POINT-BY-POINT RESPONSES TO THE REVIEWERS' COMMENTS

Akiho et al. (Manuscript ID : 20796)

Title: Promising biological therapies for ulcerative colitis: A review of the literature.

We are very grateful for the comments, suggestions raised by the reviewers which we found to be insightful and very constructive. The followings are our responses to the reviewers' comments. We believe we have adequately answered all of the questions raised by the reviewers and the editor. The excerpts from the revised manuscript in this letter are shown in blue.

Response to the comments raised by the Reviewer #1

Comment 1: It is recommended the title: Promising Biological Therapies For Ulcerative Colitis: A review of the literature.

Answer: According to the reviewer's suggestion, we have changed the title as follows.

Promising biological therapies for ulcerative colitis: A review of the literature.

Comment 2: It is recommended that the authors, to describe the chemical formula of the novel drugs to treat UC; to explain details of the pharmacokinetics and pharmacodynamics for each drug. It is advisable to develop a figure or scheme in which the display mechanism by which these novel drugs act in patients with UC; also present the different patterns of absorption, metabolism and distribution and their mechanisms of action.

Answer: According to the reviewer's suggestion, we have included additional sentences in the "ANTI-TNF-α AGENTS" section as follows.

TNF has been known to play a pivotal role in the pathogenesis of IBD [8]. When released by active macrophages and T lymphocytes, TNF initiates multiple biological reactions below: modulates immune cell function, drives adaptive immune responses, triggers epithelium apoptosis and breaks epithelial barrier, induces endothelium expressing adhesion molecules such as intercellular adhesion molecule 1 to recruit immune cells, and regulates matrix

metalloproteinase expression to induce tissue degradation and damage [9,10].

According to the reviewer's suggestion, we have included additional sentences in the "*Infliximab*" section as follows.

As the first monoclonal TNF antibody approved for human treatment, infliximab is a purified, recombinant DNA-derived chimeric human-mouse IgG monoclonal antibody and contains murine heavy (H) and light (L) chain variable regions (VH and VL, resp.), ligated to genomic human heavy and light chain constant regions [11,12]. Infliximab can quickly form stable complexes with the human soluble or the membrane form of TNF and terminate the biological activity and signals of TNF [13]. With a serum half-life of 9.5 days and still detectable in serum of IBD patients 8 weeks after infusion treatment, infliximab provides a useful strategy to neutralize TNF and to inhibit immune responses of IBD [14].

According to the reviewer's suggestion, we have included additional sentences in the "*Adalimumab*" section as follows.

Adalimumab is a complete human IgG1 anti-TNFα monoclonal Ab that has been generated through repertoire cloning. It binds to the soluble and transmembrane forms of TNFα with high affinity, thereby preventing TNFα from binding to its receptors. In vitro studies have also demonstrated its effect on the induction of cell lysis and apoptosis [15]. It is generally administered at a dose of 40 mg subcutaneously every 2 weeks, or at higher doses administered once a week. It is indicated for use in rheumatoid arthritis, psoriasis, ankylosing spondylitis, and moderate to severe Crohn's disease .

According to the reviewer's suggestion, we have included additional sentences in the "*Golimumab*" section as follows.

The affinity of golimumab for soluble TNFα was similar to that of etanercept and greater than those of infliximab and adalimumab (2.4-fold and 7.1-fold, respectively). A similar pattern was observed regarding golimumab neutralization of soluble TNFα in the cytotoxicity and endothelial cell activation assays. The IC_{50} values for golimumab were comparable to those for etanercept and ranged from 2.5- to 5.7-fold lower than those for infliximab and adalimumab. These in vitro bioassays suggest that a lower serum concentration of golimumab, compared with infliximab or adalimumab, would provide similar pharmacological effects in patients [19].

According to the reviewer's suggestion, we have included additional figure.

Figure 1

A mechanism of action that works to reduce inflammation in the gastrointestinal (GI) tract.

Vedolizumab selectively inhibits the movement of a discrete subset of T lymphocytes that preferentially migrate into inflamed GI tissue.

