles,²⁷ but none of these formulations have been tested in IBD.

The use of intramuscular MTX in adult CD is supported by controlled studies of induction and maintenance of remission, in specific clinical scenarios (see below). The intramuscular formulation is associated with side effects, such as neuropathy, local irritation, pain, bleeding, fibrosis, abscesses, gangrene, and local contractures. However, the subcutaneous formulation of MTX is a viable

alternative for parenteral use; although not used in controlled studies in adult CD, subcutaneous injections have shown similar pharmacokinetics to the intramuscular form, with comparable serum drug concentrations.^{28,29} In addition, the subcutaneous formulation is burdened with less local toxicity at the injection site and is suitable for self-administration by the patient.^{29,30}

However, it is believed by some authors that the dose is more important than the formulation in determining the efficacy of MTX. In this regard, few studies comparing the various doses of MTX did not stratify the patient by the route of administration or indication for treatment. Egan *et al.*,⁹ in a small single-blind study, randomized patients with steroid-dependent IBD to two different doses of intramuscular MTX (25 *versus* 15 mg) plus steroids, but the authors included both patients with CD and UC in the analysis, thus not providing a picture for the specific scenario of CD.

Finally, no protocols for therapeutic monitoring of MTX are currently available.^{31,32} MTX is metabolized to active polyglutamates that accumulate in cells. The intracellular level of polyglutamates in red blood cells reflects systemic

exposure, but two small studies described little correlation between these concentrations and the control of IBD.^{9,33}

Efficacy in intestinal indications

In 1989, Kozarek *et al.*¹ were the first authors to describe the use of MTX in the induction of a clinical response in active CD. This was an open-label, pilot study, with 14 patients refractory to various therapies at that time (steroids, salazopyrin and metronidazole), which were treated with intramuscular MTX at a dose of 25 mg/week for 12 weeks, then switched to an oral maintenance dose of 15 mg/week in case of initial response; an unspecified proportion of these patients had also failed immunosuppressants. In total, 79% of patients reported a clinical response defined by the reduction in clinical activity index and significant reduction in steroid dose, but the steroid-free remission dropped to 50% at 12 weeks.

Several subsequent studies have described the use of MTX in other patients series (Tables 1–5), which are very heterogeneous in terms of disease behaviour (steroid-dependent or -refractory disease, intolerance or refractoriness to thiopurines, previous or concomitant biologic therapy), treatment regimen (formulation, dose and duration of therapy) and outcomes analyzed (response *versus* remission), thus providing some confusion in the generalization of results. Controlled studies are even few (Table 1), and published meta-analyses suffer from the limitations of the included studies, without focusing on the specific indication for treatment.^{34–38} Therefore, we report the available evidence sorted, where possible, by type of patient and indication for MTX use.

Induction of remission in steroid-dependent or refractory CD. Steroid-dependency is the only indication for which MTX currently has evidence from RCTs in CD, all performed in the pre-biological era (Table 1).^{10,39–43} Many other uncontrolled studies, mostly retrospective (Tables 2 and 3),^{9,44–65} are also available, but they have included heterogeneous populations of both steroid-refractory and steroid-dependent patients, thus preventing a clear interpretation of the performance of the various formulations of MTX in these two distinct clinical scenarios. In particular, the efficacy of MTX in steroid-refractory patients is not analyzed in any specific study.

In steroid-dependent CD, the placebo-controlled, double-blind, multicenter study by Feagan *et al.*³⁹ concerned a group of patients, *naïve* to immunosuppressants and biologics, who according to a now ‘dated’ definition can be defined as steroid-dependent,⁶⁶ as they were active after at least one attempt to withdraw the steroid and therefore maintained on treatment with at least 12.5 mg of prednisolone/day. The authors demonstrated superiority of intramuscular MTX at a dose of 25 mg/week compared with placebo, with remission achieved at 16 weeks in more than one-third of patients ($p = 0.025$).

Opposite results were presented by two other placebo-controlled studies using oral MTX and lower doses ranging 12.5–22.5 mg/week, once again in steroid-dependent CD.^{41,42}

Two further RCTs compared MTX with thiopurines, without significant differences as steroid-sparing agents in steroid-dependent CD.^{10,40} MTX was used, in addition to steroids, at the oral dose of 15 mg/week, in the first study by Matè-Jimenez¹⁰ (induction of remission at 30 weeks = 80% MTX *versus* 93.7% 6-MP *versus* 14% mesalazine), while it was used at intravenous doses of 25 mg/week for 3 months, followed by the 25 mg/week oral dose for another 3 months, in the second study by Ardizzone *et al.*⁴⁰ (steroid-free remission = 44% MTX *versus* 33% AZA at 3 months; 56% MTX *versus* 63% AZA at 6 months).

The onset of clinical benefit with MTX, in terms of significant steroid reduction, is reported with variable latency, averaging 12 weeks.⁶⁷

Second-line immunosuppressive therapy in patient’s failures to thiopurines. No prospective controlled trials using MTX in patient’s failures to thiopurines monotherapy and *naïve* to biologics are available. In adults, only eight retrospective studies, most using parenteral MTX, have described case series specifically limited to patients defined as failures to thiopurines,^{46,50,53,54,56,61,63,65} with variable remission rates: 30–86% at 6 months, 10–77% at 1 year and 20% at 5 years (Table 2); however, the outcome was not clearly stratified by the type of failure (ineffectiveness *versus* intolerance) and by immunosuppressant indication (steroid-dependency *versus* refractoriness), except in two cases. Domènech *et al.*,⁵³ in 22

Table 1. Prospective controlled studies using methotrexate in Crohn's disease.

Indication	Comparator	No. of patients	MTX formulation	MTX dosage (mg/week)	Concomitant steroids (% of patients)	Previous thiopurines	Outcome
Feagan <i>et al.</i> ³⁹	Placebo	141 (MTX = 94; placebo = 47)	i.m.	25	100%	Naive	16-week clinical remission; 39.4% MTX versus 19.1% placebo ($p = 0.025$)
Oren <i>et al.</i> ⁴¹	Placebo	84 (MTX = 26; 6-MP = 32; placebo = 26)	os	12.5	80% (MTX) versus 79% (6-MP) versus 73% (placebo)	12% (MTX), 27% (6-MP), 15% (placebo). Patients had to be off immunosuppressors for at least 3 months at the time of trial entry	9-month clinical remission; 39% MTX versus 41% 6-MP versus 46% placebo ($p = n.s.$)
Arora <i>et al.</i> ⁴²	Placebo	28 (MTX = 13; placebo = 15)	os	15–22.5	26.7% taking at least 20 mg/day prednisone (versus 55.6% in placebo group)	46.7% (MTX), 22.2% (placebo)	1-year clinical relapse; 46% MTX versus 80% placebo ($p = n.s.$)
Mate-Jimenez <i>et al.</i> ¹⁰	6-MP 1.5 mg/kg/die, mesalazine 3g/die	38 (MTX = 15; 6-MP = 16; placebo = 7)	os	15	100%	Naive	30-week clinical remission; 93.7% 6-MP versus 80% MTX ($p = n.s.$) versus 14% 5-ASA ($p = 0.01$). 76-week remission (in early responders); 53.3% 6-MP versus 66.6% MTX ($p = n.s.$) versus 0% 5-ASA ($p < 0.001$)
Feagan <i>et al.</i> ⁴³	Placebo	76 (MTX = 40; placebo = 36)	i.m.	15	0%	2%	40-week clinical remission; 65% MTX versus 39% placebo ($p = 0.04$)
Ardizzone <i>et al.</i> ⁴⁰	AZA	54 (MTX = 27; AZA = 27)	i.v. for 3 months, then os for 3 months	25	100%	15% (MTX), 7% (AZA). Patients had to be off immunosuppressors for at least 3 months at the time of trial entry	3-month clinical remission; 44% MTX versus 33% AZA ($p = n.s.$) 6-month clinical remission; 56% MTX versus 63% AZA ($p = n.s.$)

Black-coloured cells, negative outcomes; CD, Crohn's disease; grey-coloured cells, positive outcomes; i.m., intramuscular; i.v., intravenous; MTX, methotrexate; n.s., not significant.

