New and emerging biologics in the treatment of inflammatory bowel disease: quo vadis?

Quelles biothérapies pour demain dans les maladies inflammatoires chroniques intestinales ?

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Summary
Inflammatory bowel diseases (IBD) are pathological conditions characterized by chronic inflammation that is primarily the consequence of dysregulation of the immune response. Over the last decade, the advances in the pathophysiology of IBD have paved the way for the development of a number of biological agents that selectively target specific molecules and/or pathways involved in gut inflammation. Although numerous, so far, the only biological therapeutics that are approved for the treatment for IBD are monoclonal antibodies against tumor necrosis factor α. This paper systematically reviews the mechanism-of-action, efficacy, short-term and, where available, long-term safety of biological agents that target molecules other than tumor necrosis factor α, in IBD.

Résumé
Les maladies inflammatoires chroniques intestinales (MICI) sont caractérisées par une inflammation chronique de la muqueuse digestive résultant d’une dérégulation de la réponse inflammatoire et immunitaire intestinale. Au cours des dix dernières années, l’identification croissante des molécules et des voies biologiques impliquées dans la physiopathologie des MICI a permis le développement de nombreux agents biologiques ayant potentiellement des propriétés thérapeutiques en inhibant sélectivement ces molécules ou ces voies de signalisation cibles. Malgré leur nombre, jusqu’ici, seuls les anticorps dirigés contre le tumor necrosis factor α (TNF) ont démontré leur efficacité clinique et obtenu une autorisation de mise sur le marché. Dans cette revue, nous exposons de manière systématique les modes d’action, l’efficacité, les données concernant la tolérance à court terme (ou à long terme si celles-ci existent), des agents biologiques autres que les molécules anti-TNF développées dans les MICI.

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The inflammatory bowel diseases (IBD) are pathological conditions characterized by chronic inflammation that is primarily the consequence of dysregulation of the immune response. Impairment of the innate immune system, which recognizes bacterial products and cellular signalling via pathogen-associated molecular patterns (PAMPs), can cause subsequent persistent activation of the adaptive immune system. In patients with CD the production of interleukin (IL)-12 and IL-18 by antigen-presenting cells (APCs) and macrophages results in a T helper (Th)1-type polarization and increased secretion of pro-inflammatory cytokines, such as interferon-γ (IFNγ). IL-2 and tumor necrosis factor α (TNF-α). These cytokines in turn stimulate APCs, macrophages and endothelial cells to produce other pro-inflammatory cytokines, including TNF-α, IL-1, IL-6, IL-8, IL-12, and IL-18, leading to the creation of a self-sustaining cycle [1-3]. Conversely, in UC patients, an atypical Th2-like immune response is observed, with increased production of IL-5 but not IL-4, and reduced levels of IFN-γ [2]. Recently, another CD4 T-cell lineage has been recognized, i.e., Th17, commitment to which is promoted by TGF-β in the presence of pro-inflammatory [4-5]. IL-23, which plays a pivotal role in driving intestinal inflammation in murine models [6], is also able to expand and maintain the Th17 cell population [7] and to induce the secretion of IL-17 by cell types other than T cells in an inflammatory environment. Another essential stage in the genesis and maintenance of intestinal inflammation is trafficking of leukocytes into the gut and the recruitment of immune cells to the site of inflammation by up-regulation of the expression of adhesion molecules. These include selectins, integrins and endothelial cells adhesion molecules such as mucosal addressin cell adhesion molecule (MadCAM)-1, vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1 and -2 [8].

Over the last decade the advances in our knowledge of the pathogenic mechanisms underlying chronic inflammation in the gut, together with the increasing progresses in biotechnology have led to the development of a number of biological agents that selectively target specific molecules and pathways involved in gut inflammation. These agents, collectively known as “biological” therapeutics, include recombinant peptides or proteins, antibody-based therapy, nucleic acid-based therapies, and cell and gene therapies [9, 10]. Considering the pathogenic step they target biological agents can be classified as inhibitors of proinflammatory cytokines (including TNF-α), anti-inflammatory cytokines, inhibitors of cell adhesion molecules (CAMs), inhibitors of Th1 polarization, inhibitors of T cells activation and proliferation, growth factors, immunostimulators, MAPK (mitogen-activated protein kinases) inhibitors, immunomodulators, anti-leukocyte molecules (Table 1).

Thus far, the only biological therapeutics that have been approved for the treatment of the inflammatory bowel diseases are two tumor necrosis factor α inhibitors, i.e., the humanized monoclonal antibody infliximab and the fully human antibody adalimumab, both of which have been approved by for the treatment of Crohn’s disease and infliximab for the treatment of ulcerative colitis. The other biologicals have been mainly tested in randomized controlled trials (RCTs) with different study designs, patient populations and outcome measures, making comparisons between them difficult. Only a relatively small number of these biological agents have demonstrated any efficacy.