Vedolizumab specifically binds to the α4β7 integrin, blocking its interaction with mucosal addressin cell adhesion molecule-1 (MAdCAM-1), which is mainly expressed on gut endothelial cells.

This interaction facilitates lymphocyte homing to the gut and is an important contributor to inflammation that is a hallmark of UC.

Comment 3: Summarizing Table 1 to make it more legible and understandable. Draw up a Table 1, showing the family of each group of drugs, and present both positive and negative outcomes associated with them.

Answer: According to the reviewer's suggestion, we have divided the Table as follows.

Table 1. Clinical trials evaluating the efficacy of anti-TNF-α agents in UC patients

Table 2 Clinical trials evaluating the efficacy of JAK inhibitor and integrin antagonists in UC patients

Comment 4: Drawing conclusions based on the percentage of patients who improve with each of the drugs described; the beneficial effects and side effects described.

Answer: The other two reviewers did not mention about it, so we did not change the conclusions based on the percentage of patients who improve with each of the drugs.

Answer: According to the reviewer's suggestion, we have included additional sentences in the "Safety" section as follows.

Safety

Recent studies have shown that a few patients experience adverse events with biological agents. For adverse events, such as infections, neoplasms are related to the immunosuppressive effects of biological agents. Patients who are administered biological agents frequently develop antibodies against these drugs. This problem is more frequent with chimeric agents like infliximab than

fully humanized agents like adalimumab.

Infliximab

Infliximab is a chimeric monoclonal antibody with a protein sequence that is 75% human and 25% mouse; therefore, human antichimeric antibody formation can occur in the blood. The presence of human antichimeric antibody is associated with an increased risk of infusion reactions during administration and reduced clinical efficacy. The common adverse events of infliximab are acute infusion reaction, and infection such as reactivation of tuberculosis.

As with other immunomodulatory drugs, infliximab therapy increases the risk of developing non-serious infections (RR ~2); however, the data on serious infections are inconsistent [36]. Examples of reported serious infections include sepsis, pneumonia, cellulitis and intra-abdominal abscess [37]. Thus, infliximab should not be administered to a patient who has a clinically active infection. Patients who are at a high risk of chronic hepatitis B infection should be screened before the initiation of infliximab therapy.

Approximately 10% of infliximab infusions are associated with mild reactions such as headache, dizziness, fever, chills, chest pain, cough dyspnea or pruritus. These reactions occur within 1–2 h after infusion and can be alleviated by reducing the rate of infusion or by pretreatment with an H1-receptor antagonist [36,37]. In the ACT 1 and ACT 2 trials, 11.4% of the patients receiving infliximab experienced infusion reactions (44 of 484), compared with 9.4% of those receiving a placebo (23 of 244) [5].

For reasons that are unclear, 1 in 1000 infliximab infusions results in a serious reaction [37]. Delayed hypersensitivity-like reactions (serum sickness-like disorders) can occur 3–14 days after episodic infliximab infusions and include, but are not limited to, myalgia, fever, rash, pruritus, dysphagia, urticaria and headache [37]. In the ACT 1 and ACT 2 trials, three patients who received either 5 or 10 mg/kg infliximab had delayed hypersensitivity reactions (n=484), as compared with two patients in the placebo study group (n=244) [5].

Cases of aplastic anemia, pancytopenia, vasculitis, hepatitis, reversible mono/polyneuropathy and demyelination have been attributed to infliximab therapy [38].

At present, there is no consensus regarding the estimated lymphoma risk

for patients treated with infliximab [36]. However, most experts believe that immunosuppression does impart some small cumulative risk of malignancy. The development of hepatosplenic T-cell lymphoma, a rare malignancy, has been reported in pediatric patients receiving infliximab treatment for Crohn's disease in the United States [38,39].

Adalimumab

A total of 1010 patients received at least one dose of adalimumab in the ULTRA 1, 2 and 3 trials. The most frequently reported serious adverse event was worsening or flare of UC. Two serious events of cytomegalovirus colitis were reported. After the double-blind study period, one serious infection of tuberculosis and two treatment-emergent fatal adverse events were reported. Three events of B-cell lymphoma occurred during ULTRA 3. All three patients had a history of smoking and either previous or concomitant azathioprine use [18].