Table 2. Specific studies which included patients who failed thiopurines for intolerance or refractoriness.

Author	Study design	No. of patients	MTX dosage (weekly)	Steroid-dependent versus steroid-refractory patients (%)	Failures to thiopurines (intolerant/refractory; %)	Failures to anti-TNF α (%)	Steroid-free clinical remission	Clinical remission (%)
Vandeputte <i>et al.</i> ⁴⁶	Retrospective	20	25 mg i.m. for 12 weeks, then 12.5 mg i.m.	65%/35%	100% (25%/75%)	n.r.	Yes	20% (3 months), 30% (6 months), 10% (1 year)
Soon <i>et al.</i> ⁵⁰	Retrospective	66. Dropout 28%	Median 15 mg (5–30), os (97%)	n.r.	100% (n.r.)	n.r.	Yes	39.6% (6 months)
Domènech <i>et al.</i> ⁵³	Retrospective	22	25 mg i.m./s.c. for 16 weeks + steroids for 3–4 months, then 10–15 mg i.m./s.c. (84%) or os (16%)	100%/0%	100% (55%/45%)	n.r.	Yes	77% (1 year), 46% (2 years), 39% (3 years)
Wahed <i>et al.</i> ⁵⁴	Retrospective	131 (99 CD, 32 UC)	Mean 25 mg (7.5–25 mg) then 15 mg (15–25 mg) for median 72 weeks (7–208); i.m./s.c. 47%	n.r.	100% (71%/29%)	n.r.	Yes	Intolerant: 62% (6 months) Refractory: 60% (6 months)
Hausmann <i>et al.</i> ⁵⁶	Retrospective	63	25 mg i.m./s.c. (4.9%) or os (51%). Monotherapy 38% versus 43% combo IFX versus 19% combo steroids	n.r.	100% (4.9%/51%)	n.r.	n.r.	79% (3 months), 75% (6 months), 71% (1 year), 50% (3 years)
Seinen <i>et al.</i> ⁶¹	Retrospective	174	25 mg s.c. for 3–4 months, then 15 mg s.c. (except for 15 patients)	n.r.	100% (n.r.)	23%	Not clear (Sustained clinical benefit)	86% (6 months), 63% (12 months), 47% (24 months), 20% (60 months)
Huang <i>et al.</i> ⁶³	Retrospective	51	20 mg s.c.	100%/0%	100% (31.4%/68.6%)	31.4%	Yes	68.6% (16 weeks), 94.3% (24 weeks), 74.3% (36 weeks), 60% (48 weeks), 45.7% (60 weeks)
Wang <i>et al.</i> ⁶⁵	Retrospective	27	15 or 25 mg, i.m.	n.r.	100% (n.r.)	29.6%	Yes	48.1% (24 weeks)

CD, Crohn's disease; IFX, infliximab; i.m., intramuscular; MTX, methotrexate; n.r., not reported; s.c., subcutaneous; UC, ulcerative colitis; TNF, tumour necrosis factor.

Table 3. Other uncontrolled studies using MTX for luminal active CD.

Author	Study design	No. of patients	MTX dosage (weekly)	Steroid-dependent versus steroid-refractory patients (%)	Failures to thiopurines (intolerant/refractory; %)	Failures to anti-TNF α	Steroid-free clinical remission	Clinical remission (%)
Kozarek <i>et al.</i> ¹	Open, prospective	21 (14 CD, 7 UC)	25 mg i.m. for 12 weeks, then tapering to minimum 7.5 mg os	n.r.	48% (including UC)	n.a.	n.r.	50% (12 weeks)
Baron <i>et al.</i> ⁴⁴	Open, prospective	19 (11 CD, 8 UC)	15 mg os for 18 weeks	100%/0%	91% (n.r.)	n.a.	Yes	20% (18 weeks)
Lemann <i>et al.</i> ⁴⁵	Retrospective	39	25 mg i.m. for at least 3 months, then minimum 7.5 mg os	38%/23%	92% (41%/51%)	n.a.	Yes	51% (1 month), 72% (3 months), 41% (1 year)
Lémann <i>et al.</i> ⁴⁷	Open, prospective	49	25 mg i.m. for at least 3 months, then 7.5 mg os or 25 mg i.m.	Mixed, not specified	86% (n.r.)	n.r.	n.r.	84% (median 1.6 months, range 0–31) 59% (1 year), 49% (2 years), 43% (3 years)
Chong <i>et al.</i> ⁴⁸	Retrospective	67	Mean 20 mg i.m. or s.c. (78%) or os (22%)	6%/94%	85% (n.r.)	n.r.	Yes	37% (mean 22 weeks, range 1–81). 75% (1 year), 31% (2 years), 21% (4 years) Only parenteral: 80% (1 year), 70% (2 years), 47% (4 years)
Fraser <i>et al.</i> ⁴⁹	Retrospective	70 (48 CD, 22 UC)	Mean 20 mg (10–25 mg) os (89%). Dropout 21%	Mixed, not specified	Most patients, n.r.	n.r.	Yes	62% (timing not specified) Among responders: 99% (1 year), 73% (2 years), 51% (3 years)
Hayee and Harris ⁵¹	Retrospective	24	25 mg i.m. (87%) for 16 weeks, then 15 mg i.m.	n.r.	92%	n.r.	n.r.	79% (16 weeks), 42% (1 year)
Nathan <i>et al.</i> ³⁰	Retrospective	45	Mean 21 (10–25 mg) s.c., duration not specified	n.r.	91% (71%/20%)	33%	Yes	9% (timing not specified)
Din <i>et al.</i> ⁵²	Retrospective	39	25 mg i.m. for 19 weeks, then 15 mg os	53%/10%	97% (61%/36%)	61%	n.r.	26% (16 weeks), 22% (50 weeks)
Parker <i>et al.</i> ⁵⁶	Retrospective	37	Median 20 mg (15–25), os 95%	n.r.	n.r.	n.r.	n.r.	response 78% (12–18 weeks)

(Continued)

Table 3. (Continued)

Author	Study design	No. of patients	MTX dosage (weekly)	Steroid-dependent versus steroid-refractory patients (%)	Failures to thiopurines (intolerant/refractory; %)	Failures to anti-TNF α	Steroid-free clinical remission	Clinical remission (%)
Saibeni <i>et al.</i> ⁵⁸	Retrospective	112 (89 CD, 23 UC)	Median 20 mg (7.5–25) i.m. (82%), os (16%), s.c. (0.9%), i.v. (0.9%), including UC	50%/5%	76% (62%/15%)	n.r.	n.r.	'response' 64.1% (timing not specified)
Chande <i>et al.</i> ⁵⁷	Retrospective	79	Median cumulative dose 1727 mg, i.m. or s.c. (87%)	n.r.	53%	38%	n.r.	51% (timing not specified)
Suares <i>et al.</i> ⁵⁹	Retrospective	66	25 mg s.c. for 4 months, then 15 mg s.c. (72.5%, range 7.5–25 mg; os or s.c.)	n.r.	92.4% (27%/65%)	27%	n.r.	'response' 89.5% (4 months)
González-Lama <i>et al.</i> ⁶⁰	Retrospective	77 (62 UC, 15 CD)	Mean 21 mg, i.m. or s.c. (67%) or os (33%)	94%/6%	88% (61%/27%)	n.r.	Yes	28% (timing not specified)
Kopylov <i>et al.</i> ⁶²	Retrospective	118	Induction: 23.75–25 os (31.4%) or parenteral (68.6%). Maintenance: 18 mg (15–25 mg), os 49.1%	84%/n.r.	49% (29%/20%)	33.6%	Yes	Induction 37.2% (timing not specified) Maintenance 63.6% (median 12 months, range 3.5–18.5)
Mesonero <i>et al.</i> ⁶⁸	Retrospective	110	25 mg/week (81%), 20 mg/week (4.5%), 15 mg/week (14.5%); parenteral 94%	n.r./n.r. (49% on steroids)	77% (52%, 25%)	100% (one drug 39%, two drugs 55.6%)	Yes	30.9% (12–16 weeks) In initial responders, 82% (12 months), 74% (24 months)

CD, Crohn's disease; i.m., intramuscular; i.v., intravenous; MTX, methotrexate; n.a., not available; n.r., not reported; s.c., subcutaneous; UC, ulcerative colitis; TNF, tumour necrosis factor.