In the following pages we briefly review the mechanism-of-action, efficacy, the short-term and, where available long-term safety of biological agents (excluding the TNFα inhibitors), in patients with CD or UC. This review will focus on published RCTs selected from a search of the available literature (PubMed, Embase) and from congress proceedings (Digestive Disease Week, United European Gastroenterology Week) published in the last decade.

### Anti-adhesion molecule therapeutics

The pivotal role played by the trafficking of leukocytes into the gut and the recruitment of the immune cells is mediated by several endothelial adhesion molecules, e.g., E-selectin, ICAM-1, ICAM-2, VCAM-1, mucosal addressin Mad-CAM-1. These adhesion molecules interact with specific integrins on leukocytes, allowing them to adhere to and transmigrate across the blood vessels to the site of inflammation [8, 11]. Blockade of these interactions therefore has the ability to prevent this important step in the genesis and maintenance of intestinal inflammation.

### Natalizumab

Natalizumab (Tysabri, Antegren) is a humanized (95% human-derived) IgG4 monoclonal antibody that selectively targets...
the human α₄-subunit of integrins, which is therefore able to inhibit both the VCAM-1/α₄β₁ and MadCAM-1/α₄β₇ pathways of leukocyte adhesion and transmigration through the endothelium.

Patients with CD

In the first RCT, in which 30 patients with mild to moderately active CD were enrolled, natalizumab showed short-term efficacy after a single 3 mg/kg intravenous dose vs. placebo, with seven (39%) of the patients in the natalizumab group achieving remission at week 2 compared with one (8%) of the placebo-treated patients. A significant increase in circulating B and T lymphocytes were also detected after the administration of natalizumab. No significant adverse events were reported [12].

The second RCT was a phase II trial in which 248 patients with moderately to severely active CD were randomized to receive, 4 weeks apart: (i) two infusions of placebo, (ii) one infusion of 3 mg/kg natalizumab and one of placebo, (iii) two infusions of 3 mg/kg natalizumab, or (iv) two infusions of 6 mg/kg natalizumab. At week 6, none of the natalizumab treatment regimens achieved a significantly higher remission rate than placebo, although a high placebo remission rate (27%) could have influenced this failure. On the other hand, all patients in the treatment groups that received two infusions of natalizumab (both the 3 and 6 mg/kg doses) achieved significantly higher remission and response rates vs. placebo group at all other time points (from 4 to 12 weeks). The safety profile was very good and anti-natalizumab antibody formation was only observed in 7% of patients [13].

In a large phase III clinical trial (ENACT-1: Evaluation of Natalizumab in Active Crohn’s disease Therapy-1), 905 patients with active CD received infusions of natalizumab (300 mg) or placebo at weeks 0, 4, and 8. At 10 weeks, there was no significant difference in the responses to natalizumab or placebo (56% vs. 49%, respectively; p = 0.05), or in the rates of remission (37% and 30%, respectively; p = 0.12). Again an unexpectedly high rate of response was reported in the placebo group. Serious adverse events occurred in 7% of patients in both the treatment and placebo groups [14]. In a second trial (ENACT-2) [14], 339 patients who responded to natalizumab in the ENACT-1 trial were randomly reassigned to receive as maintenance therapy 300 mg of natalizumab or placebo, every 4 weeks for 56 weeks. At week 36, a significant higher proportion of patients in the natalizumab-treated group maintained response (61% vs. 28% in the placebo group; p < 0.001) and remission (44% vs. 26% in the placebo group; p = 0.003) [14]. However, in an open-label extension study, a patient treated with natalizumab died as a result of progressive multifocal leukoencephalopathy (PML) associated with the JC virus [14].

The safety of a combination of natalizumab and infliximab was assessed on 79 patients with CD who were not responsive to the infliximab therapy (5 mg/kg every 8 weeks for at least 10 weeks prior to randomization and throughout this study). Patients were randomized (2:1) to receive three intravenous infusions infusions of natalizumab (300 mg; n = 52) or placebo (n = 27), one every 4 weeks. The rate of adverse events rate was similar in the two groups, with no severe adverse events reported. Two (4%) patients developed anti-natalizumab antibodies, while ten patients (14%) developed anti-infliximab antibodies [15].

In an open label study on 38 patients, natalizumab demonstrated efficacy in the treatment of adolescent patients with moderate-to-severe CD [16]. Later on, in the ENCORE (Efficacy of Natalizumab in Crohn’s Disease Response and Remission) trial, 59 patients with moderate-to-severe CD received intravenous injections of natalizumab (300 mg) or placebo at weeks 0, 4, and 8. More patients administered natalizumab showed an early (at week 8) and sustained (at week 12) response compared with those in the placebo group (46% vs. 32%, respectively; p < 0.001). The rate of adverse events was similar in the two groups [17].