Golimumab

The most commonly observed adverse events in golimumab- and placebo-treated patients were headache and nasopharyngitis. Overall, the incidences of serious adverse events (3.0% vs 6.1%), including serious infections (0.5% vs 1.8%), were also similar, respectively, for golimumab- and placebo-treated patients. The most common serious adverse event was the exacerbation of UC, reported by eight (1.1%) golimumab-treated and eight (2.4%) placebo-treated patients. The only serious infection reported by more than one patient was pneumonia (one receiving 200/100 mg golimumab and one placebo patient). One patient (400/200 mg) died from peritonitis and sepsis after surgical complications related to an ischiorectal abscess and subsequent bowel perforation after surgery; this patient was receiving concomitant 20 mg prednisolone. One patient (400/200 mg) had a demyelinating disorder reported after the patient completed PURSUIT-SC induction and subsequently was randomized to placebo in the maintenance study. Two opportunistic infections were reported up to week 6: esophageal candidiasis (400/200 mg golimumab) and cytomegalovirus infection (placebo). Neither event was reported as serious. No patient developed active tuberculosis [20].

Tofacitinib

The most commonly reported adverse events related to infection were influenza and nasopharyngitis (in six patients each). Two patients receiving 10 mg tofacitinib twice daily had serious adverse events from infection (postoperative abscess in one and anal abscess in the other). There was a dose-dependent increase in both LDL and HDL cholesterol concentrations at 8 weeks with tofacitinib, which reversed after discontinuation of the study drug. During the study period, the absolute neutrophil count was <1500 cells/mm^3 in three patients receiving tofacitinib (one at a dose of 10 mg twice daily and two at a dose of 15 mg twice daily); it was <1000 cells/mm^3 in none of the patients [21].

Vedolizumab

In the large GEMINI I study, no significant difference was observed among the study groups for the most commonly reported adverse events: namely, flare of UC, headache, nasopharyngitis and arthralgia. Serious infections were no more common with vedolizumab than with placebo. No cases of PML occurred. No significant differences in hematological or serum chemical profiles or liver function test results were identified among the study groups.

Clinically important infusion reactions were rare; three cases (two with detectable anti-vedolizumab antibodies) resulted in drug discontinuation. No cases of anaphylaxis or serum sickness were observed [32].

Etrolizumab

Patients in the 100 mg etrolizumab group had higher rates of rash, influenza-like illness, and arthralgia than did those in the placebo or 300 mg etrolizumab plus loading dose (LD) groups; all of these events were regarded as mild to moderate in severity. Serious adverse events were reported in 12 patients; five of these were related to UC (two in the 100 mg etrolizumab group; one in the 300 mg etrolizumab plus LD group; and two in the placebo group; Appendix). No serious opportunistic infections were reported. Mild injection site reactions occurred in four patients in the 300 mg etrolizumab plus LD group and in two patients in the placebo group [35].

Comment 5: It is recommended that authors increase the number of articles that support the conclusions presented in this manuscript.

Answer: According to the reviewer's suggestion, we have included additional 14 references.

Response to the comments raised by the Reviewer #2

Comments 1: Should not mention Crohn's disease as the review is only about ulcerative colitis.

Answer: According to the reviewer's suggestion, we have deleted mention about Crohn's disease.

Comments 2: Faecal calprotectin can, and SHOULD, be used in monitoring.

Answer: According to the reviewer's suggestion, we have included faecal calprotectin in the "INTRODUCTION" section as follows.

including biomarkers such as C-reactive protein, faecal calprotectin, and the histological resolution of active inflammation in UC [3,4].

Comments 3: More detail on important side effects and limitations would be helpful.

Answer: According to the reviewer's suggestion, we have included additional sentences in the "Safety" section.

Comments 4: I found the discussion of the ULTRA 1 and 2 trials confusing.

Answer: ULTRA 1 was an 8-week clinical trial investigating the use of adalimumab as induction therapy. ULTRA 2 was a 52-week clinical trial investigating the use of adalimumab as maintenance therapy.

According to the reviewer's suggestion, we have included additional sentences in the "*Adalimumab*" section as follows.