Table 4. Mucosal healing and MTX.

Author (year)	Study design	No. of patients	MTX formulation	MTX dosage	Mucosal healing
Kozarek <i>et al.</i> ¹	Prospective, open	14	Intramuscular	25 mg/week	Total 5/11 (45%), only in colonic disease.
Manosa <i>et al.</i> ⁷¹	Retrospective	8, steroid-dependent	Parenteral (s.c./i.m.)	25 mg/week for 16 weeks, then 15 mg/week on maintenance	Complete in 3/8 (37.5%), partial in 2/8 (25%)
Huang <i>et al.</i> ⁶³	Retrospective	31	Subcutaneous	20 mg/week	Complete in 47.4% at 36 weeks
Laharie <i>et al.</i> ⁷⁰	Prospective, comparing MTX, AZA and IFX	51, quiescent	Parenteral	15–25 mg/week	Absence of ulcers in 11% MTX <i>versus</i> 50% AZA <i>versus</i> 60% IFX ($p=0.008$ <i>versus</i> MTX)
Rouiller-Braunschweig <i>et al.</i> ⁶⁴	Retrospective	93	n.r.	n.r.	11.8% if MTX < 3 months 9.5% if MTX > 3 months
Vasudevan <i>et al.</i> ⁷²	Retrospective, comparing IFX/ADA and MTX/thiopurines	269 (77 MTX, 192 thiopurines, 156 IFX, 113 ADA)	Not clear: s.c. in 58% of patients on at least 20 mg/week	Median 20 mg, IQR 10–25 mg/week. 71% of patients on at least 15 mg/week, 61% on at least 20 mg/week	58% anti-TNF + thiopurines 17% anti-TNF + MTX ($p < 0.01$) at 12 months

CD, Crohn's disease; IFX, infliximab; IQR, interquartile range; MTX, methotrexate.

steroid-dependent patients (10 refractory and 12 intolerant to thiopurines), reported a steroid-free clinical remission with MTX (used parenterally in 84% of cases) of 77% at 16 weeks in the entire case series, which was maintained in 72%, 46% and 39% of cases at 1, 2 and 3 years, respectively. Even more specifically, Huang *et al.*⁶³ in a recent case series of 51 steroid-dependent CD patients reported steroid-free clinical remission at 16 weeks in 65.7% and 75% of patients refractory or intolerant to thiopurines, respectively.

Third-line therapy in patient's failures to biologics.

No controlled studies have been published for this clinical scenario. Some uncontrolled studies have included patients treated with MTX after failure of anti-TNF α drugs (Tables 2 and 3)^{30,52,56,57,59,61–63,65} but did not provide the outcomes of this specific subgroup. Recently, a retrospective multicenter Spanish study from the ENEIDA registry, specifically described 110 patients, with previous failure to at least one anti-TNF α agent, who had switched to MTX monotherapy.⁶⁸ Before switching to MTX, 77% of patients had already received a

thiopurine; 54 patients (49%) were taking concomitant steroids. The induction dose of MTX was predominantly 25 mg/week parenteral. Short-term clinical remission (week 16) was achieved in 30.9% of cases; of these responders, long-term effectiveness was maintained in 82% and 74% at 12 and 24 months, respectively. In the multivariate analysis, non-remission at short-term was associated with long-term failure.

No studies are available on the use of MTX as rescue therapy after the failure of VDZ or UST.

Maintenance of remission. Once again, Feagan was the first author of the only randomized, placebo-controlled study that demonstrated the efficacy of parenteral MTX for the maintenance of steroid-induced clinical remission, specifically in steroid-dependent CD.⁴³

In this multicenter study on 76 patients, an induction dose of 25 mg/week intramuscular for 16–24 weeks was used, followed by a maintenance dose of 15 mg/week intramuscular, for 40 weeks.

Table 5. Studies using MTX in combination with anti-TNF α agents.

	Biological drug	Study design	No. of patients	MTX dosage and formulation	Previous anti-TNF (%)	Combo (MTX versus AZA)	Clinical benefit with combined treatment
Schröder <i>et al.</i> ⁸³	IFX	Open, controlled	19	20 mg/week for 5 weeks, then 20 mg/week os	0	100% (n.r.)	Yes
Sokol <i>et al.</i> ⁸⁰	IFX	Prospective, registry (MICISTA)	121	n.r.	86.8	100% (n.r.)	No
Feagan <i>et al.</i> ⁷⁹	IFX	RCT	126	10 mg/week s.c., up to 25 mg/week	0	100% (100%/0%)	No
Colman and Rubin ⁸⁴	IFX	Retrospective	73 IBD (54 CD)	< 12.5 mg/week versus > 12.5 mg/week. Formulation not reported.	n.r.	100% (100%/0%)	Same results
Borren <i>et al.</i> ⁸⁵	IFX	Retrospective	222 IBD (163 CD)	12.5 mg/week os versus > 12.5 mg/week, parenteral	76	100% (100%/0%)	Same results
Targownik <i>et al.</i> ⁸¹	IFX/ADA	Retrospective, population-based database	852 (617 IFX, 235 ADA)	n.r.	n.r.	52% (92%/8%)	Yes for some outcomes
Targownik <i>et al.</i> ⁸²	IFX/ADA	Retrospective, population-based database	8129 (5050 IFX, 3079 ADA)	n.r.	n.r.	39.1% (84%/16%)	No
Cosnes <i>et al.</i> ⁸⁶	IFX/ADA	Retrospective, multicentric (MICISTA), comparing IFX/ADA combo versus mono thiopurines, 77 MTX]	906 [IFX 587 (374 combo), ADA 319 (152 combo), 442 thiopurines, 77 MTX]	15 mg/week os	0	58% (16% versus 84%)	Yes
Hanauer <i>et al.</i> ⁸⁷ (CLASSIC-I)	ADA	RCT	225	n.r.	0	29.8% (3.5%/26%)	No
Sandborn <i>et al.</i> ⁸⁸ (CLASSIC-II)	ADA	RCT	241	n.r.	0	34% (2.5%/27.8%)	No
Sandborn <i>et al.</i> ⁸⁹ (GAIN)	ADA	RCT	159	n.r.	100	46% (n.r.)	No

ADA, adalimumab; AZA, azathioprine; black-coloured cells, negative outcomes; CD, Crohn's disease; grey-coloured cells, positive outcomes; IBD, inflammatory bowel disease; IFX, infliximab; MTX, methotrexate; n.r., not reported; RCT, randomized controlled trial; TNF, tumour necrosis factor.

Of note, no other CD-related medication was allowed in the maintenance phase.