Finally, a recent Cochrane systematic review considered the pooled data from the studies discussed above, and concluded that natalizumab (300 mg or 3-4 mg/kg) effectively induces remission in patients with CD. In particular, patients with raised levels of C reactive protein (CRP) seem to show a greater response rate, as well as those who have previously received conventional immunosuppressive drugs and/or infliximab [18].

Patients with UC

In an uncontrolled pilot study, ten patients with active UC, defined by a Powell-Tuck score > 4, received a single infusion of 3 mg/kg natalizumab. In these patients, the median Powell-Tuck score decreased from 10 at baseline to 6 at week 2. Two patients achieved true remission, defined as a score of 0. Adverse events were infrequent and probably not related to the infusion of natalizumab [19].

Safety

The identification of a limited number of cases of PML among patients treated with natalizumab for multiple sclerosis and/or CD [20-22] has led to the withdrawal of natalizumab from the market [23].

A retrospective evaluation on 3417 patients who received natalizumab was performed to assess the risk of PML in natalizumab-treated patients. Only the three previously reported cases were confirmed, with a risk of PML of 1.0 to 1.000 patients administered natalizumab for a median time of 17.9 months [24]. This risk was not considered sufficient to justify keeping the drug off the market. In early June, 2006, natalizumab was reintroduced only for patients with multiple sclerosis under a careful risk management program (The TOUCH™ [Tysabri® Outreach: Unified Commitment to Health] Prescribing Program) whose goals were to assess risk of PML in patients administered natalizumab. For multiple sclerosis, natalizumab may only be administered as a monotherapy “to patients who have had an inadequate response to current treatments or who are unable to tolerate other therapies for multiple sclerosis”. In addition, a magnetic resonance image must be obtained to give a baseline prior to initiating treatment with natalizumab [25-27].

MLN-02

The humanized IgG1 monoclonal antibody MLN-02 (LDP-02) is a biological agent that targets the α₄β₇ integrin, specifically inhibiting its interaction with Mad-CAM-1.
Patients with CD
In a placebo-controlled study that has only been published as an abstract, 185 patients with mild-to-moderate CD received intravenous infusions of 0.5 mg/kg or 2 mg/kg MLN-02 or placebo at day 1 and 29, and were then followed for 6 months. The primary endpoint (statistically significant decrease of > 70 points in CDAI score at 8 weeks vs. placebo) was not achieved, but statistically significant clinical remission was observed in the 2 mg/kg group (36.9% vs. 20.7% of placebo; p < 0.05) [28].

Patients with UC
In multicenter, phase II, double-blind, placebo-controlled trial, 181 patients with active mild-to-moderate UC were randomized intravenous infusions of 0.5 mg/kg or 2 mg/kg MLN-02 or placebo at day 1 and 29. At week 6, a significantly higher proportion of patients in the groups administered MLN-02 achieved clinical remission (32-33% in both MLN-02 groups vs. 14% in the placebo group; p < 0.03) and endoscopic remission (28% in 0.5 mg/kg MLN-02 group vs. 12% in 2 mg/kg MLN-02 group vs. 8% in placebo group; p = 0.007) compared with the placebo group. Three serious adverse events, cytomegalovirus infection, pneumonia and an infusional reaction with angioedema, were reported [29].

Anti-ICAM-1 therapy
Alicaforsen (ISIS 2302) is a 20-base phosphorothioate oligodeoxy nucleotide that hybridizes to a sequence in the 3‘-untranslated region of the mRNA that encodes ICAM-1 in humans. This results in a reduction of the levels of ICAM that are synthesized.

Patients with CD
In a placebo-controlled pilot study in which alicaforsen was administered intravenously to 20 patients with chronically active CD, a rapid and persistent clinical beneficial effect was observed [30]. On the other hand, two large multi-center placebo-controlled trials, the first on patients with moderately active steroid-resistant CD administered alicaforsen subcutaneously [31] and the second on with steroid-dependant patients with CD administered 2 mg/kg alicaforsen intravenously three times/week [32], failed to show any efficacy. To address a potential suboptimal dosage of the drugs in these two studies, an open label high-dose study of intravenous alicaforsen was subsequently conducted on 22 patients with active CD. The intravenous administration of the drug (three times/week for 4 weeks) achieved clinical remission in nine patients (41%), with an overall response rate of 41% at week 8 and of 47% at week 12. A total of five patients dropped out due to infusion-related adverse events, and a moderate, but not clinically relevant increase in aPTT was observed 2 to 4 hours post-infusion [33].

More recently 331 patients with active CD received intravenous infusions of alicaforsen (n = 221) or placebo (n = 110) three times/week for 4 weeks. No significant difference in achieving primary and endpoint (remission at week 12) was demonstrated between the two groups of patients. Alicaforsen group showed only a higher infusion reaction rate than placebo group, and no other adverse events were reported [34].