In ULTRA 2, a 52-week randomized controlled study investigating the use of adalimumab as maintenance therapy

Comments 5: What is NONRESPONDER IMPUTATION?

Answer: According to the reviewer's suggestion, we have included additional sentences describing nonresponder imputation in the "*Adalimumab*" section as follows.

Nonresponder imputation method is used for dichotomous ("yes or no") or categorical variables, if a subject drops out of a study, that subject is assumed to be a non-responder, regardless of whether or not the subject was responding to treatment at the time of dropout.

Comments 6: Is etrolizumab gut specific?

Answer: Yes.

Answer: According to the reviewer's suggestion, we have included "in the intestine" in the "*Etrolizumab*" section as follows.

Etrolizumab is an IgG1 humanized monoclonal antibody that selectively binds the subunit of the α4β7 and the $\alpha_E\beta7$ integrin heterodimers in the intestine.

Response to the comments raised by the Reviewer #3

Comments 1: Additional description is needed about side effects of biological therapies.

Answer: According to the reviewer's suggestion, we have included additional sentences in the "Safety" section.

Response to the comments raised by Editor in chief:

Comments: The authors need to state the outcome of the studies mentioned with regards to infliximab, adalimumab, golimumab, etc....

Answer: According to editor in chief's suggestion, we have included additional sentences describing nonresponder imputation in the ***"infliximab", "Adalimumab", "Golimumab", "Tofacitinib", "Vedolizumab", "Etrolizumab"*** section as follows.

infliximab In ACT 1, 69.4% of patients who received 5 mg infliximab and 61.5% of those who received 10 mg had a clinical response at week 8, as compared with 37.2% of those who received placebo ($P < 0.001$ for both comparisons with placebo). In ACT 2, 64.5% of patients who received 5 mg infliximab and 69.2% of those who received 10 mg had a clinical response at week 8, as compared with 29.3% of those who received placebo ($P < 0.001$ for both comparisons with placebo). In both studies, patients who received infliximab were more likely to

have a clinical response at week 30 ($P \leq 0.002$ for all comparisons). In ACT 1, more patients who received 5 or 10 mg infliximab had a clinical response at week 54 (45.5% and 44.3%, respectively) than did those who received placebo[5]. The results of ACT 1 and ACT 2 showed that infliximab had superior clinical efficacy compared with placebo, both in induction and maintenance phases.

Adalimumab ULTRA 1 was an 8-wk clinical trial investigating the use of adalimumab as induction therapy in patients with moderate to severe UC despite conventional therapy[17]. In this trial, 576 patients were divided into 160/80 mg and 80/40 mg groups, based on the loading dose, and then compared with the placebo group. At the end of 8 wk, the clinical remission rate of patients receiving adalimumab was twice that of the placebo group (P = 0.031). There was no significant difference in remission rates between patients receiving adalimumab 80/40 mg and placebo (P = 0.833). In ULTRA 2, a 52-wk randomized controlled study investigating the use of adalimumab as maintenance therapy, 494 patients were divided into 160/80 mg adalimumab and placebo groups. Overall rates of clinical remission at week 8 were 16.5% on adalimumab and 9.3% on placebo (P = 0.019); corresponding values for week 52 were 17.3% and 8.5% (P = 0.004). Among anti-TNF-α-naïve patients, rates of remission at week 8 were 21.3% on adalimumab and 11% on placebo (P = 0.017); corresponding values for week 52 were 22% and 12.4% (P = 0.029). Among patients who had previously received anti-TNF-α agents, rates of remission at week 8 were 9.2% on adalimumab and 6.9% on placebo (P = 0.559); corresponding values for week 52 were 10.2% and 3% (P = 0.039). Importantly, on sub-analysis, it was observed that the anti-TNF-α-naïve group exhibited approximately two times higher clinical remission rates at week 8 and week 52, compared with the placebo group. Though it is not direct comparison, infliximab is more likely to induce a favorable clinical outcome than adalimumab. The dose of adalimumab trough level might not enough to induce remission and maintenance for ulcerative colitis. More date are needed for dose escalation of adalimumab.