In responders at week 16, the study reported a steroid-free remission rate of 65% at 40 weeks in the MTX group, compared with 39% in the placebo group ($p=0.04$). Interestingly, about half of the patients who relapsed on MTX 15 mg/week regained remission after reinduction with the 25 mg/week intramuscular dose (along with prednisone).

Comparing MTX with thiopurines as maintenance therapy in CD, three RCTs, in a total of 77 patients (mostly steroid-dependent) for 24–76 weeks, did not conclude for the superiority of one drug over the other.^{10,40,41}

A further plethora of studies, mostly retrospective, have been published describing small case series of patients treated with maintenance MTX, with variable dosages, durations of treatment and percentages of patients failures to thiopurines or IFX (Tables 2 and 3): the maximum follow-up described is 5 years, with a maintained clinical remission in 20% of patients.⁶²

From these case reports, although heterogeneous, it is clear that, even for MTX, secondary loss of response becomes a problem over time, with remission rates progressively decreasing over the years. While the steroid-free remission is described in a range of 10–80% of patients at 1 year^{42,43,46,50,51,53,61,63} and 46–70% at 2 years,^{10,48,53,61} the same falls to 39% at 3 years⁵³ and 20% at 5 years.⁶¹

Current guidelines do not define the duration of therapy with MTX. However, a retrospective study, which included 19 patients with CD and UC, reported a high relapse rate after discontinuation, most often within 1 year. Remission rate after treatment withdrawal at 6, 12 and 18 months were 42%, 21% and 16%, respectively.⁴⁹ There is more evidence on the withdrawal of MTX in patients with rheumatoid arthritis, where better outcomes have been found by distancing the weekly doses to every 2 weeks than with stopping the drug.⁶⁹

Mucosal healing. No controlled studies have analyzed the endoscopic and histological healing of MTX as primary endpoints in CD. The ability of MTX to induce mucosal healing in CD is reported

in two prospective uncontrolled studies^{1,70} and in four small retrospective studies,^{63,64,71,72} ranging from 11% to 45% according to different definitions of complete endoscopic remission. The most favourable data have been described with parenteral formulations, starting with doses of 20–25 mg/week (Table 4). However, the only prospective comparison study by Laharie *et al.*,⁷⁰ reported significantly better mucosal healing rates both with AZA or IFX than with parenteral MTX (50%, 60% and 11%, respectively).

Another recent, retrospective, study compared the mucosal healing rate induced by combination therapy with anti-TNF α and AZA *versus* anti-TNF α and MTX, reporting rates of 58% and 17% ($p < 0.01$), respectively, at 12 months.⁷² This difference was even more evident when the anti-TNF α drug used in combination was IFX rather than ADA: endoscopic remission at 12 months was not achieved in any of the 11 patients treated with MTX + ADA compared with 4/12 (33%) patients treated with MTX + IFX.⁷²

Regarding histologic response, only the Laharie's study is available; however, no significant differences between MTX, AZA and IFX were described, using D'Haens' score.⁷⁰

Fistulizing CD. The efficacy of MTX *monotherapy* in fistulizing CD has been evaluated in small retrospective studies (ranging from 4 to 29 patients), which included various types of fistulas, both perianal and internal.^{45,50,59,73,74} The anatomic type of fistula and its complexity is not always specified and its response to previous therapies. When reported, complete closure of fistulas occurred in 22–50% of patients.^{45,50,74}

The only RCT by Ardizzone *et al.*,⁴⁰ controlled to thiopurines, was not designed for this purpose, but in six patients with CD it reported the complete closure of perianal fistulas in 50% of patients at 1 month and 67% at 3 and 6 months.

The response to a *combined* approach with MTX plus IFX and surgery led to complete closure rates ranging from 33% to 74% of patients.^{75–77} In the study by Roumeguère *et al.*, an initial drainage with possible seton placement was performed and a second surgical step with seton removal (as well as possible procedures, such as fibrin glue and/or reconstructive flap) was planned between

the second and third infusion of IFX. Parenteral MTX 25 mg/week was administered 2–3 months after the first surgical step, followed by IFX 1 week later.⁷⁶

Prevention of postoperative recurrence. The use of MTX in the prevention of postoperative recurrence of CD has so far been described by only one small prospective study that analyzed the combination of IFX 5 mg/kg and low doses of oral MTX (10 mg/week), the latter given to reduce the immunogenicity of IFX (see below).⁷⁸ The authors compared 7 patients on combo treatment with 16 control patients treated with oral mesalazine. No patients on combination therapy experienced endoscopic recurrence at 2 years, compared with 12/16 patients on mesalazine.

Combination therapy with biologics: clinical efficacy

Infliximab. IFX + MTX combination therapy has not demonstrated favourable efficacy results to date, either in a RCT using high parenteral doses,⁷⁹ or in some registries using MTX at unspecified dosages (Table 5).^{80–82}

The only exception was an early small randomized, open-label study by Schröder *et al.*,⁸³ which compared IFX 5 mg/kg monotherapy ($n=8$) versus IFX + MTX 20 mg/week ($n=11$) for 48 weeks in 19 patients refractory or intolerant to AZA. MTX was infused intravenously for the first 5 weeks then administered per os. Clinical remission at 48 weeks was 71% in the combination group, compared with 33% on monotherapy.

Some years later, the COMMIT trial did not confirm these good impression using smaller doses (10 mg, increased to 25 mg/week at week 5) of subcutaneous MTX in 126 patients who were immunosuppressors-naïve and received prednisone 6 weeks before.⁷⁹ Steroid-free remission was comparable at both 14 weeks (76% versus 78%) and 50 weeks (56% versus 57%) in combination therapy compared with IFX monotherapy, respectively. However, the potential effect of concomitant steroids in the induction phase of both arms makes uncertain this short-term outcome. Another bias might have been the lower dose of MTX used by Feagan compared with Schroeder. However, discordant conclusions have been provided in two retrospective studies, aimed by the research of the best dose of MTX to combine with IFX (≤ 12.5 mg/week or higher).^{84,85}

Adalimumab. Table 5 summarizes the outcomes of studies describing MTX in combination with ADA. A recent meta-analysis of 24 studies in CD, regarding the efficacy of adding an immunosuppressor to ADA treatment, did not conclude in favour of combination therapy in terms of improved remission rates or clinical response.⁹⁰ However, this meta-analysis and other studies^{86–89} did not stratify outcomes according to the type of immunosuppressor (MTX versus AZA) or previous exposure to biologics (*naïve versus failures*). Data regarding MTX are really limited in terms of number of patients included and heterogeneity of interpretation and, therefore, no firm conclusions can be drawn.

Regarding the most appropriate dose of MTX when combined with ADA, there are not comparative studies in IBD. In rheumatoid arthritis, however, there is even a large RCT that examined four different oral dosages (2.5, 5, 10 and 20 mg), in biologic-naïve patients: similar benefit-risk profile was found for 10 and 20 mg/week of MTX, with increased ADA trough levels.⁹¹

Certolizumab pegol. The effect of combination therapy with CZP and an immunosuppressant, including MTX, is unknown. The PRECISE trials, in fact, have not reported data about the clinical efficacy of combination therapy.^{92,93} A retrospective single-centre study of 222 IBD patients (163 with CD) treated with MTX in combination with various anti-TNF α drugs had included 6 patients on CZP but did not report the specific outcomes.⁸¹

Vedolizumab. Also for VDZ, we do not have prospective controlled studies, specifically designed to evaluate the effect of combination therapy with MTX (Table 6).