Patients with UC
An enema formulation of alicaforsen has also been used for the treatment of pouchitis or active left-sided UC in a number of studies, with limited efficacy but a good safety profile [35-38]. In an open-label, uncontrolled trial, 12 patients with unremitting pouchitis received 240 mg alicaforsen enema nightly for 6 weeks. After 6 weeks of nightly treatment, the pouchitis disease activity index (PDAI) decreased from 11.42 points at baseline to 6.83 points (p = 0.001). By week 6, a statistically significant remission rate (7/12 patients, 58%) was also observed in association with a reduction of the endoscopic score [35].

In a first dose-escalating RCT, 40 patients with mild to moderately active distal UC were randomized to four dosing cohorts. In each cohort, two patients received placebo while eight patients received 60 ml of the alicaforsen enema (0.1, 0.5, 2, or 4 mg/ml respectively) once daily for 28 consecutive days. At day 29 of treatment, the disease activity index (DAI) score was significantly decreased in all alicaforsen groups, in a dose-dependent manner (overall p = 0.003 vs. placebo) [36].

In a phase II RCT, 120 patients with left-sided active UC were randomized to one of five treatment arms for 6 weeks; (i) 120 mg alicaforsen enema daily for 10 days and then every other day, (ii) 240 mg alicaforsen enema every other day, (iii) 240 mg alicaforsen enema daily for 10 days and then every other day, (iv) 240 mg alicaforsen enema daily, and (v) a placebo enema daily. The primary endpoint, a mean percentage change in DAI at week 6, was not achieved [37].

Finally, 190 patients with active left-sided UC (n = 159) or pancolitis (n = 31) were randomized to receive an 120 mg alicaforsen enema (n = 65), a 240 mg alicaforsen enema (n = 62) or a 4 g mesalazine enema (n = 63) nightly for 6 weeks. The alicaforsen enema failed to demonstrate superior efficacy to the mesalazine enema in this study [38].

Immunostimulators
Starting from the observation that CD-like gastrointestinal lesions may occur in some forms of genetically-dependent immunodeficiency such as glycogen storage disease Ib [39, 40], some researchers have supported the hypothesis that a defect of the innate immune system may play a pivotal role in the pathogenesis of CD. Therefore, stimulators of the innate immune system such as recombinant human granulocyte-colony stimulating factors (rhuG-CSF, filgrastim) and recombinant human granulocyte monocyte-colony stimulating factors (rhuGM-CSF, sargramostim) have been tested in patients with CD.

Recombinant Human G-CSF
After preliminary uncontrolled positive experiences [41, 42], 20 patients with active luminal CD received daily subcutaneous administrations of rhuG-CSF (300 μg) in a 12 week open-label study. At week 12, 25% of patients achieved clinical remission, while 55 and 35% achieved a decrease of at least 70 and 100 points of the CDAI score, respectively. Furthermore, three out of four patients with fistulae responded. The only adverse event reported was
an excessive neutrophil count (more than 35 × 10⁹/L) in two patients, while the most common reported side effect was a mild transient bone pain [43].

**Recombinant Human GM-CSF**

After a preliminary open-label experience in 15 patients with moderate-to-severe CD [44], rhuGM-CSF has been recently tested in a phase II placebo-controlled trial on 124 patients with moderate-to-severe CD [45]. In this study, patients were randomly assigned using a 2:1 ratio to receive 6 μg/kg of rhuGM-CSF subcutaneously or placebo daily for 56 days. The primary endpoint, defined as a statistically significant decrease of CDAI score of at least 70 points at day 57, was not achieved. On the other hand, all secondary endpoints, i.e., a significant remission rate and a decrease of the CDAI during treatment of at least 100 points (at days 15, 29 and 57) and through the 30 days of follow-up, were achieved. A high placebo remission rate may have contributed to the failure to achieve the primary endpoint. Injection-side reaction (90% of patients) and bone pain (37% of patients) were reported in the patients administered rhuGM-CSF, while severe adverse events (migraine, demyelinating syndrome, ischemic heart disease) were observed in three patients [45].

**Inhibitors of Th1 polarization**

In patients with CD, the production of IL-12 and IL-18 by APCs and macrophages results in a Th1 polarized immune response and increased secretion of pro-inflammatory cytokines such as IFN-γ, IL-2 and TNF-α. Antibody blockade of IL-12 and IFN-γ has therefore been tested in patients with CD.

**Anti-IL-12**

In a phase II placebo-controlled study, 79 CD patients received an IgG1 full-human monoclonal antibody that recognises IL-12 or placebo [46]. Patients were divided into three groups and administered seven weekly subcutaneous injections of 1 or 3 mg/kg of anti-IL-12 or placebo, with a four week interval between the first and the second injection (Cohort 1) or with only a 1 week interval (Cohort 2). Safety was the primary endpoint of the study, and no significant difference in adverse events was observed, apart from injection site reactions and the detection of anti-drug antibodies in three patients. For the secondary endpoints of response and remission rate only the 3 mg/kg group in the second cohort obtained a statistically significant response rate at week 7 (75% vs. 25% of placebo; p = 0.03), and at 18 weeks of follow-up significance was not maintained (p = 0.08). Remission rate at the end of the treatment and through the follow-up was not significantly different among the groups in either cohort. The secretion of IL-12, IFN-γ and TNF-α by mononuclear cells of the colonic mucosa appeared to decrease after administration of the anti-IL-12 antibodies [46].