Up to 4 years of data for adalimumab-treated patients from ULTRA 1 and 2, and the open-label extension ULTRA 3 have been presented[18]. A total of 600/1094 patients enrolled in ULTRA 1 or 2 were randomized to receive

adalimumab and induced in the intent to treat analyses. Of these, 199 patients remained on adalimumab after 4 years follow-up. Rates of remission according to partial Mayo score, remission according to inflammatory bowel disease questionnaire score, mucosal healing, and corticosteroid discontinuation at week 208 were 24.7%, 26.3%, 27.7% (nonresponder imputation), and 59.2% (observed), respectively. Of the patients who were followed up in ULTRA 3 (588/1094), a total of 360 patients remained on adalimumab 3 years later. Remission according to partial Mayo score and mucosal healing after ULTRA 1 or 2 to year 3 of ULTRA 3 were maintained by 63.6% and 59.9% of patients, respectively (nonresponder imputation). Nonresponder imputation method is used for dichotomous ("yes or no") or categorical variables, if a subject drops out of a study, that subject is assumed to be a non-responder, regardless of whether or not the subject was responding to treatment at the time of dropout.

Golimumab In PURSUIT-SC, 774 patients were randomized to receive golimumab at week 6. The clinical response and remission rates showed a significant change in both the golimumab 200/100 mg and 400/200 mg groups ($P < 0.0001$)[10]. In PURSUIT-M, 464 patients who had responded to golimumab induction therapy in PURSUIT-SC were randomized to receive placebo or golimumab 50/100 mg every 4 weeks for 52 weeks. Clinical response was maintained through week 54 in 47.0% of patients receiving 50 mg golimumab, 49.7% of patients receiving 100 mg golimumab, and 31.2% of patients receiving placebo ($P = 0.010$ and $P < 0.001$, respectively). At weeks 30 and 54, a higher percentage of patients who received 100 mg golimumab were in clinical remission and had mucosal healing (27.8% and 42.4%) than patients given placebo (15.6% and 26.6%; $P = 0.004$ and $P = 0.002$, respectively) or 50 mg golimumab (23.2% and 41.7%, respectively)[7]. Though PURSUIT-M had included only persons who responded to induction in its maintenance phase, golimumab is more likely to induce a favorable clinical outcome than adalimumab.

Tofacitinib The primary outcome, clinical response at 8 wk, occurred in 32%, 48%, 61% and 78% of patients receiving tofacitinib at a dose of 0.5 mg ($P = 0.39$), 3 mg ($P = 0.55$), 10 mg ($P = 0.10$), and 15 mg ($P < 0.001$), respectively, as

compared with 42% of patients receiving placebo. Clinical remission at 8 wk occurred in 13%, 33%, 48% and 41% of patients receiving tofacitinib at a dose of 0.5 mg (P = 0.76), 3 mg (P = 0.01), 10 mg (P < 0.001), and 15 mg (P < 0.001), respectively, as compared with 10% of patients receiving placebo[21]. Though the study population is small, 15 mg of tofacitinib showed most superior clinical response rate in induction phase than the other biological agents for ulcerative colitis.

Vedolizumab Response rates at week 6 were 47.1% and 25.5% among patients in the vedolizumab and placebo groups, respectively (difference with adjustment for stratification factors, 21.7% points; 95%CI: 11.6–31.7; P < 0.001). At week 52, 41.8% of patients who continued to receive vedolizumab every 8 wk and 44.8% of patients who continued to receive vedolizumab every 4 wk were in clinical remission (Mayo Clinic score ≤ 2 and no subscore > 1), as compared with 15.9% of patients who switched to placebo [adjusted difference, 26.1% points for vedolizumab every 8 wk *vs* placebo (95%CI: 14.9–37.2; P < 0.001) and 29.1% points for vedolizumab every 4 wk *vs* placebo (95%CI: 17.9–40.4; P < 0.001)]. The frequency of adverse events was similar between the vedolizumab and placebo groups.

Etrolizumab Clinical remission occurred at week 10 in 20.5% of patients in the etrolizumab 100 mg group (P = 0.004), 10.3% of patients in the etrolizumab 420 mg loading dose group (P = 0.048), and no patients in the placebo group. The study population is so small, more studies are needed to confirm these data.