A population pharmacokinetic modelling showed that MTX had no clinically relevant effect on VDZ linear clearance.¹¹⁵ Regarding clinical efficacy, the placebo-controlled, phase III, GEMINI-2 and GEMINI-3 trials did not analyze the specific role of MTX as combination therapy.^{94,116}

Further open-label real-life experiences, which are often limited by the retrospective design and low numbers of patients included (Table 4), have not described significant differences between VDZ monotherapy and immunosuppressant combination therapy,^{95–99,101–112,114} except in two studies.^{100,113}

Table 6. Studies using MTX in combination with VDZ.

	Study design	No. of patients	MTX dosage and formulation	Previous anti-TNF α (%)	Combo (MTX versus AZA)	Clinical benefit with combined treatment
Sandborn <i>et al.</i> ⁸⁴ (GEMINI2)	RCT	967	n.r.	59.5	52% (n.r.)	No
Shelton <i>et al.</i> ⁹⁵	Retrospective, multicenter	107	n.r.	97.1	31.7% (17.3%/14.9%)	No
Dulai <i>et al.</i> ⁹⁶ (VICTORY Consortium)	Retrospective, multicenter	215	n.r.	91	40% (n.r.)	No
Baumgart <i>et al.</i> ⁹⁷	Prospective, multicenter	97	n.r.	94.8	80.4% (n.r.)	No
Stallmach <i>et al.</i> ⁹⁸	Prospective, multicenter	67	n.r.	91	14.9% (n.r.)	No
Amiot <i>et al.</i> ⁹⁹	Prospective, multicenter	173	25 mg/week parenteral	100	25% (n.r.)	No
Allegretti <i>et al.</i> ¹⁰⁰	Retrospective, bicentre cohort	96 completing 14-week induction VDZ	n.r.	n.r.	54% (n.r.)	Yes
Amiot <i>et al.</i> ¹⁰¹ (OBSERV-IBD)	Prospective, multicenter	161 responders to 14-week induction VDZ	25 mg/week parenteral	99.4% (90.7% more than two previous biologics)	25% (n.r.)	No
Gouynou and Peyrin-Biroulet ¹⁰²	Case series	2, non-responders to 14-week induction VDZ	25 mg/week sc for 3 months, then 15 mg/week	n.r.	2 (100%)	No
Samaan <i>et al.</i> ¹⁰³	Retrospective, two-centre	27 CD + 23 UC	n.r.	76% both diseases	42% (2%/40%) both diseases	No
Eriksson <i>et al.</i> ¹⁰⁴	Retrospective (SWIBREG registry) multicenter	147	n.r.	86	35% (n.r.)	No
Kopylov <i>et al.</i> ¹⁰⁵	Multicentre, prospective	130	n.r.	92.6	24.6% (n.r.)	No
Kopylov <i>et al.</i> ¹⁰⁶	Retrospective, multicenter,	50	n.r.	0	10% (n.r.)	No
Lenti <i>et al.</i> ¹⁰⁷	Retrospective, multicenter	135	n.r.	95.5	51.8% (n.r.)	No
Macaluso <i>et al.</i> ¹⁰⁸	Retrospective, multicenter	84	n.r.	76.1	8% (n.r.)	No
Iborra <i>et al.</i> ¹⁰⁹	Retrospective, multicenter	30	n.r.	90	37% (n.r.)	No
Ylisaakko-Oja <i>et al.</i> ¹¹⁰	Retrospective, multicenter	108	n.r.	95.4	46% (n.r.)	No
Macaluso <i>et al.</i> ¹¹¹	Prospective database	1	n.r.	100	100%	No
Hoffmann <i>et al.</i> ¹¹²	Retrospective, single centre	28	n.r.	89.3	67.9% (4%/11%)	No
Ylisaakko-Oja <i>et al.</i> ¹¹³	Retrospective	23	15.7 \pm 6.8 mg/week, formulation not reported	95	100%	No
Hu <i>et al.</i> ¹¹⁴	Retrospective	53	n.r.	n.r.	n.r.	No

AZA, azathioprine; black-coloured cells, negative outcomes; CD, Crohn's disease; grey-coloured cells, positive outcomes; IBD, inflammatory bowel disease; MTX, methotrexate; n.r., not reported; RCT, randomized controlled trial; TNF, tumour necrosis factor; UC, ulcerative colitis; VDZ, vedolizumab.

Similar to other biologic agent trials, major limitations of these uncontrolled case series are the inability to differentiate the specific contribution of MTX compared with other immunosuppressants (especially thiopurines) and the limited number of patients included in MTX arms. Only three studies, all referring to patient's predominant (91–95%) failures to anti-TNF α agents, analyzed the specific contribution of MTX, without observing a significant effect of the combined treatment, with regard to intestinal clinical endpoints.^{97,98,111} However, Macaluso *et al.*,¹¹¹ in a small case series of four patients (three CD and one UC) in whom MTX (15–25 mg/week, unspecified formulation) was added to VDZ monotherapy in case of persistent joint manifestations, reported an unspecified joint response in two cases at the 15 mg/week dose.

Two studies, including biologic-naïve patients, did not report benefit from combination therapy but results were not stratified for type of immunosuppressive drug.^{109,106}

Ustekinumab. Current data from literature do not suggest superiority of combination therapy with UST and immunosuppressants, including MTX. As with other biologics, however, there are no controlled trials specifically designed (Table 7).

Phase II and III trial (UNITI-1, UNITI-2 and IM-MUNITI) sub-analyses did not show benefit from combination treatment, but these are small subgroups that render these analysis underpowered and do not provide specific data for MTX.^{117,118,120}

Uncontrolled published trials are also limited by the retrospective design, the small number of included patients (range 2–8) and the inability to stratify the immunosuppressant drug.^{119,121–127}

Combination therapy with biologics: immunomodulatory effects and drug optimization. In the COMMIT study,⁷⁹ and in a prospective study by Vermeire *et al.*,¹²⁸ the combination of MTX + IFX was associated with significantly lower levels of anti-IFX antibodies compared to IFX monotherapy as well as with higher circulating levels of IFX: it is known that these parameters influence long-term outcomes, such as secondary loss of response to IFX and the development of infusion reactions.^{129,130} Both studies used parenteral MTX, with doses ranging from 10–15 mg/week.

Other studies, both in CD and rheumatoid arthritis, confirm the ability of MTX to affect the immunogenicity not only of IFX^{131–133} but also of ADA^{88,134–137} and VDZ,¹³⁸ especially by reducing the development of anti-drug antibodies.

Few studies, limited to the anti-TNF α treatment, have analyzed whether this immunomodulatory effect is matched by a clinical benefit. A recent meta-analysis focused on the clinical response associated with the addition of an immunosuppressant (MTX or AZA) to anti-TNF α therapy (four studies), without specifying MTX ($n=19$) versus AZA ($n=30$) outcomes.¹³⁹

Concerning IFX, two small retrospective studies have provided some clinical data, with positive results:^{140,141} MTX use rather than AZA was significantly associated with the risk of relapse (HR 3.37, 95% CI 1.14–9.96) in 43 patients who stopped combination therapy,¹⁴⁰ while both MTX ($n=2$) and AZA ($n=3$) restored clinical response in five patients with secondary loss of response to IFX.¹⁴¹ The addition of parenteral MTX was useful also in small series (range 5–21) of patients who lost clinical response to ADA, without differences with AZA.^{135,137,142}

Finally, Kennedy *et al.* performed the largest prospective study of anti-TNF α therapy in IBD, by enrolling 1610 patients with active luminal CD treated with IFX or ADA. Clinical variables that were associated with treatment failure were week 14 drug concentrations and immunogenicity. Combination therapy with a thiopurine or MTX mitigated this risk. MTX was used in 59/955 patients treated with IFX and in 30/655 patients treated with ADA. No difference was measured in terms of immunogenicity between thiopurines or MTX.¹³⁶

Overall, these studies may suggest specific synergistic and/or additive effects between MTX and IFX or ADA, favouring the sustainability of long-term efficacy of the anti-TNF α drug. No studies, on the contrary, are available on MTX use as rescue therapy in failures to VDZ or UST monotherapy.