**Anti-IFN-γ antibody**

Fontolizumab (HuZAF) is a subcutaneously administered humanized monoclonal antibody against IFN-γ. In a first RCT, 45 patients with moderate-to-severe CD received single doses of fontolizumab (0.1, 1.0 or 4.0 mg/kg) or placebo. By day 29, patients responding to the single initial dose were randomised again to receive three further doses of one half their initial dose of fontolizumab or placebo at 4 weekly intervals [47]. The primary outcome of this study was safety and tolerability. There was a slight increase in the chills and flu-like symptoms reported among the patients administered 1.0 and 4.0 mg/kg fontolizumab. Two serious adverse events where there was worsening of CD were reported. No difference in the CD activity was noted among the different treatment groups [47]. In a larger RCT, 133 patients with moderate-to-severe CD were randomized to receive 1 (n = 42) or 2 infusions of fontolizumab (4 or 10 mg/Kg) at days 0 and 28 (n = 91) [48]. The primary endpoint in this trial was clinical response at day 28, which was not achieved. However, a post-hoc analysis showed that patients in the double-infusions groups with a CRP higher than or equal to 10 mg/dl achieved statistically significant higher remission and response rates than placebo. Two grade 3 adverse events that were considered to be related to CD were reported [48].

**Anti-inflammatory cytokines**

As the inflammatory bowel diseases result from aberrant or excessive activation of the immune response, researchers have investigated the efficacy of boosting the effects of anti-inflammatory cytokines as a therapeutic strategy.

**IL-10**

IL-10-deficient mice develop a CD-like chronic intestinal inflammation. As IL-10 is an anti-inflammatory cytokine, the efficacy of administration of recombinant human IL-10 (rhuIL10) has been tested.

**Patients with CD**

In the first clinical trial, 46 patients with active steroid-resistant CD were randomized to receive intravenous rhuIL10 (0.5, 1.0, 5.0, 10, or 25 μg/kg) or placebo once daily for 7 consecutive days. During a 3-week follow-up period, 50% of patients in the IL-10 group experienced remission compared with 23% in the placebo group. No serious adverse events were reported [49].

In a subsequent trial, the safety and the efficacy of subcutaneous administration of rhuIL10 (1.0, 4.0, or 8.0 μg/kg) were evaluated in 329 patients with steroid-refractory CD. No significant differences in the remission rate were observed between rhuIL10 and placebo in this trial [50]. Subcutaneous administration of rhuIL10 (1.0, 5.0, 10, or 20 μg/kg) also failed to demonstrate efficacy in an RCT involving 95 patients with active mild-to-moderate CD who were not on corticosteroids, immunosuppressive drugs or mesalamine. Furthermore, in this trial, reversible anemia and thrombocytopenia were observed at the higher doses of rhuIL10 [51].

In a phase II trial involving 65 CD patients with previous ileal or ileo-colonic resection, patients were administered intravenous rHuIL10 at dose of 4 μg/kg once daily (n = 22) or 8 μg/kg twice daily (n = 21) versus placebo (n = 22). Neither of these treatment regimens prevented endoscopic...
recurrence [52]. Finally, in a preliminary trial on 10 CD patients with no placebo control, oral administration of genetically modified lactococcus bacteria secreting IL-10 has been tested obtaining good preliminary results in terms of safety and efficacy [53].

**Patients with UC**

In a phase II trial that has only been published in abstract form, rHuIL10 failed to demonstrate efficacy in 94 patients with mild to moderately active UC [54].

**IL-11**

IL-11 is produced by mesenchymal cells, and has both anti-inflammatory and mucosal protective effects [55]. In a preliminary evaluation, 76 patients with active CD were randomized to receive subcutaneous rhuIL11 (5, 16, or 40 μg/kg) or placebo, two or five times/week for 3 weeks [56]. A good safety profile was observed for rhuIL11, with the only effect observed being an increase in platelet count, especially in patients administered the higher doses of rhuIL11, i.e., 40 μg/kg for two or five weekly doses, or 16 microg/kg for 5 weekly doses. The highest response rate was observed in the groups of patients administered 16 μg/kg rhuIL11 (33–42% vs. 7% in the placebo group).

In a second RCT, 148 patients with mild to moderately active CD were randomized to receive 15 μg/kg rhuIL11 once weekly (n = 49), 7.5 microg/kg rhuIL11 twice weekly (n = 50) or placebo (n = 49), over a period of 6 weeks. Patients were not permitted to take steroids for the duration of the study. Patients administered the single weekly dose of 15 μg/kg rhuIL11 achieved a higher remission rate than patients in the placebo group (36.7% vs. 16.3%, respectively). Overall the treatment showed a good safety profile, although evidence of a dose-related thrombocytosis is a cause for some concern [57].

More recently, a comparison was made between subcutaneous rhuIL11 and prednisolone in a double-blind RCT involving 51 patients with active CD [58]. Patients were randomized to receive either subcutaneous rhuIL11 (1 mg once weekly) and prednisolone placebo tablets, or active prednisolone (60 mg/d) and rhuIL11 placebo, for 12 weeks. Prednisolone active or placebo tablets were tapered after 1 week. A significantly inferior response rate and rate of short-term remission was observed in patients administered rhuIL11 compared with prednisolone [58].

**Inhibitors of T cell activation (anti-CD40 L)**

Expression of CD40 ligand (CD40L) on the surface of T cells is triggered by interaction with an APC [59]. In addition, activated platelets can express CD40L and are the major source of the soluble form of CD40L (sCD40L) in IBD patients [60]. Both forms of CD40L can activate mesenchial cells (endothelial cells, fibroblasts), leading to production of cytokines and expression of adhesion molecules [61,62].

Testing of a humanized anti CD40L antibody (IDEC-131) had reached phase II clinical trials in patients with CD, but the trial was discontinued due to the occurrence of thromboembolic events [63].

**Anti-CD4 therapy**

Activation of CD4-positive T cells (T-helper cells) has been observed in patients with IBD. Three monoclonal antibodies against CD4 have been developed: cM-T412, MAX.16H5 and BF-5. They have been tested in four phase I studies on UC and CD patients with encouraging preliminary results [64,65], although two of the studies have only been published in abstract form (Deusch K et al., Gastroenterology 1993; 104: A691 and Emmrich J et al., Gastroenterology 1995; 108: A815). The occurrence of significant reduction of CD4+ cells in the blood, although not apparently accompanied by a significant increase in opportunistic infections, led to the discontinuation of investigation of these agents.

**Growth Factors**

Growth factors have been shown to decrease mucosal permeability of the bowel and to favor mucosal healing after damage has occurred [66].

**Growth hormone**

In a small RCT, 37 adult patients with moderate-to-severe CD were randomized to receive subcutaneous administration of growth hormone (GH; n = 19; 5 mg/d as loading dose for one week, followed by a maintenance dose of 1.5 mg/d) or placebo (n = 18) for 4 months. At the end of this period, a significant decrease in CDAI score from baseline was observed in the group administered GH compared with placebo [67].

**Keratinocyte growth factor-2**

In a phase II RCT, 88 patients with active UC were randomized to receive intravenous administration of the recombinant keratinocyte growth factor-2 (KGF-2) repifermin (1.0, 5.0, 10, 25, or 50 microg/kg) or placebo for 5 consecutive days. No differences were observed in the response rate and the rate of adverse events was observed between the two treatment groups after 4 weeks of treatment [68].

**Epidermal growth factor**

Patients with mild-to-moderate left-sided UC (n = 24) were randomized to receive daily enemas of epidermal growth factor (EGF; n = 12; 5 μg in 100 ml of an inert carrier), or enema with carrier alone (n = 12) for 14 days. Primary endpoint was clinical remission at week 2, defined by a St. Mark score of 4 or less without endoscopic signs of inflammation. At week 2, 10 of the 12 patients in the group that received the EGF enemas were in remission (primary endpoint) compared with 1 of the 12 patients in the control group (83% vs. 8%, p < 0.001). The potential neoplastic risk related to the proliferative stimulus induced by this agent remains to be assessed [69].
Immunomodulators

Interferons

Patients with CD

Only preliminary uncontrolled studies on a small number of patients with CD patients have been performed with either IFN-α-2a or IFN-α-2b [70-72]. No further controlled trials on interferons in CD patients have been described in the published literature.

Patients with UC

In the first uncontrolled study, 28 patients with moderate (29%) or severe (71%) UC received subcutaneous injection of IFN-α-2a. Complete clinical and endoscopic remission was achieved within 15 days and was maintained after 6 months of therapy in 23/28 (82%) patients [73]. In a subsequent open-label randomized trial, 32 active left-sided UC patients were randomized to receive subcutaneous injection of IFN-α-2a or prednisolone enemas for 30 days. Both treatment groups experienced significant improvements in the Powell-Tuck index and rectal histology score [74].

In an RCT, 60 patients with active UC (CAI > 6) were randomized to receive 0.5 μg/kg or 1 μg/kg of pegylated-IFN-α-2b or placebo once a week for 12 weeks. At week 12, IFN-α-2b failed to induce significantly higher clinical remission rates than placebo [75].