Cross indications

Arthropathies. The most common extraintestinal manifestation in IBD patients is the articular one. In a simplified way, we can distinguish the axial

Table 7. Studies reporting MTX use in combination UST in CD.

Author	Study design	No. of patients	MTX dosage and formulation	Previous anti-TNF α (%)	Combo (MTX versus AZA)	Clinical benefit with combined treatment
Sandborn <i>et al.</i> ¹¹⁷	RCT	131	n.r.	58	37.4% (6.9%/30.5%)	No
Sandborn <i>et al.</i> ¹¹⁸ (CERTIFI)	RCT	394	n.r.	100	24.4% (n.r.)	No
Kopylov <i>et al.</i> ¹¹⁹	Retrospective, single centre	38	n.r.	100	10.5% (5.3%/5.3%)	No
Feagan <i>et al.</i> ¹²⁰	RCT	741 (UNITI-1) + 628 (UNITI-2) + 397 (IM-UNITI)	n.r.	UNITI-1 100%, UNITI-2 and IM-UNITI not available	UNITI-1 30.8% (?), UNITI-2 34.9% (?), IM-UNITI 36.4% (?)	No
Khorrami <i>et al.</i> ¹²¹	Retrospective, multicenter	116	n.r.	100	36.2% (n.r.)	No
Wils <i>et al.</i> ¹²²	Retrospective, multicentre	122	n.r.	100	15% (5.7%/9%)	Yes (AZA and MTX)
Ma <i>et al.</i> ¹²³	Retrospective, multicenter	167	n.r.	95.2	43.7% (n.r.)	No
Ma <i>et al.</i> ¹²⁴	Retrospective, multicenter	104	n.r.	92.3	42.3% (n.r.)	Yes (AZA and MTX)
Battat <i>et al.</i> ¹²⁵	Retrospective, single centre	62	n.r.	100	25.8% (16.1%/9.7%)	No
Greenup <i>et al.</i> ¹²⁶	Retrospective, single centre	69	n.r.	99	42% (n.r.)	No
Wils <i>et al.</i> ¹²⁷	Retrospective	88 responders to 1-year UST	n.r.	100	14.8% (6%/9%)	No
Hu <i>et al.</i> ¹¹⁴	Retrospective	63	n.r.	n.r.	n.r.	No

AZA, azathioprine; black-coloured cells, negative outcomes; grey-coloured cells, positive outcomes; MTX, methotrexate; n.r., not reported; RCT, randomized controlled trial; TNF, tumour necrosis factor; UST, ustekinumab.

form, characterized by sacroileitis and spondylitis, from the peripheral form marked by arthritis and/or dactylitis and/or entesitis.

While MTX is considered the anchor drug of rheumatoid arthritis treatment and the most commonly prescribed conventional synthetic disease-modifying anti-rheumatic drug (DMARD), either as monotherapy or in combination with biologic or targeted synthetic DMARDs,^{143,144} there are not specific prospective controlled trials for the treatment of IBD-associated arthritis.

The use of MTX monotherapy in the axial forms of enteropathic arthritis is not supported by a Cochrane meta-analysis,¹⁴⁵ and it is not endorsed by international guidelines,^{146,147} which instead

favour anti-TNF α therapies. Notably, in the Cochrane review,¹⁴⁵ three small RCTs with a total of 116 patients were analyzed.^{148–150} MTX doses ranged from 7.5–10 mg/week orally for 12–24 weeks, while parenteral use was not explored. Instead, a small open-label study by Haibel consisting of 20 patients using MTX 20 mg/week subcutaneously demonstrated an ASAS20 response of 25%, which is similar to placebo response rates in some studies with anti-TNF α agents.¹⁵¹

In peripheral form of enteropathic arthritis, MTX showed its efficacy according to treatment guidelines for spondyloarthritis,¹⁵² although there is no evidence derived from *ad hoc* studies. Responses to the drug, variously defined, have been described

in cases of spondylitis associated with peripheral involvement, either as monotherapy or in combination with salazopyrin.^{151,153–157}

Also, the use of MTX in combination with anti-TNF α agents, which is reported in treatment of other rheumatic diseases,^{158,159,160} lacks *ad hoc* studies in IBD-associated arthropathies. Conflicting data on the impact of MTX co-treatment on anti-TNF α survival are present in literature, with some observational cohort studies showing positive results,^{161–164} and a number of other large studies which demonstrated no benefits.^{165–168}

While, a single prospective monocentre study in 65 patients with CD and 15 patients with UC demonstrated MTX efficacy in patients with paradoxical articular manifestations during anti-TNF α treatment, without reporting its formulation.¹⁶⁹

Psoriasis and psoriasis induced by anti-TNF α agents. MTX remains as one of the first-line treatments used in patients with psoriasis, despite its lower efficacy compared with ADA and IFX.^{170,171} In the context of IBD, one proposed – but unsuccessful – use for MTX is its combination with anti-TNF α agents to control treatment-induced psoriatic lesions. The available literature is limited to small series or case reports.

In the first published report, Chu *et al.* described a case of palmoplantar pustular psoriasis that appeared during ADA treatment and was refractory to topical steroids but sensitive to cyclosporine. Not only MTX (in an unspecified formulation) but also various other agents failed to switch to an alternative maintenance therapy to cyclosporine in this notoriously difficult-to-treat form of psoriasis.¹⁷²

Buisson *et al.* described the effect of MTX in the treatment of psoriasiform lesions that arose during anti-TNF therapy in seven patients with CD. Six patients received 25 mg/week of MTX, whereas only one patient received 7.5 mg/week; the formulation was parenteral in all but one patient. At the time of MTX introduction, some were continuing anti-TNF ($n=2$), some switched to other anti-TNF ($n=3$) and some discontinued the drug ($n=2$). After a follow-up of 20–45 months (median 29 months), only one patient had a response, that lasted 42 weeks and then relapsed.¹⁷³

In the most recent study by Mazloom *et al.* in eight patients treated with MTX (only one case in monotherapy and the other seven in combination with topical therapy), only 4/8 showed an unspecified improvement, whereas the other four, including the patient in monotherapy, had no improvement. These patients belonged to a larger case series of 102 cases of anti-TNF-induced psoriasis, and the indications for anti-TNF were heterogeneous, including not only IBD but also rheumatologic patients; moreover, treatment outcome was not stratified by pathology or by type of psoriasis. The most useful MTX dose, when effective, was greater than 15 mg per week, whereas no patient treated with dosages below 10 mg had a benefit; the formulation was not specified.¹⁷⁴

Regarding treatment *ab initio* in patients with both psoriasis and CD, only a recent safety analysis of UST in the various phase II/III registration trials is available, which reports no different outcomes between UST monotherapy and UST in combination with MTX.¹⁷⁵

Other cutaneous manifestations. With regard to other cutaneous manifestations in CD, the use of MTX is only anecdotally described in small case series of pyoderma gangrenosum (PG) and erythema nodosum (EN), refractory to steroids,^{176,177} and in one case report of metastatic CD in combination with IFX.⁷⁴ RCTs for PG or EN are not available.

No studies have described the use of MTX in Sweet's syndrome and in oral CD; on the contrary, oral ulcerations can occur as a side effect of MTX therapy.

Schmidt *et al.*, in describing the favourable outcome of 16 patients treated with pulse cyclophosphamide in combination with AZA or MTX, reported a 'substantial improvement' (in terms of pain and regression in size and/or number of lesions) within 8 weeks, in all eight patients with PG ($n=5$) or EN ($n=3$), refractory to steroids, four of whom treated with MTX (not specifying the type of skin lesion in this treatment group). After up to 30-month follow-up, all patients has achieved and maintained complete remission of their skin lesions, but the authors did not describe how many of these remained on MTX therapy.¹⁷⁶

More recently, Duarte-Chang and Visueti¹⁷⁷ presented the case of a young man with PG in the

setting of active CD, refractory to systemic steroid, who recovered a full clinical response 4 weeks after the addition of MTX, at a dose of 25 mg/week subcutaneously for 16 weeks, followed by 15 mg/week of maintenance treatment.

Very few other case reports of MTX used for PG, not associated with CD, showed mixed results (favourable with oral,¹⁷⁸ unfavourable with unspecified formulations).¹⁷⁹

In another case report by Tonkovic-Capin *et al.*,¹⁸⁰ low doses of oral MTX had a beneficial effect on orofacial swelling in a case of cheilitis granulomatosa accompanied by CD with recurrence despite systemic glucocorticoids. Cheilitis granulomatosa is a rare idiopathic condition with painless lip swelling, characterized by non-necrotizing granulomatous inflammation which may precede the presentation of CD even after long-term follow-up. MTX 5 mg orally once weekly was initiated. Within 2 months, there was a marked reduction in the patient's facial swelling; increasing MTX dose to 10 mg orally once weekly yielded almost complete resolution of facial swelling. This beneficial response has been maintained for 16 months, continuing MTX at the same dosage.

Equally anecdotal is the case of a 35-year-old woman with severe fistulizing CD presented with pyostomatitis vegetans affecting both the mouth and the vulva. Pyostomatitis vegetans is a rare non-microbial neutrophilic disease of the oral mucosa, associated with IBD. Three injections of IFX and maintenance therapy with MTX (25 mg weekly) resulted in rapid and complete regression of both the pyostomatitis vegetans and the CD, during 15 months of follow-up.¹⁸¹

Ocular manifestations. MTX has been frequently employed to treat ocular inflammatory diseases, including uveitis, scleritis, and orbital inflammatory disease.¹⁸² While the use of MTX is advocated at the forefront of paediatric guidelines for the treatment of children with chronic anterior uveitis and juvenile idiopathic arthritis requiring systemic immunosuppression (after failure/intolerance of topical steroids),^{183–186} there are not evidence-based guidelines or specific case series about its use in the treatment of adult IBD-associated uveitis.

In general, the therapeutic approach to uveitis has, however, differed minimally for different

non-infectious etiologies. Most clinical trials for uveitis enrolled patients with a specific anatomic location for the uveal inflammation but not a specific etiology.¹⁸² Most forms of anterior uveitis respond particularly well to topical steroids, which are not adequate to treat intermediate and posterior uveitis. If topical steroids are not adequate, the treating physicians will usually embark on a trial of oral corticosteroids.

The early use of corticosteroid-sparing immunosuppression has been advocated by a Delphi panel.¹⁸⁷ Traditionally, MTX was the most popular immunosuppressive for this indication.

The first report on the use of MTX in uveitis was published in 1965 by Wong and Hersh,¹⁸⁸ who described positive effects in 9 of 10 patients with a diagnosis of 'cyclitis' who were refractory to systemic steroid therapy. Since then, small series have reported MTX to be effective for ocular inflammation in general,¹⁸⁹ and for specific ocular inflammatory conditions, including uveitis associated to juvenile idiopathic arthritis,^{190–193} sarcoidosis,¹⁹⁴ Behcet's disease,¹⁹⁵ mucous membrane pemphigoid,¹⁹⁶ and rheumatoid arthritis.¹⁹⁷

A recent systematic review analyzed the adult literature regarding the treatment of anterior uveitis, both idiopathic and associated with systemic disorders (mainly ankylosing spondylitis).¹⁹⁸ with regard to MTX, a single-centre prospective study in 19 patients,¹⁹⁹ and three retrospective studies in 36, 104 and 160 patients, respectively, are available.^{200–202} Another retrospective study in 46 patients with acute anterior uveitis associated with HLA-B27-positive ankylosing spondylitis (and UC in one patient) has been recently published.²⁰³ The majority of these studies described the efficacy of MTX in patients predominantly *naïve* to immunosuppressants and biologics, significantly reducing the number of relapses and uveitis activity, increasing the interval between relapses and reducing steroid consumption. The dose of MTX in these patients ranged from 7.5 to 25 mg/week per os or subcutaneously.

Primary sclerosing cholangitis. Both uncontrolled open-label studies and one RCT failed to demonstrate the efficacy of oral MTX in the treatment of primary sclerosing cholangitis (PSC).^{204,205} The empiric use of MTX in patients with PSC is therefore not recommended. The same authors of the controlled trial suggested continuing studies

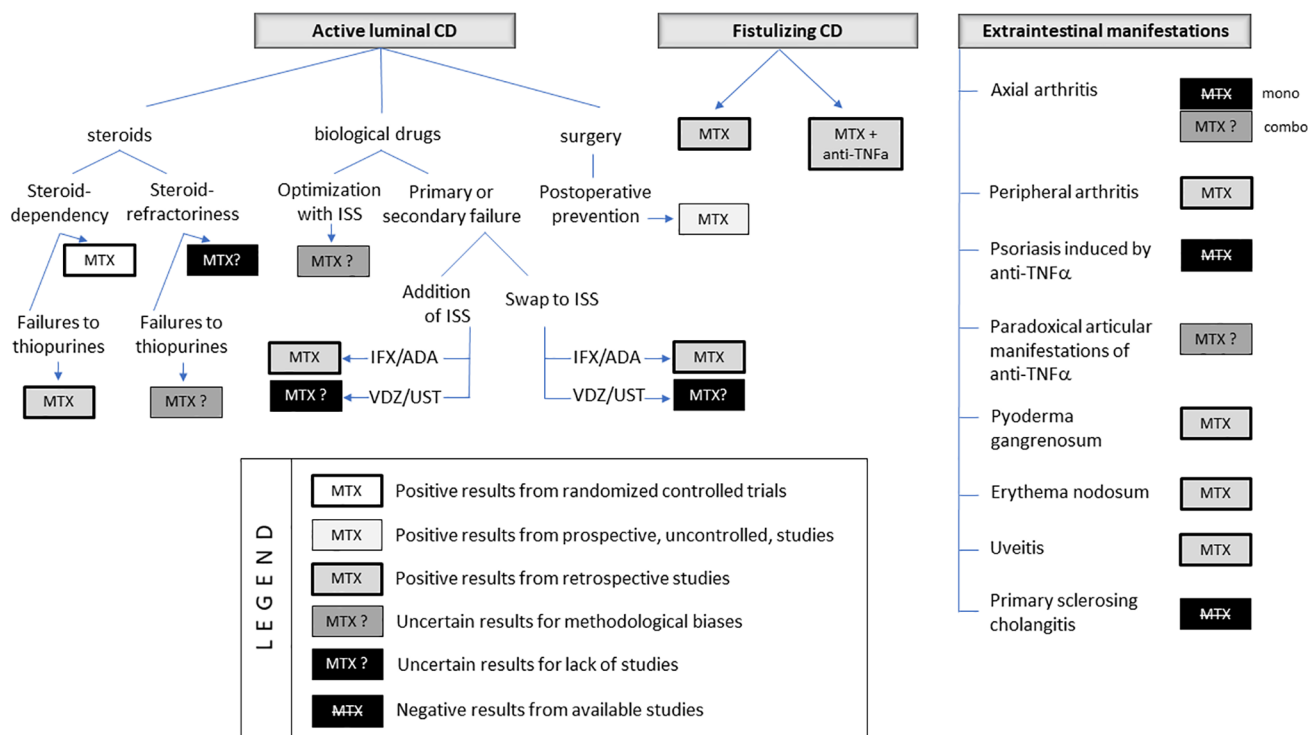


Figure 2. Positioning MTX use according to intestinal and extraintestinal indications in CD.

in patients with precirrhotic disease, without the signs of portal hypertension or liver failure, based on their previous small series of two patients,²⁰⁶ and a preliminary study in 10 patients treated with low doses of oral MTX, which described biochemical and histologic improvement.²⁰⁷ However, no further controlled studies followed in this specific setting.