In a preliminary open study, 25 patients with active UC refractory to steroidal therapy received intravenous doses of 0.5 MIU human natural IFN-β daily or 1 MIU recombinant IFN-β-1a subcutaneously. Clinical remission was achieved by 22 of the 25 (88%) patients, with flu-like symptoms and hair loss as the most frequent adverse events [76]. Finally, in two RCT on 17 patients with moderately active UC [77] and 91 patients with active steroid-refractory UC [78], respectively, subcutaneous IFN-β-1a did not achieve greater clinical and endoscopic remission or steroid withdrawal compared with placebo.

MAPK inhibitors

The mitogen-activated protein kinases (MAPK) are a class of proteins that can activate the transcription factor NF-κB, leading to the production of TNF-α and other pro-inflammatory signalling molecules. They include p38 MAPK, JNK and ERK, which are strong intracellular mediators of inflammation [10]. Specific inhibitors of MAPK have been evaluated with variable results in small trials with patients with IBD.

RDP58

RDP58 is a nine D-amino acid peptide that blocks p28 MAPK and JNK, which is orally administered and is not systemically bioavailable. In two multicenter parallel RCTs, RDP58 was tested on a total of 127 patients with mild-to-moderate UC. In the first trial, 34 patients were randomized (1: 2 ratio) to receive placebo (n = 13) or RDP58 100 mg (n = 21). At 28 days, only 29% of the patients in the RDP58 100 mg group achieved clinical response (a CDAI score of < 3) vs. 46% in placebo group (p = 0.46). In the second trial, 93 patients were randomized (1: 1: 1 ratio) to receive daily administrations of placebo (n = 30), 200 mg RDP58 (n = 31), or 300 mg RDP58 (n = 32). Patients in both the RDP58 groups achieved significantly higher remission rates compared with placebo (71% and 72%, respectively, versus 43%, p = 0.016). No difference in the incidence of side effects was observed between the RDP58 and placebo groups [79]. In a subsequent phase II trial in patients with CD, RDP58 failed to demonstrate efficacy [80].

BIRB-796

BIRB-796 is a specific inhibitor of p38 MAPK [81]. In a recent multicenter RCT, 284 patients with moderate-to-severe CD were randomized to receive placebo or BIRB-796 (10, 20, 30, or 60 mg) twice daily for 8 weeks. BIRB 796 failed to achieve the primary and secondary endpoints, including clinical response, clinical remission and improvement in quality-of-life [82].

CNI-1493

CNI-1493 is a guanyldihydrazone small molecule that inhibits both p38 MAPK and JNK, and suppresses the activation of dendritic cells [83]. In a small trial, 12 patients with severe CD were randomized to receive 8 or 25 mg/m² CNI-1493 daily for 12 days. After 4 weeks, clinical response and remission were achieved by 67% and 25% of patients, respectively. After 8 weeks, 58% of the patients achieved clinical response and 42% clinical remission. No serious adverse events were reported, although liver toxicity was noted [84].

Inhibitors of transcription factors

A selective inhibitor of the transcription factor p65, which belongs to NF-κB, has been tested on 11 patients with steroid-resistant UC or colonic CD. Each patient received a single dose of a topical formulation of an antisense NF-κB oligonucleotide, while continuing mesalazine, antibiotics and/or azathioprine therapy. Of the patients in the treatment group, 71% of patients achieved clinical, endoscopic and histological response compared with 25% of the placebo group [85].

Inhibitors of pro-inflammatory cytokine receptors

Anti-IL6 receptor

A humanized monoclonal antibody that targets the IL-6 receptor (anti-IL6R, MRA) has been tested in a small trial involving 36 patients with active CD refractory to conventional therapies. Patients were randomized to receive twice weekly intravenous infusions of 8 mg/kg MRA, placebo, or MRA/placebo alternately for 12 weeks. The biweekly administration of 8 mg/Kg MRA resulted in a response rate of 80% compared with 31% of the patients administered placebo. At 2 weeks, a significant decrease in serum level of the acute phase reactants was also observed. The incidence of adverse events was similar across all the treatment groups [86].
Inhibitors of T cell proliferation

IL-2 is secreted by activated T-cells and interacts with its high affinity receptor IL-2R, thereby promoting cell survival and proliferation. Expression of the IL-2R α-chain (CD25) is upregulated in activated T cells, while it is virtually absent on resting T cells. Monoclonal antibodies that target CD25 or the ε chain of CD3 (the invariant chain of the T-cell antigen receptor complex) have recently been tested in patients with UC, having previously demonstrated efficacy in the treatment of graft-versus-host disease.

Basiliximab

Basiliximab (Simulect®) is a chimeric IgG1 monoclonal antibody that targets CD25. The blockade of CD25 is not associated with cytokine-release syndrome, due to the structure of CD25, which has no cytoplasmic tail. In an open label study, 10 patients with active UC despite steroid treatment received a single intravenous bolus of basiliximab (40 mg) plus steroids. Clinical remission at 8 weeks was achieved by 9 of the 10 (90%) patients, but of these, all but one relapsed within a median time of 9 weeks [87].