Discussion

Our systematic review describes the evidences available to support the use of MTX in specific clinical scenarios of CD, with the aim to critically discuss the current indications described by the most recent guidelines, as well as its clinical off-label use, which is increasingly proposed as rescue therapy or optimization strategy in different clinical settings.

Our review shows that, despite more than one hundred published studies, there are very few evidences on the efficacy of MTX derived from RCTs. Moreover, several studies are limited by some methodological biases or were performed

many years ago, according to different criteria of patient selection and treatment efficacy.

The latest guidelines recommend the use of MTX in patients with active CD as immunomodulator in the scenarios of steroid-dependency, steroid-failure, intolerance to thiopurines or in association with anti-TNF α treatment as combination therapy.²⁻⁵ In our review, we show that steroid-dependency is the only scenario supported by RCT so far.

On the contrary, the other ‘classic’ indications for MTX, indicated in previous guidelines and deleted or conditionally granted in more recent editions,^{2-5,208,209} do not find support from high level of evidence: steroid-refractoriness, failure to thiopurines and combination therapy with anti-TNF α drugs, although described in single uncontrolled case series, do not find an unequivocal favourable opinion from numerous, but heterogeneous, studies. Not surprisingly, the meta-analyses published to date describe the extreme heterogeneity of study populations, treatment regimens and outcome definitions.^{28-35,210} One

seemingly redundant point, however, appears to be the better performance of the higher parenteral doses (25 mg per week) compared with the low oral doses, the first and only ones to be associated with a benefit over placebo in induction RCTs.^{39–42,210}

Regarding the maintenance of remission, parenteral MTX appears to be effective in maintaining steroid-induced remission of CD, with controlled data for at least 1 year in steroid-dependent disease, in favour of the 15 mg/week dose. This has been confirmed by some meta-analyses that, although limited by the paucity of available *ad hoc* studies, have concluded for a favourable NNT = 4, comparable to that reported in meta-analyses concerning thiopurines.^{35–38}

The role of the oral formulation as maintenance treatment remains uncertain: the unfavourable results of the two small placebo-controlled studies by Oren *et al.*⁴¹ and Arora *et al.*⁴² do not seem to support this formulation at least at the lowest dosages (12.5–15 mg) for 1 year of observation. However, the placebo-controlled performance of higher dosages (25 mg) is supported by the study of Ardizzone *et al.*⁴⁰ as an alternative to AZA but with higher rates of adverse events (asthenia, nausea and vomiting, not requiring drug withdrawal) than AZA, and without data from placebo-controlled trials.

In the most favourable studies to date,^{10,39,40} MTX was used in patients *naïve* to immunosuppressants. More uncertain remains the role of MTX in second- and third-line after the failure of a first immunosuppressant (virtually thiopurines) or at least one biologic. However, it should be noted that the studies conducted in the so-called ‘failures’ actually describe a clinically heterogeneous context. In patients with early intolerance to AZA, in whom thiopurine has often not yet reached any clinical effect, it is not known whether a second-line drug, such as MTX, can achieve better results than the patient already refractory to AZA or other therapies since no controlled study has so far stratified the clinical outcome according to these clinical characteristics. Our review shows that MTX (preferably parenteral) may have a role as a second- or third-line therapy, but not precisely quantifiable by magnitude and sustainability of its effect and without clear evidence about the best dosage.

Our review then discusses some specific scenarios, such as fistulizing disease, postoperative prophylaxis, mucosal healing and cross-indications for extraintestinal manifestations.

In fistulizing disease and postoperative prophylaxis, small uncontrolled studies seem promising and interesting, but *ad hoc* controlled trials are needed.

MTX has been periodically tested in association with almost all currently available biologic drugs to optimize their efficacy and/or immunogenicity, but through small or uncontrolled series, or according to formulations and dosages that are probably not adequate. In some scenarios there is a complete lack of data, as in the case of MTX + ADA combination in patients *naïve* to biologics. In other studies, MTX decreased the immunogenic profile of IFX, ADA and VDZ, favourably influencing levels of anti-drug antibodies and, in some cases, circulating biologic drug. Whether this immunomodulatory effect is matched by a clinical benefit remains unclear, but it appears that combination therapy does not improve the performance of biologics in terms of short-term clinical efficacy.

Instead, a challenge of current research is to understand whether MTX can be used to optimize long-term biological therapy efficacy. The possible scenarios would be its association *ab initio*, to prevent the appearance of antibodies to drugs, or during biological therapy to modulate the eventual profile of immunogenicity, for example in case of loss of response or in case of appearance of anti-drug antibodies. At the moment, we have few studies, underpowered or retrospective, and more focused on combination therapy with anti-TNF α agents. No specific data are available on the role of MTX in combination with VDZ or UST since current studies, reporting no benefit by adding immunosuppressants in general, did not stratify the outcome between MTX and thiopurines.

Even in extraintestinal manifestations, the role of MTX is mostly empirical and based on sharing similar approaches in other etiologies. In articular disease, the oral route had a bad performance, while parenteral MTX still deserves better analyses. In psoriasis induced by anti-TNF α agents, adding MTX is not useful, while oral MTX can

be an option in some rare cutaneous manifestations. Finally, the use of MTX in uveitis seems interesting, but no data came from IBD-associated uveitis series.

The other side of the coin of the clinical use of MTX is safety. Within the standard dose range (subcutaneous or intramuscular, 15–25 mg weekly), up to one-third of patients discontinues MTX because of intolerance. Nausea and flu-like symptoms after parenteral administration are common.³² At higher doses, myelotoxicity is possible, and long-term use has been associated with hepatic fibrosis that is more common in obese patients or with alcohol use.²¹¹ Allergic pneumonitis is rare. MTX is also immunosuppressive and has been associated with an increase in infectious disease (e.g. viral infections, including herpes zoster). MTX is contraindicated during pregnancy and lactation. With the use of MTX, it has been shown that there is an elevated risk of NMSC, specifically squamous cell and basal cell carcinoma, especially in those patients with a prior history of NMSC.²¹²

Finally, in Figure 2, we show a proposal of therapeutic use of MTX according to the specific evidences found in our review. Moreover, MTX could still be a therapeutic option in specific settings. First of all, there could be economic benefits. The burden of IBD is high, mostly owing to biological therapy. Hence, some health systems for which access to newer biologics is not an easy or affordable option could consider MTX therapy a viable option. Second, MTX could offer some advantages in patients with recent or active malignant disease.

In conclusion, evidence from high-quality studies in favour of MTX in CD is scarce and limited to the steroid-dependent disease, in which other drugs are the leading players today. Numerous other clinical scenarios require well-designed clinical studies in terms of patient profile, drug formulation and dosage, and criteria of efficacy.

Author contributions

Andrea Cassinotti: Conceptualization; Data curation; Formal analysis; Methodology; Project administration; Writing – original draft.

Alberto Batticciotto: Data curation; Formal analysis; Writing – review & editing.

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Simone Segato: Writing – review & editing.

Federico Zanzi: Writing – review & editing.

Antonella Cappelli: Supervision; Writing – review & editing.

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