In a more recent open study [88], 20 patients with moderate (n = 13) or severe (n = 7) active UC, despite treatment with prednisone, received a single intravenous infusion of basiliximab (40 mg). At week 8, 10 of the 20 patients (50%) were in clinical remission (7 of the 13 with moderate UC and 3 of the 7 patients with severe UC). At week 24, 9 of the 20 patients were steroid-free (45%), 13 (65%) were in clinical remission (3 of the 7 patients with severe UC and 10 of the 13 with moderate UC). Five of the patients required a colectomy (4 of the patients with severe UC, 1 of the patients with moderate UC) during follow up. Basiliximab was well tolerated, although adverse events were reported, including pyrexia, transient photosensitivity and lethargy, mild paraesthesia of the feet, loin pain and infection of the upper respiratory tract [87,88]. These results indicate that basiliximab made the patients steroid-sensitive, suggesting the opportunity of a combination therapeutic with steroids.

Daclizumab

Daclizumab (Zenapax®) is a humanized IgG1 monoclonal antibody that targets CD25. In an open-label study, ten patients with UC involving moderate colitis refractory to medical treatment received two intravenous infusions 1 mg/kg daclizumab. After 8 weeks, clinical remission was achieved by five of the ten (50%) patients, while eight of the ten (80%) patients achieved the primary endpoint of a decreased score of ≥ 4 points or remission [89]. A recently published RCT studied 159 patients with moderately active UC, who were randomized in a 1: 1: 1 ratio to receive intravenous infusion of placebo, or: (i) 1 mg/kg daclizumab, at weeks 0 and 4, alternating with placebo at weeks 2 and 6; or (ii) 2 mg/kg daclizumab at weeks 0, 2, 4, 6. At week 8, clinical remission was achieved in 2% of the patients administered 1 mg/kg daclizumab, compared with 7% of patients administered 2 mg/kg daclizumab and 10% of patients administered placebo (p = 0.11 and p = 0.73, respectively; versus placebo). The incidence of adverse events was similar between the three groups [90].

Visilizumab

Visilizumab (Nuvion®, HuM291) is a humanized IgG2 monoclonal antibody that binds to the ε chain of CD3 (the invariant chain of the T-cell antigen receptor complex), inducing the apoptosis of T cells. In a phase I study, patients with severe steroid-resistant UC received two infusions of visilizumab (15 μg/kg) one day apart. In this study, all of the patients achieved clinical and endoscopic remission [91]. In a further phase I study, 32 patients with severe steroid-refractory UC received intravenous injections of 10 or 15 μg/kg visilizumab on 2 consecutive days. On day 30, 84% of the patients had achieved clinical response, 41% were in clinical remission and 44% were in endoscopic remission. In addition, 45% of the patients did not require salvage therapy during the first year of follow-up. In both of these studies, a transient mild-to-moderate cytokine-release syndrome (chills, headache, pyrexia, fatigue and arthralgia), a transient decrease in T lymphocytes (with a recovery time of about 3 weeks) and transient elevation of the EBV viral load were routinely observed at each infusion. No opportunistic infections were reported [92]. The transient but nonetheless relevant leukopenia may limit the use of this biological agent in patients with a severe attack of UC, who are potentially at risk for the development of multiple organ dysfunction.

Conclusions

Over the last decade, advances in bio-technology together with the growth in our knowledge of the pathogenic mechanisms underlying chronic inflammation in the gut have led to the development of a large number of biological agents. Of these, only TNF-α inhibitors (infliximab, adalimumab) are currently approved for the treatment of IBD. Natalizumab has shown very promising results for the treatment of CD, but safety concerns, in particular the existence of a 1 to 1,000 risk of developing PML has limited the use and approval of this therapeutic. As is currently the case for multiple sclerosis, the use of natalizumab may be limited to patients with severe CD after failure (including intolerance) of all other available therapies. Results from small preliminary trials of antibodies against IL12 and the IL-6R in patients with CD have been promising, but further studies on a larger number of patients are required. In patients with UC, basiliximab has shown efficacy in association with steroids for the treatment of moderate steroid-refractory UC, indicating a potential role in inducing sensitivity to steroids.

No studies published thus far have described a head-to-head comparison of TNF-α inhibitors and newer biological agents belonging to other classes, despite the demonstration that some agents, such as natalizumab, show efficacy in subgroups of patients previously or contemporarily receiving infliximab. Finally, given that the efficacy, short-term and long-term safety for the newer biological therapeutics are either very limited or remain to be proven on a large number of patients, the use of such agents in patients remains to be justified, given their high costs.

Conflict of interest:

Silvio Danese and Erika Angelucci have no conflict of interest.


Emerging biologicals in IBD


